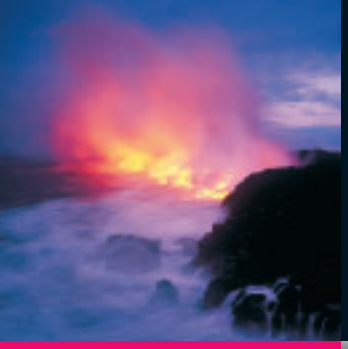

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Contents

Introduction

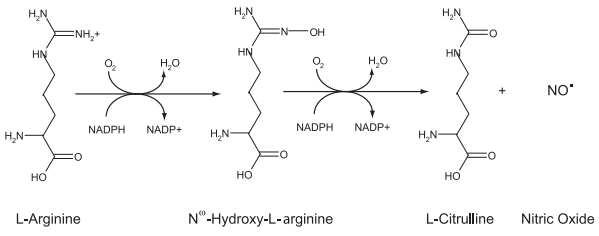
Nitric Oxide (NO•)

Nitric oxide (NO•) is a gaseous, electrically free radical which displays bioactivity. Because of its chemical properties it can easily diffuse across cell membranes and between cells. In this way it functions as an intracellular and intercellular signalling molecule. Evidence for the biological activity of NO• was first described in 1980 when Furchgott and Zawadski reported the existence of an endothelium-dependent relaxing factor for smooth muscles [1], which was identified seven years later as NO• [2-4]. Once considered to be a toxic by-product, today it is clear that NO• is important for a wide range of physiological processes, including the functionality of neurons and the immune response. NO• also contributes to certain pathophysiologies.

Nitric Oxide Synthases (NOS)

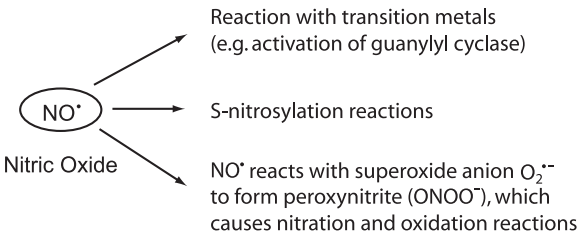
Nitric oxide synthases (NOS, EC 1.14.13.39) [5] catalyze the biosynthesis of NO• and L-citrulline from the amino acid L-arginine [5, 6]. The overall reaction occurs in two defined steps and overall involves a five-electron oxidation of L-arginine requiring NADPH and $O_2^{\bullet-}$ as co-substrates in each step. All NOS isoforms are homodimeric and bind FAD, FMN [7, 8] and protoporphyrin IX heme [9, 10]. One bound tetrahydrobiopterin (BH4) per monomer is also required for full activity [7, 11].

Three members of the NOS family are well characterized. These isoforms are known as neuronal NOS (nNOS; NOS I) [12, 13], endothelial NOS (eNOS; NOS III) [14, 15] and inducible NOS (iNOS; NOS II) [16-18]. The role and existence of mitochondrial NOS (mtNOS) [19, 20], an isoform of nNOS, is a matter of debate. While nNOS is predominantly expressed in neurons of the brain and peripheral nervous system, eNOS is mainly found in endothelial cells. Both isoforms are constitutively expressed but Ca^{2+}-calmodulin-dependent. Thus, nNOS and eNOS produce transient and short living levels of active NO•. In contrast, iNOS expression is inducible but Ca^{2+}-calmodulin-independent [21]. Hence, iNOS can constantly produce high levels of NO• to sustain physiological duties and to contribute to certain pathologies. Murine and human iNOS are both thought to be regulated primarily on the transcriptional level, while their expression respond differently to stimuli such as proinflammatory cytokines, microbial lipopolysaccharides (LPS), dsRNA, and hypoxia.



Cellular Interactions and Effects of Nitric Oxide

Three main interactions are promoted by NO•. Firstly, NO• reacts with transition metals of the prosthetic group of enzymes to modulate their activity. The most prominent example of such kind of enzymatic targets is soluble guanylyl cyclase (sGC), which mediates many effects of NO• (see page 34). Similar targets are the cyclo-oxygenase and mitochondrial cytochromes. Secondly, NO• alters the activity of certain proteins by S-nitrosylation. There is increasing attention paid to this selective modification of cysteine residues in proteins. Accordingly, S-nitrosylation has been found recently to be important for processes such as the regulation of eNOS by HSP90 [22], iNOS mediated activation of COX-2 [23], dynamin mediated endocytosis [24], CREB-regulated gene expression in neurons [25], GAPDH-mediated apoptosis [26, 27] (see also page 5), and blocking of the neuroprotective effect of protein-disulphide isomerase (PDI) during ER stress [28]. Thirdly, NO• reacts with superoxide anion ($O_2^{\bullet-}$), a reactive oxygen species (ROS) to form peroxynitrite, which exists under physiological conditions both in the protonated (ONOOH) and anionic ($ONOO^-$) form. Peroxynitrite modifies biomolecules either directly, or indirectly by radicals formed after its decomposition. Diverse damages by oxidation of proteins, lipids, and DNA, as well as damages of enzymes by nitration have been reported. High levels of peroxynitrite lead to NADH and energy depletion, swelling, calcium release, and finally necrotic cell death (see also page 5).



Literature References:

[1] The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine: R. F. Furchgott & J. V. Zawadzki; Nature **288,** 373 (1980) ▪ **[2]** Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide: L. J. Ignarro, et al.; PNAS **84,** 9265 (1987) ▪ **[3]** Endothelium-derived relaxing factor from pulmonary artery and vein possesses pharmacologic and chemical properties identical to those of nitric oxide radical: L. J. Ignarro, et al.; Circ. Res. **61,** 866 (1987) ▪ **[4]** Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor: R. M. Palmer, et al.; Nature **327,** 524 (1987) ▪ **[5]** Nitric oxide as a secretory product of mammalian cells: C. Nathan; FASEB J. **6,** 3051 (1992) ▪ **[6]** Catalysis by nitric oxide synthase: M.A. Marletta, et al.; Curr. Opin. Chem. Biol. **2,** 656 (1998) ▪ **[7]** Purification of the inducible murine macrophage nitric oxide synthase. Identification as a flavoprotein: J.M. Hevel, et al.; J. Biol. Chem. **266,** 22789 (1991) ▪ **[8]** Purification and characterization of the cytokine-induced macrophage nitric oxide synthase: an FAD- and FMN-containing flavoprotein: D.J. Stuehr, et al.; PNAS **88,** 7773 (1991) ▪ **[9]** Cloned, expressed rat cerebellar nitric oxide synthase contains stoichiometric amounts of heme, which binds carbon monoxide: K. McMillan, et al.; PNAS **89,** 11141 (1992) ▪ **[10]** Nitric oxide synthase is a cytochrome P-450 type hemoprotein: K.A. White & M.A. Marletta; Biochemistry **31,** 6627 (1992) ▪ **[11]** Ca2+/calmodulin-dependent NO synthase type I: a biopteroflavoprotein with Ca2+/calmodulin-independent diaphorase and reductase activities: H.H. Schmidt, et al.; Biochemistry **31,** 3243 (1992) ▪ **[12]** Structural organization of the human neuronal nitric oxide synthase gene (NOS1): A. V. Hall, et al.; J. Biol. Chem. **269,** 33082 (1994) ▪ **[13]** Cloned human brain nitric oxide synthase is highly expressed in skeletal muscle: M. Nakane, et al.; FEBS Lett. **316,** 175 (1993) ▪ **[14]** Cloning and expression of a cDNA encoding human endothelium-derived relaxing factor/nitric oxide synthase: S. P. Janssens, et al.; J. Biol. Chem. **267,** 14519 (1992) ▪ **[15]** Molecular cloning and characterization of human endothelial nitric oxide synthase: P. A. Marsden, et al.; FEBS Lett. **307,** 287 (1992) ▪ **[16]** Molecular cloning and expression of inducible nitric oxide synthase from human hepatocytes: D. A. Geller, et al.; PNAS **90,** 3491 (1993) ▪ **[17]** Purification and cDNA sequence of an inducible nitric oxide synthase from a human tumor cell line: P. A. Sherman, et al.; Biochemistry **32,** 11600 (1993) ▪ **[18]** Cloning, characterization, and expression of a cDNA encoding an inducible nitric oxide synthase from the human chondrocyte: I. G. Charles, et al.; PNAS **90,** 11419 (1993) ▪ **[19]** Purification and characterization of a nitric-oxide synthase from rat liver mitochondria: A. Tatoyan & C. Giulivi; J. Biol. Chem. **273,** 11044 (1998) ▪ **[20]** Immunocytochemical evidence for a mitochondrially located nitric oxide synthase in brain and liver: T. E. Bates, et al.; BBRC **213,** 896 (1995) ▪ **[21]** Calcium-dependent nitric oxide synthesis in endothelial cytosol is mediated by calmodulin: R. Busse & A. Mulsch; FEBS Lett. **265,** 133 (1990) ▪ **[22]** S-nitrosylation of Hsp90 promotes the inhibition of its ATPase and endothelial nitric oxide synthase regulatory activities: A. Martinez-Ruiz, et al.; PNAS **102,** 8525 (2005) ▪ **[23]** Inducible nitric oxide synthase binds, S-nitrosylates, and activates cyclooxygenase-2: S. F. Kim, et al.; Science **310,** 1966 (2005) ▪ **[24]** Nitric oxide regulates endocytosis by S-nitrosylation of dynamin: G. Wang, et al.; PNAS **103,** 1295 (2006) ▪ **[25]** A nitric oxide signaling pathway controls CREB-mediated gene expression in neurons: A. Riccio, et al.; Mol. Cell. **21,** 283 (2006) ▪ **[26]** S-nitrosylated GAPDH initiates apoptotic cell death by nuclear translocation following Siah1 binding: M. R. Hara, et al.; Nat. Cell Biol. **7,** 665 (2005) ▪ **[27]** Neuroprotection by pharmacologic blockade of the GAPDH death cascade: M. R. Hara, et al.; PNAS **103,** 3887 (2006) ▪ **[28]** S-nitrosylated protein-disulphide isomerase links protein misfolding to neurodegeneration: T. Uehara, et al.; Nature **441,** 513 (2006)

Selected Latest Review Articles (General)

The basics about nitric oxide: R. Bruckdorfer; Mol. Aspects Med. **26,** 3 (2005) ▪ Peroxynitrite and drug-dependent toxicity: A. Denicola & R. Radi; Toxicology **208,** 273 (2005) ▪ Nitric oxide is a signaling molecule that regulates gene expression: L.J. Hofseth, et al.; Methods Enzymol. **396,** 326 (2005) ▪ Critical overview of mitochondrial nitric-oxide synthase: K. Kato & C. Giulivi; Front. Biosci. **11,** 2725 (2006) ▪ Nitric oxide, a biological double-faced janus--is this good or bad?: T. Thippeswamy, et al.; Histol. Histopathol. **21,** 445 (2006) ▪ The regulation and pharmacology of endothelial nitric oxide synthase: D. M. Dudzinski, et al.; Annu. Rev. Pharmacol. Toxicol. **46,** 235 (2006) ▪ Nitric oxide and cell proliferation: A. Villalobo; FEBS J. **273,** 2329 (2006)

Physiological Effects of Nitric Oxide

Overview

A wide array of homeostatic processes are mediated by endogenously generated nitric oxide ($NO^{\bullet}$). Well known are i) the regulation of the vascular tone and maintenance of the vessel wall through $NO^{\bullet}$ action on solubal guanylyl cyclase (sGC), ii) development and maintenance of new blood vessels (angiogenesis) important for wound healing as well as tumor growth, iii) anti-inflammatory effects such as inhibition of platelet aggregation and reduction of leukocyte adhesion, as well as iv) the role of $NO^{\bullet}$ as a neurotransmitter in the nervous system. However, $NO^{\bullet}$-deregulation contributes to different diseases such as neurodegeneration, hypertension and stroke, heart diseases, and erectile dysfunction. For further information see selected review articles listed below.

The differences in physiological functions of the NOS isoforms

nNOS/NOS I	iNOS/NOS II	eNOS/NOS III
Neurotransmitter in GI tract	Non-specific immune response to microorganisms	Regulates blood flow
Penile erection	Part of inflammatory response	Regulates blood pressure
Sphincter relaxation		Inhibits platelet activation
Blood flow		
Synapatic plasticity		
Modulates responses to glutamate		

Selected Review Articles

Nitric oxide and resolution of inflammation: S. Hortelano, et al.; Methods Enzymol. **359,** 459 (2002) ▪ NO and angiogenesis: J.P. Cooke; Atheroscler. Suppl. **4,** 53 (2003) ▪ Nitric oxide synthase inhibition in sepsis? Lessons learned from large-animal studies: B. Hauser, et al.; Anesth. Analg. **101,** 488 (2005) ▪ Regulation of immune responses by L-arginine metabolism: V. Bronte & P. Zanovello; Nat. Rev. Immunol. **5,** 641 (2005) ▪ Nitric oxide and neurological disorders: A. J. Duncan & S. J. Heales; Mol. Aspects Med. **26,** 67 (2005) ▪ The role of nitric oxide in cardiovascular diseases: K. M. Naseem; Mol. Aspects Med. **26,** 33 (2005) ▪ Nitric oxide and reactive nitrogen species in airway epithelial signaling and inflammation: P.F. Bove & A. van der Vliet; Free Radic. Biol. Med. **41,** 515 (2006) ▪ The ubiquitous role of nitric oxide in cardioprotection: S. P. Jones & R. Bolli; J. Mol. Cell. Cardiol. **40,** 16 (2006) ▪ Nitric oxide and inflammation: G. Cirino, et al.; Inflamm. Allergy Drug Targets **5,** 115 (2006) ▪ The role of nitric oxide in tumour progression: D. Fukumura, et al.; Nat. Rev. Cancer **6,** 521 (2006)

Nitric Oxide & Angiogenesis

Formation of new blood vessels (angiogenesis) is essential for wound healing and tumor growth. $NO^{\bullet}$ has been shown to mediate angiogenesis by direct and indirect mechanisms.

- In endothelial cells, endogenous and/or exogenous $NO^{\bullet}$ triggers multiple angiogenic signalling pathways through S-nitrosylation and/or cGMP towards angiogenesis [1]. However, anti-angiogenic effects of $NO^{\bullet}$ have also been reported [2]. This discrepancy might be explained by differences in concentration and duration of $NO^{\bullet}$ exposure. Low concentrations of $NO^{\bullet}$ increase but high concentrations inhibit phosphorylation of protein kinase C (PKC), extracellular-signal-regulated protein kinase (ERK) and Jun, and the binding activity of activator protein-1 (AP-1) [3, 4].
- $NO^{\bullet}$ has been shown to mediate the function of many angiogenic factors [5] like VEGF, sphingosine-1-phosphate, angiopoietins, estrogen, shear stress and metabolic stress which activate eNOS. Furthermore, anti-angiogenic factors exert their effects through inhibition of eNOS–$NO^{\bullet}$ signalling (endostastin, thrombospondin 1) [6, 7]. Along with these findings, increased eNOS expression can be linked with increased tumor angiogenesis, high vascular permeability and metastasis. Selective inhibition of eNOS, genetically or with a pharmacological agent may inhibit tumor angiogenesis [8, 9].
- $NO^{\bullet}$ is an important modulator of the expression of endogenous angiogenic factors [10]. Additionally, $NO^{\bullet}$ activates the transcription factor hypoxia-inducible factor 1α (HIF1α), which in turn upregulates VEGF, thereby promoting angiogenesis [11]. Furthermore, $NO^{\bullet}$ inhibits the expression of endogenous anti-angiogenic factors, which leads to the conclusion that it regulates pro- and anti-angiogenic factors through multiple mechanisms.

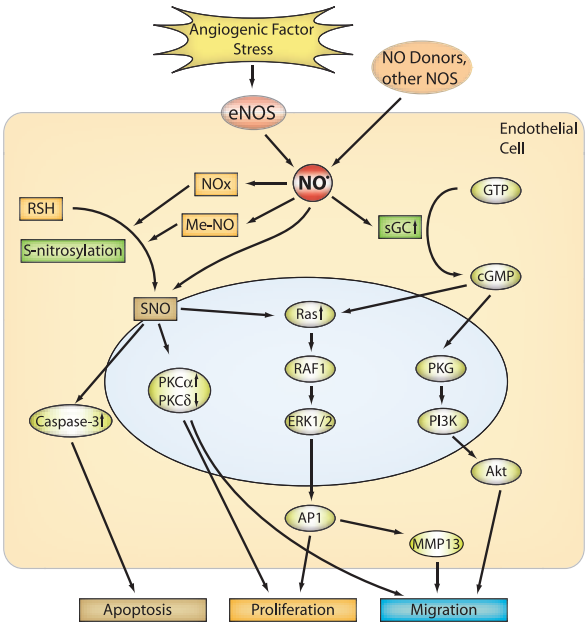
Literature References:

[1] Activation of the phosphatidylinositol 3-kinase/protein kinase Akt pathway mediates nitric oxide-induced endothelial cell migration and angiogenesis: K. Kawasaki, et al.; Mol. Cell Biol. **23,** 5726 (2003) ▪ **[2]** Nitric oxide inhibits proliferation of human endothelial cells via a mechanism independent of cGMP: R. Heller, et al.; Atherosclerosis **144,** 49 (1999) ▪ **[3]** Dual actions of nitric oxide on angiogenesis: possible roles of PKC, ERK, and AP-1: M.K. Jones, et al.; BBRC **318,** 520 (2004) ▪ **[4]** Nitric oxide regulates angiogenesis through a functional switch involving thrombospondin-1: L.A. Ridnour, et al.; PNAS **102,** 13147 (2005) ▪ **[5]** Role of nitric oxide in angiogenesis and microcirculation in tumors: D. Fukumura & R.K. Jain; Cancer Metastasis Rev. **17,** 77 (1998) ▪ **[6]** Dephosphorylation of endothelial nitric oxide synthase contributes to the anti-angiogenic effects of endostatin: C. Urbich, et al.; FASEB J. **16,** 706 (2002) ▪ **[7]** Thrombospondin-1 inhibits endothelial cell responses to nitric oxide in a cGMP-dependent manner: J.S. Isenberg, et al.; PNAS **102,** 13141 (2005) ▪ **[8]** Selective inhibition of tumor microvascular permeability by cavtratin blocks tumor progression in mice: J.P. Gratton, et al.; Cancer Cell **4,** 31 (2003) ▪ **[9]** NO mediates mural cell recruitment and vessel morphogenesis in murine melanomas and tissue-engineered blood vessels: S. Kashiwagi, et al.; J. Clin. Invest. **115,** 1816 (2005) ▪ **[10]** Induction of vascular endothelial growth factor by nitric oxide in human glioblastoma and hepatocellular carcinoma cells: K. Chin, et al.; Oncogene **15,** 437 (1997) ▪ **[11]** Regulation of the hypoxia-inducible factor 1alpha by the inflammatory mediators nitric oxide and tumor necrosis factor-alpha in contrast to desferroxamine and phenylarsine oxide: K.B. Sandau, et al.; J. Biol. Chem. **276,** 39805 (2001)

Selected Review Articles

NO and angiogenesis: J.P. Cooke; Atheroscler. (Suppl. 4), 53 (2003) ▪ Nitric oxide: a newly discovered function on wound healing: J.D. Luo & A.F. Chen; Acta Pharmacol. Sin. **26,** 259 (2005) ▪ The role of nitric oxide in tumour progression: D. Fukumura, et al.; Nat. Rev. Cancer **6,** 521 (2006) ▪ Regulators of angiogenesis and strategies for their therapeutic manipulation: M. Milkiewicz, et al.; Int. J. Biochem. Cell Biol. **38,** 333 (2006) ▪ Nitric oxide in blood: M.M. Elahi, et al.; FEBS J. **274,** 906 (2007)

eNOS-mediated Angiogenesis



Nitric Oxide & Cell Death

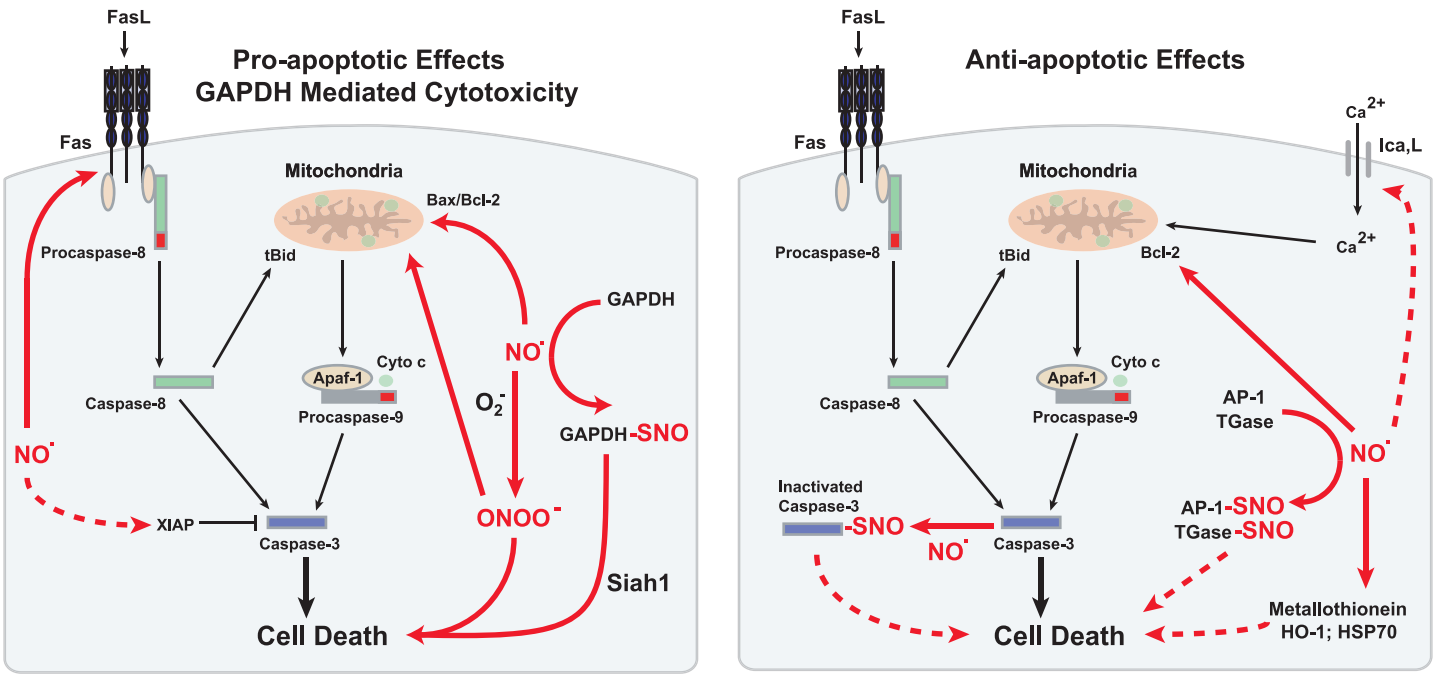


FIGURE: Pro- and anti-apoptotic mechanisms of NO: Adapted from *Modulation of apoptosis by nitric oxide: Implications in myocardial ischemia and heart failure:* H. M. Razavi, et al.; Pharmacol. Ther. **106,** 147 (2005). Solid red lines indicate stimulation, while dashed red lines indicate inhibition.

Nitric oxide ($NO^{\bullet}$) stimulates controversial and multi-faceted effects on cell viability. Oxidative stress, DNA damage, protein modification, disruption of energy metabolism, interference with calcium homeostasis and mitochondrial dysfunction can be triggered by $NO^{\bullet}$. While these forms of cell stress may lead to either necrotic or apoptotic cell death, protective effects on cellular viability have been reported for $NO^{\bullet}$ too. $NO^{\bullet}$ seems to be capable of both inducing and preventing apoptosis depending on doses and cell types.

Pro-apoptotic effects of $NO^{\bullet}$ may be executed by mechanisms acting on both, the mitochondrial and death receptor pathways. One direct effect on cell death receptor mediated apoptosis has been shown by $NO^{\bullet}$ mediated upregulation of CD95 (APO-1/Fas) and TRAIL/APO-2 ligands. Activation of death receptors leads to the activation of caspase-8 and other downstream caspases and results in apoptosis. Effecting the mitochondrial pathway, $NO^{\bullet}$ has been shown to increase the Bax to Bcl-2 ratio, facilitating Bax expression and decreasing its degradation by impeding 26S proteasome activity. It has been shown that $NO^{\bullet}$ regulates mitochondrial permeability transition and cytochrome c release in a dose dependent fashion. Cytochrome c recombines with the apoptosis associated factor-1 (Apaf-1) to recruit and activate procaspase-9. $NO^{\bullet}$ reacts rapidly with superoxide ($O_2^{\bullet -}$) to generate the potent reactive nitrogen species, peroxynitrite ($ONOO^-$). Peroxynitrite and $NO^{\bullet}$ inhibit mitochondrial superoxide dismutase, leading to the accumulation of $O_2^{\bullet -}$ and consequently to the increase of peroxynitrite, which oxidizes different biomolecules. $NO^{\bullet}$ also downregulates X-linked inhibitor of apoptosis protein (XIAP).

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as a sensor of $NO^{\bullet}$ stress has been discovered recently. Thus, GAPDH becomes S-nitrosylated by $NO^{\bullet}$ to be capable of binding E3 ubiquitin ligase Siah1.

GAPDH stabilizes Siah1. As a result of the nuclear localization signal of Siah1, the GAPDH-Siah1 complex translocates to the nucleus where substrates of Siah1 become degraded, which results in cytotoxicity.

Anti-apoptotic effects of $NO^{\bullet}$ are mediated by S-nitrosylation of inactivated caspase-3, activator protein-1 (AP-1) and tissue transglutaminase (TGase). It has been reported that $NO^{\bullet}$ inhibits L-type Ca^{2+} channels and increases expression of Bcl-2, both leading to the prevention of cytochrome c release. In addition, $NO^{\bullet}$ may upregulate protective proteins such as heme oxygenase-1 (HO-1), metallothionein, and heat shock protein 70 (HSP70).

Selected Review Articles

Nitric oxide, cell signaling and cell death: G. A. Blaise, et al.; Toxicology **208,** 177 (2005) ▪ Apoptotic volume decrease and nitric oxide: C. D. Bortner; Toxicology **208,** 213 (2005) ▪ What else has to happen for nitric oxide to induce cell death?: V. Borutaite & G. Brown; Biochem. Soc. Trans. **33,** 1394 (2005) ▪ Nitric oxide as a modulator of apoptosis: C. Q. Li & G. N. Wogan; Cancer Lett. **226,** 1 (2005) ▪ Modulation of apoptosis by nitric oxide: implications in myocardial ischemia and heart failure: H. M. Razavi, et al.; Pharmacol. Ther. **106,** 147 (2005) ▪ Nitric Oxide-GAPDH-Siah: A Novel Cell Death Cascade: M. R. Hara & S. H. Snyder; Cell. Mol. Neurobiol. **26,** 527 (2006) ▪ GAPDH as a sensor of NO stress: M. R. Hara, et al.; Biochim. Biophys. Acta **1762,** 502 (2006)

Nitric Oxide – GADPH –Siah1: A Novel Cell Death Cascade

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) participates in a cell death cascade wherein a variety of stimuli activate nitric oxide synthases (NOS) with NO nitrosylating GAPDH, conferring on it the ability to bind to Siah1, an E3-ubiquitin-ligase, whose nuclear localization signal enables the GAPDH/Siah1 protein complex to translocate to the nucleus where degradation of Siah1 targets elicits cell death.

LIT: S-nitrosylated GAPDH initiates apoptotic cell death by nuclear translocation following Siah1 binding: M.R. Hara, et al.; Nat. Cell Biol. **7,** 665 (2005) ▪ Neuroprotection by pharmacologic blockade of the GAPDH death cascade: M.R. Hara, et al.; PNAS **103,** 3887 (2006) ▪ Nitric oxide-GAPDH-Siah: a novel cell death cascade: M.R. Hara & S.H. Snyder; Cell. Mol. Neurobiol. **26,** 527 (2006)

Nitric Oxide Synthase (NOS) Proteins

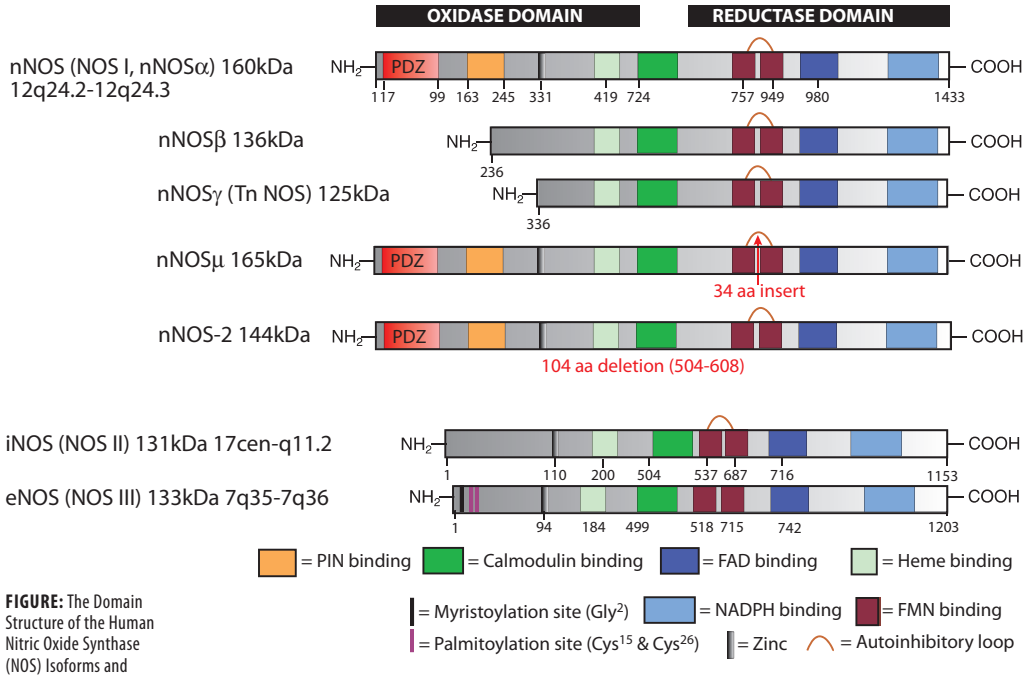


FIGURE: The Domain Structure of the Human Nitric Oxide Synthase (NOS) Isoforms and their Splice Variants.

FOR DETAILS SEE: Nitric oxide synthases: structure, function and inhibition: W.K. Alderton, et al.; Biochem. J. **357,** 593 (2001) or visit http://us.expasy.org/sprot/ and accession numbers P29475 for human NOS I, P35228 for human NOS II or P29474 for human NOS III.

Selected Review Articles

Update on mechanism and catalytic regulation in the NO synthases: D.J. Stuehr, et al.; J. Biol. Chem. **279,** 36167 (2004) ▪ Arginase: structure, mechanism, and physiological role in male and female sexual arousal: D.W. Christianson; Acc. Chem. Res. **38,** 191 (2005) ▪ Cardiac neurobiology of nitric oxide synthases: E.J. Danson & D.J. Paterson; Ann. N. Y. Acad. Sci. **1047,** 183 (2005) ▪ Cardiac nitric oxide: emerging role for nNOS in regulating physiological function: E.J. Danson, et al.; Pharmacol. Ther. **106,** 57 (2005) ▪ Mitochondrial nitric oxide synthase: P. Ghafourifar & E. Cadenas; TIPS **26,** 190 (2005) ▪ NOS:molecular mechanisms, clinical aspects, therapeutic and monitoring approaches: S.A. Kharitonov; Curr. Drug Targets Inflamm. Allergy **4,** 141 (2005) ▪ Inducible nitric oxide synthase (iNOS) in tumor biology: the two sides of the same coin: M. Lechner, et al.; Semin. Cancer Biol. **15,** 277 (2005) ▪ New insights into nNOS regulation of vascular homeostasis: G.L. Semenza; J. Clin. Invest. **115,** 2976 (2005) ▪ The regulation and pharmacology of endothelial nitric oxide synthase: D.M. Dudzinski, et al.; Annu. Rev. Pharmacol. Toxicol. **46,** 235 (2006) ▪ Endothelial nitric oxide synthase: insight into cell-specific gene regulation in the vascular endothelium: J.E. Fish & P.A. Marsden; Cell. Mol. Life Sci. **63,** 144 (2006) ▪ Amino acids, arginase and nitric oxide in vascular health: N.N. Huynh & J. Chin-Dusting; Clin. Exp. Pharmacol. Physiol. **33,** 1 (2006) ▪ Arginine: beyond protein: S.M. Morris, Jr.; Am. J. Clin. Nutr. **83,** 508S (2006) ▪ The arginine-arginase balance in asthma and lung inflammation: N. Zimmermann & M.E. Rothenberg; Eur. J. Pharmacol. **533,** 253 (2006) ▪ Subcellular targeting and trafficking of nitric oxide synthases: S. Oess, et al.; Biochem. J. **396,** 401 (2006)

eNOS [NOS III]

eNOS [NOS III] (human) (rec.)

ALX-201-070-R100 100 µl

Produced in Sf9 cells. **MW:** ~130kDa/subunit; homodimer. **PURITY:** 50,000 x g supernatant. **SPECIFIC ACTIVITY:** ≥0.1nmol/mg/min at pH 7.0, 37°C, 1mM L-arginine, 1mM NADPH, 10µM (6R)-tetrahydrobiopterin, 5µM FAD, 5µM FMN, 50nM calmodulin, 1mM $CaCl_2$ and 7mM GSH.

LIT: Endothelial nitric-oxide synthase (type III) is activated and becomes calcium independent upon phosphorylation by cyclic nucleotide-dependent protein kinases: E. Butt, et al.; J. Biol. Chem. **275,** 5179 (2000)

eNOS [NOS III] (bovine) (rec.)

ALX-201-127-U010 10 U

Produced in Sf9 cells. **MW:** ~135kDa/subunit; homodimer. **PURITY:** 100,000 x g supernatant. **SPECIFIC ACTIVITY:** ≥1 U/ mg protein. One unit is defined as the amount of enzyme that produces 1nmol nitric oxide per min. at 37°C in 50mM HEPES, pH 7.4, containing 5µM oxyhemoglobin, 1mM $CaCl_2$, 20µg/ml calmodulin, 0.1mM NADPH, 50µM arginine, 12µM tetrahydrobiopterin and 170µM DTT.

iNOS [NOS II]

iNOS [NOS II] (human) (rec.)

ALX-201-069-R150 150 µl

Produced in Sf9 cells. **MW:** ~130kDa/subunit; homodimer. **PURITY:** 50,000 x g supernatant. **SPECIFIC ACTIVITY:** ≥0.015nmol/µl/min at pH 7.0, 37°C, 1mM L-arginine, 1mM NADPH, 10µM (6R)-tetrahydrobiopterin, 5µM FAD, 5µM FMN and 7mM GSH.

LIT: Endothelial nitric-oxide synthase (type III) is activated and becomes calcium independent upon phosphorylation by cyclic nucleotide-dependent protein kinases: E. Butt, et al.; J. Biol. Chem. **275,** 5179 (2000)

NEW iNOS [NOS II] (human) (rec.) (purified)

ALX-201-260-C010 10 µg

Produced in *E. coli.* Fused to a His-tag. **MW:** ~130kDa/subunit; homodimer. **PURITY:** ≥90% (SDS-PAGE and heme spectral analysis). **SPECIFIC ACTIVITY:** ≥1'000-1'250nmol/min/mg protein. Amount assayed 0.5-1µg. Optimal conditions: 37°C, 40mM TRIS-HCl, HEPES or EPPS buffer, pH 7.6, 10µM oxyhemoglobin, 0.3mM DTT, 1mM NADPH, 4µM FAD, 4µM FMN, 4µM H4B, 100units/ml catalase, 10units/ml superoxide dismutase and 0.1mg/ml BSA in a final volume of 1ml.

iNOS [NOS II] (mouse) (rec.)

ALX-202-040-U050 50 U

Produced in *E. coli.* **MW:** ~130kDa/subunit; homodimer. **PURITY:** 100,000 x g supernatant. **SPECIFIC ACTIVITY:** One unit of enzyme produces 1nmol/mg/min at pH 7.4, 37°C in 50mM HEPES (containing 1mM L-arginine), 1mM magnesium acetate, 0.1mM NADPH, 5mM oxyhemoglobin, 12mM (6R)-tetrahydrobiopterin and 170mM DTT.

LIT: Purification of the inducible murine macrophage nitric oxide synthase: J.M. Hevel, et al.; J. Biol. Chem. **266,** 22789 (1991)▪ Nitric-oxide synthase assays : J.M. Hevel & M.A. Marletta; Meth. Enzymol. **233,** 250 (1994)▪ Nitric oxide synthases in mammals: R.G. Knowles & S. Moncada; Biochem. J. **298,** 249 (1994)

nNOS [NOS I]

NEW nNOS [NOS I] (human) (rec.) (purified)

ALX-201-261-C010 10 µg

Produced in *E. coli.* **MW:** ~160kDa/subunit; homodimer. **PURITY:** ≥90% (SDS-PAGE and heme spectral analysis). **SPECIFIC ACTIVITY:** ≥400-600nmol/mg/min (U/mg) protein. Amount assayed 0.5-1µg. Optimal conditions: 37°C, 40mM TRIS-HCl, HEPES or EPPS buffer, pH 7.6, 10µM oxyhemoglobin, 0.3mM DTT, 1mM NADPH, 4µM FAD, 4µM FMN, 4µM H4B, 100units/ml catalase, 10units/ml superoxide dismutase, 0.1mg/ml BSA, 35µl of 9mM EDTA, 35µl of 150mg/ml calmodulin and 12.5mM $CaCl_2$ in a final volume of 1ml.

nNOS [NOS I] (rat) (rec.) (purified)

ALX-201-028-R050 50 µl

Produced in Sf9 cells. **MW:** ~160kDa/subunit; homodimer. **PURITY:** ≥98% (SDS-PAGE). **SPECIFIC ACTIVITY:** ~0.7µmol L-citrulline/ mg/min at pH 7.0; 37°C; 0.1mM L-arginine, 0.2mM NADPH, 10µM (6R)-tetrahydrobiopterin, 5µM FAD/FMN, 10µg/ml calmodulin, 10-500µM $CaCl_2$.

LIT: Isolation of nitric oxide synthetase, a calmodulin-requiring enzyme: D.S. Bredt & S.H. Snyder; PNAS **87,** 682 (1990) ▪ Electron transfer in the nitric-oxide synthases. Characterization of L- arginine analogs that block heme iron reduction: H.M. Abu-Soud, et al.; J. Biol. Chem. **269,** 32318 (1994) ▪ Characterization of neuronal nitric oxide synthase and a C415H mutant, purified from a baculovirus overexpression system: M.K. Richards & M.A. Marletta; Biochemistry **33,** 14723 (1994) ▪ Evidence for a bidomain structure of constitutive cerebellar nitric oxide synthase: E.A. Sheta, et al.; J. Biol. Chem. **269,** 15147 (1994) ▪ Expression of rat brain nitric oxide synthase in baculovirus-infected insect cells and characterization of the purified enzyme: C. Harteneck, et al.; Biochem. J. **304,** 683 (1994) ▪ Molecular mechanisms of inhibition of porcine brain nitric oxide synthase by the antinociceptive drug 7-nitro-indazole [published erratum appears in Neuropharmacology 1995 Feb;34(2):243]: B. Mayer, et al.; Neuropharmacology **33,** 1253 (1994) ▪ Calmodulin controls neuronal nitric-oxide synthase by a dual mechanism. Activation of intra- and interdomain electron transfer: H.M. Abu-Soud, et al.; J. Biol. Chem. **269,** 32047 (1994)

NOS Cofactors, Regulators & Activators

Nitric oxide synthase (NOS) enzymes are dimers that require the presence of calmodulin and the cofactors (6R)-5,6,7,8-tetrahydrobiopterin (BH4), iron-protoporphyrin IX (heme), flavin-adenine dinucleotide (FAD) and flavin mononucleotide (FMN) for full activity. Two main domains can be identified in each NOS isoform: an amino-terminal oxygenase domain and a carboxy-terminal reductase domain.

Electrons donated by conversion of NADPH to NADP are transferred to the oxygenase domain through a redox chain that involves the electron carriers FAD and FMN. The oxygenase domain then uses the cofactors heme and BH4 to catalyze the reaction between oxygen (O_2) and L-arginine, which generates L-citrulline and nitric oxide ($NO^{\bullet}$). The presence of bound calmodulin is required for electron flow.

NOS Domain Binding Cofactors

Flavin Adenine Dinucleotide . disodium salt

ALX-480-084-M050 50 mg

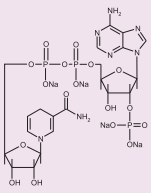
LIT: FAD and GSH participate in macrophage synthesis of nitric oxide: D.J. Stuehr, et al.; BBRC **168,** 558 (1990) ▪ Cloned and expressed nitric oxide synthase structurally resembles cytochrome P-450 reductase: D.S. Bredt, et al.; Nature **351,** 714 (1991) ▪ Purification of the inducible murine macrophage nitric oxide synthase. Identification as a flavoprotein: J.M. Hevel, et al.; J. Biol. Chem. **266,** 22789 (1991) ▪ Activation of neuronal nitric oxide synthase by flavin adenine dinucleotide: A. Hashida-Okumura, et al.; Biochem. Mol. Biol. Int. **35,** 1339 (1995)

NADPH . tetrasodium salt

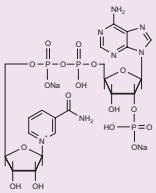
ALX-480-004-M050 50 mg
ALX-480-004-M250 250 mg
ALX-480-004-G001 1 g

NADP . disodium salt

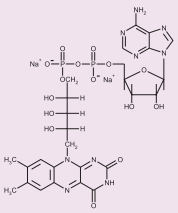
ALX-480-003-M050 50 mg
ALX-480-003-M250 250 mg
ALX-480-003-G001 1 g



NADPH . tetrasodium salt



NADP . disodium salt



Flavin Adenine Dinucleotide . disodium salt

Latest Insight

New class of eNOS (NOS III) Activator

The myristoylated pseudosubstrate of PKCζ (mPS), a synthetic myristoylated 13 amino acids peptide mimicking the endogenous PKCζ pseudosubstrate region is known as a selective cell permeable inhibitor of PKCζ. K. Krotova, et al. showed that the action of mPS is not limited to the inhibition of PKC activity, but that myristoylation of certain peptides can activate eNOS (NOS III) through Akt phosphorylation. Therefore, such myristoylated peptides can be considered a new class of nitric oxide production activators in endothelial cells.

NEW PKCζ Pseudosubstrate (Myristoylated)

Myr-Ser-Ile-Tyr-Arg-Arg-Gly-Ala-Arg-Arg-Trp-Arg-Lys-Leu-OH

ALX-260-155-M001 1 mg

Selective cell permeable inhibitor of PKCζ. Activates eNOS (NOS III) through Akt phosphorylation, and can be considered as a new class of nitric oxide production activators in endothelial cells.

LIT: Peptides modified by myristoylation activate eNOS in endothelial cells through Akt phosphorylation: K. Krotova, et al.; Br. J. Pharmacol. **148,** 732 (2006)

BH4 – A Key Cofactor of NOS

(6R)-5,6,7,8-Tetrahydro-L-biopterin (BH4) is a cofactor in enzymatic hydroxylation of aromatic rings, cleavage of glycerol-ether, cyanide oxidation and nitric oxide biosynthesis. BH4 functions exclusively as a one-electron donor during reductive activation of the oxyferrous complex of heme-dioxy enzymes. For more information on BH4 visit: www.bh4.org.

Selected Review Articles

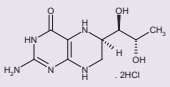
Tetrahydrobiopterin biosynthesis, regeneration and functions: B. Thony, et al.; Biochem. J. **347,** 1 (2000) ▪ Tetrahydrobiopterin in nitric oxide synthesis: a novel biological role for pteridines: A.C. Gorren & B. Mayer; Curr. Drug Metab. **3,** 133 (2002) ▪ Tetrahydrobiopterin biosynthesis, utilization and pharmacological effects: G. Werner-Felmayer, et al.; Curr. Drug Metab. **3,** 159 (2002) ▪ The role of tetrahydrobiopterin in superoxide generation from eNOS: enzymology and physiological implications: J. Vasquez-Vivar, et al.; Free Radic. Res. **37,** 121 (2003) ▪ Tetrahydrobiopterin radical enzymology: C.C. Wei, et al.; Chem. Rev. **103,** 2365 (2003) ▪ Tetrahydrobiopterin and nitric oxide: mechanistic and pharmacological aspects: E.R. Werner, et al.; Exp. Biol. Med. **228,** 1291 (2003) ▪ Tetrahydrobiopterin: regulator of endothelial nitric oxide synthase in vascular disease: K.M. Channon; Trends Cardiovasc. Med. **14,** 323 (2004) ▪ Structure-function studies on nitric oxide synthases: H. Li & T.L. Poulos; J. Inorg. Biochem. **99,** 293 (2005) ▪ Ligand-protein interactions in nitric oxide synthase: D.L. Rousseau, et al.; J. Inorg. Biochem. **99,** 306 (2005)

(6R)-5,6,7,8-Tetrahydro-L-biopterin . 2HCl

[(6R)-BH_4 . 2HCl]

ALX-440-001-M005 5 mg
ALX-440-001-M025 25 mg

Cofactor of nitric oxide synthases (NOS).



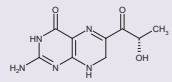
LIT: Brain nitric oxide synthase is a biopterin- and flavin-containing multi-functional oxido-reductase: B. Mayer, et al.; FEBS Lett. **288,** 187 (1991) ▪ Tetrahydrobiopterin, a cofactor for rat cerebellar nitric oxide synthase, does not function as a reactant in the oxygenation of arginine: J. Giovanelli, et al.; PNAS **88,** 7091 (1991) ▪ Purification and characterization of particulate endothelium-derived relaxing factor synthase from cultured and native bovine aortic endothelial cells: J.S. Pollock, et al.; PNAS **88,** 10480 (1991) ▪ Stimulation of human nitric oxide synthase by tetrahydrobiopterin and selective binding of the cofactor: P. Klatt, et al.; FEBS Lett. **305,** 160 (1992) ▪ Macrophage nitric oxide synthase: relationship between enzyme-bound tetrahydrobiopterin and synthase activity: J.M. Hevel & M.A. Marletta; Biochemistry **31,** 7160 (1992) ▪ Mechanistic probes of N-hydroxylation of L-arginine by the inducible nitric oxide synthase from murine macrophages: R.A. Pufahl, et al.; Biochemistry **31,** 6822 (1992) ▪ Tetrahydrobiopterin synthesis. An absolute requirement for cytokine- induced nitric oxide generation by vascular smooth muscle: S.S. Gross & R. Levi; J. Biol. Chem. **267,** 25722 (1992) ▪ **For a comprehensive bibliography please visit our website.**

Related Product

L-Sepiapterin

ALX-440-004-M010 10 mg
ALX-440-004-5010 5x10 mg

Key intermediate in the pterin salvage pathway, tetrahydrobiopterin biosynthesis.



LIT: Biosynthesis and metabolism of tetrahydrobiopterin and molybdopterin: C. Nichol, et al.; Ann. Rev. Biochem. **54,** 729 (1985) ▪ Tetrahydrobiopterin synthesis. An absolute requirement for cytokine- induced nitric oxide generation by vascular smooth muscle: S.S. Gross & R. Levi; J. Biol. Chem. **267,** 25722 (1992)

Latest Insight

Controlling the Pain

Tetrahydrobiopterin (BH4) is an essential cofactor for catecholamine, serotonin and nitric oxide production. I. Tegeder, et al. recently reported that GTP cyclohydrolase (GCH1), the rate-limiting enzyme for BH4 synthesis, is a key modulator of peripheral neuropathic and inflammatory pain. Their findings propose that BH4 is an intrinsic regulator of pain sensitivity and that the GTP cyclohydrolase haplotype is a marker for these traits.

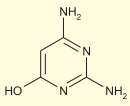
LIT: GTP cyclohydrolase and tetrahydrobiopterin regulate pain sensitivity and persistence: I. Tegeder, et al.; Nat. Med. **12,** 1269 (2006)

Suppressor of NOS

2,4-Diamino-6-hydroxypyrimidine

ALX-270-005-M250 250 mg
ALX-270-005-G001 1 g

Selective inhibitor of GTP cyclohydrolase I, which is the rate-limiting enzyme for *de novo* tetrahydrobiopterin synthesis. Thus it suppresses the activity of nitric oxide synthase (NOS).



LIT: 2,4-Diamino-6-hydroxypyrimidine, an inhibitor of GTP cyclohydrolase I, suppresses nitric oxide production by chicken macrophages: Y.J. Sung, et al.; Int. J. Immunopharmacol. **16,** 101 (1994) ▪ 2,4-Diamino-6-hydroxypyrimidine, an inhibitor of tetrahydrobiopterin synthesis, downregulates the expression of iNOS protein and mRNA in primary murine macrophages: C. Bogdan, et al.; FEBS Lett. **363,** 69 (1995) ▪ GTP cyclohydrolase I inhibition by the prototypic inhibitor 2, 4-diamino-6-hydroxypyrimidine. Mechanisms and unanticipated role of GTP cyclohydrolase I feedback regulatory protein: L. Xie, et al.; J. Biol. Chem. **273,** 21091 (1998) ▪ The mechanism of potent GTP cyclohydrolase I inhibition by 2,4-diamino-6-hydroxypyrimidine: requirement of the GTP cyclohydrolase I feedback regulatory protein: M.A. Kolinsky & S.S. Gross; J. Biol. Chem. **279,** 40677 (2004)

Calmodulin & Nitric Oxide Synthases

Calmodulin (CaM) is a ubiquitous, calcium-binding protein that can bind to and regulate a multitude of different protein targets, thereby affecting many different cellular functions. Calmodulin mediates processes such as inflammation, metabolism, apoptosis, nerve growth and the immune response.

Calmodulin is an important nitric oxide synthase (NOS)-binding regulatory protein. The constitutive NOSs (nNOS/NOS I and eNOS/NOS III) are regulated by the reversible binding of calmodulin through changes in the intracellular Ca^{2+} concentration. iNOS/NOS II is expressed with calmodulin bound permanently and remains active in the absence of free Ca^{2+}. In all three isoforms, calmodulin binds to the protein linker connecting the reductase with the oxygenase domain and regulates the activity of the enzyme by activating FMN-to-heme electron transfer.

LIT: Calmodulin-dependent regulation of mammalian nitric oxide synthase: S. Daff; Biochem. Soc. Trans. **31,** 502 (2003)

Characteristics of Nitric Oxide Synthases

Enzyme	Gene	No. of Residues	Regulation
nNOS	NOS I	1429-1433	Ca^{2+}/CaM
iNOS	NOS II	1144-1153	Cytokine-inducible / Ca^{2+}-independent
eNOS	NOS III	1203-1205	Ca^{2+}/CaM

Calmodulin Proteins & Antibodies

NEW Calmodulin (pig)

ALX-202-062-M001 Purified 1 mg
ALX-202-062B-C100 Biotin 100 µg
ALX-202-062L-C100 Fluorescein 100 µg
ALX-202-062RH-C100 Rhodamine 100 µg

Isolated from pig brain.

LIT: Trifluoperazine binding to porcine brain calmodulin and skeletal muscle troponin C: L. Massom, et al.; Biochemistry **29,** 671 (1990)

NEW Calmodulin (pig) (Agarose Immobilized)

ALX-212-015-L002 2 ml

Isolated from pig brain. Immobilized to aldehyde activated agarose.

Calmodulin (human brain) (high purity)

ALX-200-005-C500 500 µg

Isolated from human brain.

Calmodulin (bovine brain) (high purity)

ALX-202-024-M001 1 mg

Isolated from bovine brain.

LIT: Conformational transition accompanying the binding of Ca2+ to the protein activator of 3',5'-cyclic adenosine monophosphate phosphodiesterase: C.B. Klee; Biochemistry **16,** 1017 (1977) ▪ Large-scale purification and characterization of calmodulin from ram testis: its metal-ion-dependent conformers: F. Autric, et al.; Biochim. Biophys. Acta **631,** 139 (1980)

Calmodulin (bovine brain) (high purity) (Biotin)

ALX-209-001-C050 Biotin 50 µg

Isolated from bovine brain.

LIT: A rapid and sensitive method for detection and quantification of calcineurin and calmodulin-binding proteins using biotinylated calmodulin: M.L. Billingsley, et al.; PNAS **82,** 7585 (1985) ▪ Preparation of fluorescent, cross-linking, and biotinylated calmodulin derivatives and their use in studies of calmodulin-activated phosphodiesterase and protein phosphatase: R.L. Kincaid, et al.; Meth. Enzymol. **159,** 605 (1988)

Calmodulin (human) (recombinant)

JBS-PR-390 100 µg

Produced in *E. coli.*

Antibodies

MAb to Calmodulin (6D4)

ALX-804-017-R200 200 µl

CLONE: 6D4. **ISOTYPE:** Mouse IgG1. **IMMUNOGEN:** Purified calmodulin from *Dictyostelium discoideum strain.* **SPECIFICITY:** Recognizes rat, bovine, chicken, chlamydomonas and dictyostelium calmodulin. Detects a band of ~17kDa by Western blot. Does not cross-react with parvalbumin, troponin, S-100 and myosin light chain kinase (MLCK). **APPLICATION:** ELISA, ICC, WB.

LIT: Production and specificity of monoclonal antibodies against calmodulin from Dictyostelium discoideum: D. Hulen, et al.; Cell Motility & Cytoskeleton **18,** 113 (1991)

MAb to Calmodulin (2D1)

ALX-804-018-R200 200 µl

CLONE: 2D1. **ISOTYPE:** Mouse IgG1. **IMMUNOGEN:** Purified calmodulin from *Dictyostelium discoideum strain*. **SPECIFICITY:** Recognizes rat, bovine, chicken brain, chlamydomonas and dictyostelium calmodulin. In competition assays it reacts with the SDS denatured, but not native form of bovine brain calmodulin. Detects a band of ~17kDa by Western blot. Does not cross-react with parvalbumin, troponin, S-100 and myosin light chain kinase. **APPLICATION:** ELISA, ICC, WB.

LIT: Production and specificity of monoclonal antibodies against calmodulin from Dictyostelium discoideum: D. Hulen, et al.; Cell Motility & Cytoskeleton **18,** 113 (1991) ▪ Calmodulin and the contractile vacuole complex in mitotic cells of Dictyostelium discoideum: Q. Zhu, et al.; J. Cell Sci. **104,** 1119 (1993)

Wheat germ Calmodulin

NEW

Ideal for applications requiring both calmodulin activation and residue specific tagging.

Wheat germ (*Triticum aestivum*) calmodulin (CaM) sequence is unique in that it contains cysteine at residue position 26 (Cys^{26}). In contrast to mammalian CaMs, which lack cysteine, the preparation featured here is ideal for applications requiring both calmodulin activation and residue specific tagging. The single sulfhydryl moiety at Cys^{26} can be labelled using a variety of sulfhydryl (SH) reactive protein-modification reagents such as fluorescent maleimide derivatives. Additionally, since wheat germ CaM contains only a single tyrosine residue (Tyr^{138}) versus two tyrosine residues found in mammalian CaMs, Tyr^{138} serves as a mono-specific C-terminal region labelling site.

NEW Calmodulin (wheat) (*for duo site labelling*)

ALX-204-005-C250 Purified 250 µg
ALX-204-005-M001 Purified 1 mg

Labelled Calmodulin (wheat)

ALX-204-005B-C050 Biotin 50 µg
ALX-204-005L-C050 Fluorescein 50 µg
ALX-204-005RH-C050 Rhodamine 50 µg

Isolated from wheat germ (*Triticum aestivum*). Wheat germ calmodulin sequence is unique in that it contains cysteine (Cys^{26}) and only a single tyrosine (Tyr^{138}), in contrast to mammalian CaMs, which have two tyrosine residues and lack cysteine. This allows wheat germ CaM to be independently derivatized at both N- and C-terminal regions. Therefore it is ideal for applications requiring both calmodulin activation and residue specific tagging. Wheat germ CaM also has no tryptophan resulting in a distinctive absorbance spectrum when compared to mammalian CaM sequences. The conjugated formats are labelled at Cys^{26}.

LIT: Site-specific derivatives of wheat germ calmodulin. Interactions with troponin and sarcoplasmic reticulum: G.M. Strasburg, et al.; J. Biol. Chem. **263,** 542 (1988)

NEW Calmodulin (wheat) (Immobilized High Loading)

ALX-212-013-R200 200 µl

Isolated from wheat germ (*Triticum aestivum*). Coupled primarily through SH group (Cys^{26}) to epoxy activated resin (non-agarose).

LIT: Site-specific derivatives of wheat germ calmodulin. Interactions with troponin and sarcoplasmic reticulum: G.M. Strasburg, et al.; J. Biol. Chem. **263,** 542 (1988)

Resin for Efficient Capture of Calmodulin Binding Proteins

NEW Calmodulin (wheat & pig) (Immobilized High Loading)

ALX-212-014-R200 200 µl

Calmodulins isolated from wheat germ (*Triticum aestivum*) and pig brain. Both immobilized via SH group (Cys^{26}) (wheat) and amino group (pig) to epoxy activated resin (non-agarose). Thus allowing multiple orientations of bound calmodulin for efficient capture of calmodulin binding proteins.

LIT: Site-specific derivatives of wheat germ calmodulin. Interactions with troponin and sarcoplasmic reticulum: G.M. Strasburg, et al.; J. Biol. Chem. **263,** 542 (1988)

More Information? Please visit

www.axxora.com

Nitric Oxide Inducing Compounds

iNOS/NOS II is induced in response to bacterial components or other inflammatory compounds, such as LPS, IFN-γ, IL-1β or TNF-α. As a reaction iNOS constitutively produces sustained µM levels of NO• that functions as a critical inflammatory mediator. A highly produced NO• level by iNOS is critical in non-specific host defense against infection from pathogenic microbes, and linked to the tissue and organ damage of septic shock. An influence was also observed in neurodegenerative disease, where it may be involved in neuronal cytotoxicity (e.g. Alzheimer's disease) or in apoptosis, a critical event for removal of inflammatory cells.

Selected Review Articles

Nitric oxide and the immune response: C. Bogdan; Nat. Immunol. **2,** 907 (2001) ▪ Inflammatory neurodegeneration mediated by nitric oxide, glutamate, and mitochondria: G.C. Brown & A. Bal-Price; Mol. Neurobiol. **27,** 325 (2003) ▪ Nitric oxide and superoxide in inflammation and immune regulation: T.J. Guzik, et al.; J. Physiol. Pharmacol. **54,** 469 (2003) ▪ Nitric oxide: a key regulator of myeloid inflammatory cell apoptosis: E.L. Taylor, et al.; Cell Death Differ. **10,** 418 (2003) ▪ iNOS-mediated nitric oxide production and its regulation: F. Aktan; Life Sci. **75,** 639 (2004) ▪ Biochemical aspects of nitric oxide: S. Mariotto, et al.; Curr. Pharm. Des. **10,** 1627 (2004) ▪ The nitric oxide theory of aging revisited: S.M. McCann, et al.; Ann. N. Y. Acad. Sci. **1057,** 64 (2005)

Nitric Oxide Inducing Compounds

IFN-γ (human) (rec.)
BMS303 110 µg

IFN-γ (mouse) (rec.)
BMS326 100 µg

IL-1β (human) (rec.) (cell culture grade)
ALX-520-001-C010 10 µg

IL-1β (mouse) (rec.)
BMS332 10 µg

IL-1β (rat) (rec.)
BMS333 10 µg

TNF-α (human) (rec.) (cell culture grade)
ALX-520-002-C010 10 µg
ALX-520-002-C050 50 µg

TNF-α, Soluble (human) (rec.)
ALX-522-008-C050 50 µg

TNF-α, Soluble (human) (rec.) Set
ALX-850-060-KI01 1 Set

TNF-α, Soluble (mouse) (rec.) Set
ALX-850-061-KI01 1 Set

Technical Note

To guarantee consistent and authentic results, the use of endotoxin-free ddWater or PBS is recommended for solubilization. These products have been subjected to multiple rounds of LPS removal by adsorption with activated charcoal.

LIT: The removal of 14C labeled endotoxin by activated charcoal: A.S. Pegues, et al.; Int. J. Artif. Organs **2,** 153 (1979)

NEW PBS (endotoxin-free)
ALX-505-007-LD15 1.5 ml
Sterile and endotoxin-free PBS for use with TLR*grade*™ or endotoxin-free *grade* reagents.

NEW ddWater (endotoxin-free)
ALX-505-008-LD15 1.5 ml
Sterile, double distilled and endotoxin-free water for use with TLR*grade*™ or endotoxin-free *grade* reagents.

Product Highlight

Ultrapure TLR*grade*™ LPS – ready-to-use

- **Purity ≥99.9%; Concentration 1 mg/ml**
- **Sterile, water-clear, pyrogen-free ready-to-use formulation**
- **No hazardous handling**
- **No problems with dissolution resulting in excellent reproducibility especially when used in animal models**

Key Literature References:

[1] A new method for the extraction of R lipopolysaccharides: C. Galanos, et al.; Eur. J. Biochem. **9,** 245 (1969)

[2] Isolation and purification of R-form lipopolysaccharides: C. Galanos & O. Luderitz; Meth. Carbohyd. Chem. **9,** 11 (1993)

[3] Electrodialysis of lipopolysaccharides and their conversion to uniform salt forms: C. Galanos & O. Luderitz; Eur. J. Biochem. **54,** 603 (1975)

LPS from *E. coli*, Serotype EH100 (Ra) (TLR *grade*™) (liquid)
ALX-581-010-L002 2 ml
Isolated and purified from *E. coli* EH100 (Ra-mutant) by a modification of the PCP extraction method [1,2], converted to the uniform sodium salt form [3] and dissolved in sterile distilled pyrogen-free water.
LIT: see above

LPS from *E. coli*, Serotype R515 (Re) (TLR*grade*™) (liquid)
ALX-581-007-L002 2 ml
Rough (R)-form LPS isolated and purified from *E. coli* R515 (Re-mutant) by a modification of the PCP extraction method [1,2], converted to the uniform sodium salt form [3] and dissolved in sterile distilled pyrogen-free water.
LIT: see above

LPS from *E. coli*, Serotype 0111:B4 (TLR*grade*™) (liquid)
ALX-581-012-L002 2 ml
Smooth (S)-form LPS, isolated and purified from *E. coli* O111:B4 by a modification of the phenol water extraction and PCP extraction method [1,2], converted to the uniform sodium salt form [3] and dissolved in sterile pyrogen-free distilled water.
LIT: see above

LPS from *Salmonella abortus equi* S-form (TLR*grade*™) (liquid)
ALX-581-009-L002 2 ml
Smooth (S)-form LPS, isolated and purified from *Salmonella abortus equi* by modification of the phenol water extraction and PCP method [1,2], converted to the uniform salt form [3] and dissolved in sterile distilled pyrogen-free water.
LIT: see above

NEW LPS from *Salmonella minnesota* R595 (Re) (TLR*grade*™) (liquid)
ALX-581-008-L002 2 ml
Rough (R)-form LPS, isolated and purified from *Salmonella minnesota* R595 (Re-mutant) by a modification of the PCP extraction method [1,2], converted to the uniform sodium salt form [3] and dissolved in sterile distilled pyrogen-free water.
LIT: see above

NEW LPS, from *Salmonella typhimurium* S-form (TLR *grade*™) (liquid)
ALX-581-011-L002 2 ml
Smooth (S)-form LPS, isolated and purified from *Salmonella typhimurium* by modification of the phenol water extraction and PCP extraction method [1,2], converted to the uniform salt form [3] and dissolved in sterile distilled pyrogen-free water.
LIT: see above

Antibodies to Nitric Oxide Synthases

Product	Immunogen	Specificity	Lit.	Application	Prod No.	Size
Antibodies to nNOS (NOS I)						
PAb to nNOS	Synthetic peptide corresponding to aa 1422-1433 (E^{1422}SKKDTDEVFSS^{1433}) of human neuronal nitric oxide synthase (nNOS; NOS I).	Recognizes human, mouse and rat nNOS (NOS I). Does not cross-react with iNOS (NOS II) or eNOS (NOS III). Detects a band of ~160kDa by WB.		ICC, IHC (PS) IP, WB	ALX-210-510-1	1 Vial
PAb to nNOS	Purified neuronal nitric oxide synthase (nNOS; NOS I) from pig brain.	Recognizes mammalian nNOS (NOS I). Does not cross-react with iNOS (NOS II) or eNOS (NOS III).	[1-3]	IHC	ALX-210-501-R025 ALX-210-501-5025	25 µl 5x25 µl
PAb to nNOS	Synthetic peptide corresponding to aa 724-739 (T^{724}KRRAIGFKKLAEAVK^{739}) of the calmodulin binding domain of rat neuronal nitric oxide synthase (nNOS; NOS I).	Recognizes mouse, rat and bovine nNOS (NOS I). Does not cross-react with iNOS (NOS II) or eNOS (NOS III). Detects a band of ~155kDa by WB.	[16]	IHC (FS), WB	ALX-210-513-R100	100 µl
PAb to nNOS (rat)	Synthetic peptide corresponding to aa 237-250 (I^{237}QVDRDLDGKSHKA^{250}) of human neuronal nitric oxide synthase (nNOS; NOS I).	Recognizes rat nNOS (NOS I). Does not cross-react with iNOS (NOS II) or eNOS (NOS III).	[16]	WB	ALX-210-514-R100	100 µl
PAb to nNOS (human)	Synthetic peptide corresponding to aa 1411-1425 (C^{1411}NRLRSESIAFIEESK^{1425}) of human neuronal nitric oxide synthase (nNOS; NOS I).	Recognizes human nNOS (NOS I). **Blocking Peptide:** ALX-163-013.		WB	ALX-210-529-C100	100 µg
PAb to nNOS (rat) (phosphorylated) (pSer^{1412})	Synthetic peptide corresponding to aa 1411-1425 (C^{1411}NRLRSE(pS)IAFIEESK^{1425}) of human neuronal nitric oxide synthase (nNOS; NOS I).	Recognizes rat Ser^{1412}-phosphorylated nNOS (NOS I). **Blocking Peptide:** ALX-163-012.		WB	ALX-210-528-C100	100 µg
Antibodies to iNOS (NOS II)						
PAb to iNOS	Purified inducible nitric oxid synthase (iNOS; NOS II) from cytokine activated mouse macrophages.	Recognizes human, mouse and rat iNOS (NOS II). Cross-reacts slightly with nNOS (NOS I). Does not cross-react with eNOS (NOS III).		WB	ALX-210-503-C050	50 µg
PAb to iNOS (mouse)	Synthetic peptide corresponding to aa 1131-1144 (K^{1131}KGSALEEPKATRL^{1144}) of mouse inducible nitric oxide synthase (iNOS; NOS II).	Recognizes mouse iNOS (NOS II). Does not cross-react with eNOS (NOS III) and nNOS (NOS I). Detects a band of ~130kDa by WB.	[11–14, 16, 21, 22]	IHC (PS), WB	ALX-210-504-R100	100 µg
PAb to iNOS	Synthetic peptide corresponding to aa 17-31 (D^{17}LKEEKDINNVKKT^{31}) of mouse inducible nitric oxide synthase (iNOS; NOS II).	Recognizes human, mouse and rat iNOS (NOS II). Detects a band of ~135kDa by WB	[24, 31, 32]	ICC, WB	ALX-210-515-R200	200 µl
Antibodies to eNOS (NOS III)						
PAb to eNOS	Synthetic peptide corresponding to aa 597-611 (P^{597}YNSSPRPEQHKSYK^{611}) of bovine endothelial nitric oxide synthase (eNOS; NOS III).	Recognizes human, mouse, rat and dog eNOS (NOS III). Does not cross-react with iNOS (NOS II) or nNOS (NOS I). Detects a band of ~140kDa by WB.	[7, 8, 10, 16, 25]	ICC, WB	ALX-210-505/1-R100	100 µl
PAb to eNOS	Synthetic peptide corresponding to aa 1185-1201 (R^{1185}GAVPWAFDPPGSDTNS^{1201}) of C-terminal human endothelial nitric oxide synthase (eNOS; NOS III).	Recognizes human and bovine eNOS (NOS III). Does not cross-react with nNOS (NOS I) or iNOS (NOS II).		WB	ALX-210-511-R100	100 µl
MAb to eNOS (phosphorylated) (pSer^{1177}) **Clone:** 15E2 **Isotype:** Mouse IgG1	Synthetic phosphopeptide from human endothelial nitric oxide synthase (eNOS; NOS III).	Recognizes human Ser^{1177}-phosphorylated eNOS (NOS III). Does not cross-react with unphosphorylated (eNOS (NOS III) or other unrelated serine-phosphorylated proteins.		WB	ALX-804-396-C100	100 µg
MAb to eNOS **Clone:** H32 **Isotype:** IgG2a	Purified endothelial nitric oxide synthase from cultured bovine aortic endothelial cells.	Recognizes human, bovine, baboon, pig, rat and mouse eNOS (NOS III). Does not cross-react with nNOS (NOS I) or iNOS (NOS II)	[4, 6]	IHC, IP, WB, ELISA	ALX-804-651-C100	100 µg
Antibodies to NOS (Universal)						
MAb to NOS **Clone:** NOS-3F7-B11-B5 **Isotype:** Mouse IgM	Purified neuronal nitric oxide synthase (nNOS; NOS I) from bovine brain.	Recognizes iNOS (NOS II), eNOS (NOS III) and nNOS (NOS I) in mouse, rat and bovine samples.	[5, 26]	IHC (FS), WB	ALX-804-029-R200	200 µl
PAb to NOS	Synthetic peptide corresponding to aa 1113-1122 (Q^{1113}KRYHEDIFG^{1122}) of mouse inducible nitric oxide synthase (nNOS; NOS II). and neuronal nitric oxide synthase (nNOS; NOS I). This sequence is highly conserved between different NOS isoforms.	Recognizes mouse iNOS (NOS II), rat, bovine and pig nNOS (NOS I) and human, pig and bovine eNOS (NOS III). Detects NOS in human endothelial cells, macrophages and peripheral neural tract cells.	[20, 23, 27–30, 34]	IHC (FS), WB	ALX-210-508-R100	100 µl

LIT: [1] Nitric oxide synthase in cardiac nerve fibers and neurons of rat and guinea pig heart: L. Klimaschewski, et al.; Circ. Res. **71,** 1533 (1992) ▪ **[2]** Nitric oxide synthase in VIP-containing vasodilator nerve fibres in the guinea-pig: W. Kummer, et al.; Neuroreport **3,** 653 (1992) ▪ **[3]** Nitric oxide synthase-containing neural processes on large cerebral arteries and cerebral microvessels: C. Ladecola, et al.; Brain Res. **606,** 148 (1993) ▪ **[4]** Characterization and localization of endothelial nitric oxide synthase using specific monoclonal antibodies: J.S. Pollock, et al.; Am. J. Physiol. **265,** C1379 (1993) ▪ **[5]** Distribution of NOS in normoxic vs. hypoxic rat lung: upregulation of NOS by chronic hypoxia: C. Xue, et al.; Am. J. Physiol. **267,** L667 (1994) ▪ **[6]** Nitric oxide synthase isozymes antibodies: J.S. Pollock, et al.; Histochem. J. **27,** 738 (1995) ▪ **[7]** Physiological dilation of uteroplacental arteries in the guinea pig depends on nitric oxide synthase activity of extravillous trophoblast: A. Nanaev, et al.; Cell Tissue Res. **282,** 407 (1995) ▪ **[8]** Reduced gene expression of vascular endothelial NO synthase and cyclooxygenase-1 in heart failure: C.J. Smith, et al.; Circ. Res. **78,** 58 (1996) ▪ **[9]** Endothelin-1 production and decreased endothelin B receptor expression in advanced prostate cancer: J.B. Nelson, et al.; Cancer Res. **56,** 663 (1996) ▪ **[10]** Inducible and endothelial nitric oxide synthase expression during development of transplant arteriosclerosis in rat aortic grafts: L.M. Akyurek, et al.; Am. J. Pathol. **149,** 1981 (1996) ▪ **[11]** Induction of nitric oxide synthase in the human cardiac allograft is associated with contractile dysfunction of the left ventricle: N.P. Lewis, et al.; Circulation **93,** 720 (1996) ▪ **[12]** Experimental allergic encephalomyelitis in the rat is inhibited by aminoguanidine, an inhibitor of nitric oxide synthase: W. Zhao, et al.; J. Neuroimmunol. **64,** 123 (1996) ▪ **[13]** Localization of nitric oxide synthases and nitric oxide production in the rat mammary gland: M. Onoda & H. Inano; J. Histochem. Cytochem. **46,** 1269 (1998) ▪ **[14]** Specificity of antibodies to nitric oxide synthase isoforms in human, guinea pig, rat, and mouse tissues: W. Coers, et al.; J. Histochem. Cytochem. **46,** 1385 (1998) ▪ **[15]** Decreased endothelial nitric oxide synthase in gastric mucosa of rats with chronic renal failure: M. Tomikawa, et al.; Am. J. Physiol. **274,** F1102 (1998) ▪ **[16]** Granulated metrial gland cells contain nitric oxide synthases during pregnancy in the rat: S.M. Sladek, et al.; Placenta **19,** 55 (1998) ▪ **[17]** Limb reduction defects in endothelial nitric oxide synthase-deficient mice: A.R. Gregg, et al.; Am. J. Physiol. **275,** H2319 (1998) ▪ **[18]** Interaction between caveolin-1 and the reductase domain of endothelial nitric-oxide synthase. Consequences for catalysis: S. Ghosh, et al.; J. Biol. Chem. **273,** 22267 (1998) ▪ **[19]** Nitric oxide synthase in cardiac sarcoplasmic reticulum: K.Y. Xu, et al.; PNAS **96,** 657 (1999) ▪ **[20]** Nitric oxide and cyclic GMP regulate retinal patterning in the optic lobe of Drosophila: S.M. Gibbs & J.W. Truman; Neuron **20,** 83 (1998) ▪ **[21]** Alterations of nitric oxide synthase expression and activity during rat lung transplantation: M. Liu, et al.; Am. J. Physiol. Lung Cell Mol. Physiol. **278,** L1071 (2000) ▪ **[22]** Differential inducible nitric oxide synthase expression in systemic and pulmonary vessels after endotoxin: E.J. Pulido, et al.; Am. J. Physiol. Regul. Integr. Comp. Physiol. **278,** R1232 (2000) ▪ **[23]** Neurons involved in nitric oxide-mediated cGMP signaling in the tobacco hornworm, Manduca sexta: R.M. Zayas, et al.; J. Comp. Neurol. **419,** 422 (2000) ▪ **[24]** Upregulation of nitric oxide synthase in mice with severe hypoxia-induced pulmonary hypertension: K.A. Fagan, et al.; Respir. Res. **2,** 306 (2001) ▪ **[25]** Decreased expression of myocardial eNOS and caveolin in dogs with hypertrophic cardiomyopathy: A. Piech, et al.; Am. J. Physiol. Heart Circ. Physiol. **282,** H219 (2002) ▪ **[26]** Nitric oxide protects cardiac sarcolemmal membrane enzyme function and ion active transport against ischemia-induced inactivation: K.Y. Xu, et al.; J. Biol. Chem. **278,** 41798 (2003) ▪ **[27]** Interactions between epithelial nitric oxide signaling and phosphodiesterase activity in Drosophila: K.E. Broderick, et al.; Am. J. Physiol. Cell Physiol. **285,** C1207 (2003) ▪ **[28]** Inducible peroxidases mediate nitration of anopheles midgut cells undergoing apoptosis in response to Plasmodium invasion: S. Kumar, et al.; J. Biol. Chem. **279,** 53475 (2004) ▪ **[29]** Nitric oxide inhibits the rate and strength of cardiac contractions in the lobster Homarus americanus by acting on the cardiac ganglion: A. Mahadevan, et al.; J. Neurosci. **24,** 2813 (2004) ▪ **[30]** Cardioprotection during the final stage of the late phase of ischemic preconditioning is mediated by neuronal NO synthase in concert with cyclooxygenase-2: Y. Wang, et al.; Circ. Res. **95,** 84 (2004) ▪ **[31]** Myocyte nitric oxide synthase 2 contributes to blunted beta-adrenergic response in failing human hearts by decreasing Ca2+ transients: M.T. Ziolo, et al.; Circulation **109,** 1886 (2004) ▪ **[32]** Endocardial endothelium in the avascular frog heart: role for diffusion of NO in control of cardiac O2 consumption: A. Adler, et al.; Am. J. Physiol. Heart Circ. Physiol. **287,** H14 (2004) ▪ **[33]** Gonadal hormones and frontocortical expression of vascular endothelial growth factor in male stroke-prone, spontaneously hypertensive rats, a model for attention-deficit/hyperactivity disorder: S. Jesmin, et al.; Endocrinology **145,** 4330 (2004) ▪ **[34]** Midgut epithelial responses of different mosquito-Plasmodium combinations: the actin cone zipper repair mechanism in Aedes aegypti: L. Gupta, et al.; PNAS **102,** 4010 (2005) ▪ **[35]** Acetylcholine induces contractile and relaxant effects in canine nasal venous systems: M. Wang & M.A. Lung; Eur. Respir. J. **28,** 839 (2006)

Inhibitors of Nitric Oxide Synthases

Product Name	Product No.	Size		µM	IC_{50} and K_i values in µM nNOS		iNOS		eNOS		Lit. Ref.
1400W . 2HCl [N-(3-(Aminomethyl)benzyl)acetamidine . 2HCl]	ALX-270-073-M005 ALX-270-073-M025	5 mg 25 mg		IC_{50} IC_{50} K_i	3.2 7.3 2	(h) (h) (h)	0.794 0.23 0.007	(h) (h) (h)	49 1000 50	(h) (h) (h)	[50] [53] [35]
(4S)-N-(4-Amino-5[aminoethyl]aminopentyl)-N'-nitro-guanidine . 3 TFA	ALX-270-408-M001 ALX-270-408-M005	1 mg 5 mg		K_i	0.12	(r)	39	(m)	314	(b)	[54]
L-NAA . HCl [N^G-Amino-L-arginine . HCl]	ALX-106-014-M005	5 mg		IC_{50} K_i K_i	3 0.3 1.2	(r) (b) (r)	7.4 3 1.7	(m) (m) (r)	2.5	(b)	[1] [33] [14]
AET . 2HBr [S-(2-Aminoethyl)-ITU . 2HBr]	ALX-270-030-M010 ALX-270-030-M050	10 mg 50 mg		IC_{50} K_i	14.5 1.8	(h) (h)	7.9 0.59	(h) (h)	28.8 2.1	(h) (h)	[50] [13]
Aminoguanidine . bicarbonate	ALX-420-004-M100 ALX-420-004-M500	100 mg 500 mg		IC_{50} IC_{50}	631 41	(h) (b)	126 5	(h) (m)	794 255	(h) (b)	[50] [39, 44]
Aminoguanidine . HCl	ALX-420-006-M100 ALX-420-006-M500	100 mg 500 mg		IC_{50} IC_{50}	30.3 3300	(h) (b)	6.4 3.2	(h) (m)	22.1	(h)	[27] [27]
Aminoguanidine . $½H_2SO_4$	ALX-420-021-M100 ALX-420-021-M500 ALX-420-021-G005 ALX-420-021-G010	100 mg 500 mg 5 g 10 g		K_i K_i K_i K_i	3300 160 830	(r) (r)	700 5.4 16 100 32	(m) (m) (m) (h) (m)	>8000	(b)	[39] [7] [26] [13]
2-Amino-4-methylpyridine [2-Amino-4-picoline]	ALX-270-449-G001	1 g		IC_{50} IC_{50} IC_{50}	0.1 0.068	(h) (h)	0.04 0.006 0.08	(h) (m) (h)	0.09 0.112	(h) (h)	[27] [27] [50]
S-(3-Aminopropyl)-ITU . 2HBr	ALX-270-029-M010 ALX-270-029-M050	10 mg 50 mg		IC_{50} K_i	4.1 0.61	(r) (h)	 0.46	 (h)	 1.2	 (h)	[30, 47] [13]
4-Amino-(6R)-BH4 . 2HCl [4-Amino-(6R)-5,6,7,8-tetrahydro-L-biopterin . 2HCl]	ALX-440-046-M005	5 mg		IC_{50}	1	(r)*	7.2	(m)**	14.8	(b)***	[32*, 37**, 48***]
AMT . HCl [2-Amino-5,6-dihydro-6-methyl-4*H*-1,3-thiazine . HCl]	ALX-270-033-M010 ALX-270-033-M050	10 mg 50 mg		IC_{50} IC_{50} K_i	0.008 0.034	(h) (r)	0.008 0.004 0.04	(h) (m) (m)	0.025 0.15	(h) (b)	[50] [24] [24]
Aprotinin (bovine) (Competitive Protein Inhibitor of NOS)	BCO-5018-1	10 mg		K_i	0.05	(r)	0.078	(r)			[42]
3-Bromo-7-nitroindazole	ALX-270-009-M005 ALX-270-009-M025 ALX-270-009-M050	5 mg 25 mg 50 mg		IC_{50}	0.17	(r)	0.29	(r)	0.86	(b)	[21]
3-Bromo-7-nitroindazole . Na	ALX-270-199-M005 ALX-270-199-M025	5 mg 25 mg									
ADMA . 2HABS (asymmetrical) [N^G, N^G-Dimethyl-L-arginine . 2HABS]	ALX-106-005-M005 ALX-106-005-M025	5 mg 25 mg		IC_{50} K_i	~2 0.67	(r)* (r)	~40	(m)**			[22*, 5**] [56]
ADMA . 2HCl (asymmetrical) [N^G, N^G-Dimethyl-L-arginine . 2HCl]	ALX-106-006-M005 ALX-106-006-M025	5 mg 25 mg		K_i	>300	(r)	>300	(r)			[14]
SDMA . 2HABS (symmetrical) [N^G, N'^G-Dimethyl-L-arginine . 2HABS]	ALX-106-007-M005 ALX-106-007-M025	5 mg 25 mg		Control compound.							[5]
SDMA . 2HCl (symmetrical) [N^G, N'^G-Dimethyl-L-arginine . 2HCl]	ALX-106-008-M005 ALX-106-008-M025	5 mg 25 mg									
Diphenyleneiodonium chloride	ALX-270-003-M025 ALX-270-003-M100	25 mg 100 mg		IC_{50}			0.05	(m)	0.3	(p)	[4]
S-Ethyl-ITU . HBr [S-Ethylisothiourea . HBr]	ALX-270-025-M050 ALX-270-025-M250	50 mg 250 mg		IC_{50} IC_{50} K_i K_i	0.123 0.015 0.25 0.029	(h) (b) (r) (h)	0.056 0.02 0.013 0.019	(h) (m) (m) (h)	0.166 0.28 0.37 0.039	(h) (b) (b) (h)	[50] [39] [24] [13]
ETPI . HCl [S-Ethyl-N-[4-(trifluoromethyl)phenyl]isothiourea . HCl]	ALX-270-182-M005 ALX-270-182-M025	5 mg 25 mg		IC_{50} IC_{50} K_i	0.093 3.6 0.32	(r) (h) (h)	8.6 >100 37	(m) (h) (h)	5.2 50 9.4	(b) (h) (h)	[51] [50] [38]
GED . bicarbonate [Guanidinoethyldisulfide . $2H_2CO_3$]	ALX-270-276-M010	10 mg		K_i	25	(b)	4.3	(m)	18	(b)	[31]
GGA [α-Guanidinoglutaric acid]	ALX-270-023-M010 ALX-270-023-M050	10 mg 50 mg		IC_{50} K_i	64 2.7	(r) (r)					[19] [19]
GW 274150 [(S)-2-Amino-(1-iminoethylamino)-5-thioheptanoic acid] *(Due to patent restrictions not sold by ALEXIS® Biochemicals)*	ALX-270-269-M001	1 mg		IC_{50} IC_{50} IC_{50} K_i	145 177 206 4.6	(h) (h) (r) (h)	1.4 2.2 4.5 1.12	(h) (h) (r) (h)	466 544 185	(h) (h) (h)	[52, 53] [59] [59!] [59]
HMN-1180	ALX-270-457-M001 ALX-270-457-M005	1 mg 5 mg		K_i	5.4	(r)					[45]
L-HOArg . AcOH [N^G-Hydroxy-L-arginine . AcOH]	ALX-106-004-M005 ALX-106-004-M025	5 mg 25 mg		K_i	15.2	(r)	6.9	(r)			[14]
2-Iminobiotin [Guanidinobiotin]	ALX-430-008-M010 ALX-430-008-M050	10 mg 50 mg		Ki	37.5	(r)	21.8	(m)			[16]
2-Imino-4-methylpiperidine . AcOH	ALX-270-211-M005 ALX-270-211-M025	5 mg 25 mg		IC_{50}	0.2	(h)	0.1	(h)	1.1	(h)	[43]
S-Isopropyl-ITU . HBr [S-Isopropylisothiourea . HBr]	ALX-270-028-M010 ALX-270-028-M050	10 mg 50 mg		IC_{50}	0.009 0.037	(b) (h)	0.008 0.010	(m) (h)	0.075 0.022	(b) (h)	[39] [13]
1,5-Isoquinolinediol [5-Hydroxy-1(2*H*)-isoquinolinone]	ALX-480-039-M005 ALX-480-039-M025	5 mg 25 mg		IC_{50} IC_{50}			9.3	(m)			[60]

Purified (PF) = Purified (Preservative free); FC = Flow Cytometry; ICC = Immunocytochemistry; IP = Immunoprecipitation; IHC = Immunohistochemistry (FS = Frozen Sections, PS = Paraffin Sections); WB = Western blot; BP = Blocking Peptide

Inhibitors of Nitric Oxide Synthases *continued*

IC_{50} and K_i values in µM

Product Name	Product No.	Size	µM	nNOS		iNOS		eNOS		Lit. Ref.
MEG . sodium succinate [Mercaptoethylguanidine . sodium succinate]	ALX-270-252-M010	10 mg	IC_{50}	60	(r)	11.5	(r)	110	(b)	[31]
S-Methyl-ITU . H_2SO_4 [SMT . H_2SO_4; S-Methylisothiourea . H_2SO_4]	ALX-270-027-M050	50 mg	IC_{50}	0.51	(h)	0.5	(h)	0.6	(h)	[50]
	ALX-270-027-M250	250 mg	K_i	0.16	(h)	0.12	(h)	0.2	(h)	[13]
S-Methyl-L-thiocitrulline . 2HCl	ALX-106-012-M010	10 mg	IC_{50}	0.31	(r)			5.4	(r)	[11]
	ALX-106-012-M050	50 mg	IC_{50}	1.05	(r)	2.2	(r)			[25]
			IC_{50}	0.002	(r)	0.01	(m)	0.012	(b)	[51]
			IC_{50}	0.04	(h)	0.2	(h)	0.115	(h)	[50]
			K_i	0.001	(h)	0.04	(h)	0.011	(h)	[11]
			K_i	0.05	(r)	0.84	(r)			[25]
L-NAME . HCl	ALX-105-003-G005	5 g	IC_{50}	2.5	(r)	20	(r)			[8]
[N^G-Nitro-L-arginine-methyl ester . HCl]	ALX-105-003-G025	25 g	IC_{50}	0.76	(h)	31.6	(h)	1.51	(h)	[50]
D-NAME . HCl	ALX-105-004-M250	250 mg	Control compound.							
[N^G-Nitro-D-arginine-methyl ester . HCl]	ALX-105-004-G001	1 g								
	ALX-105-004-G005	5 g								
L-NOARG	ALX-105-001-G005	5 g	IC_{50}	0.022	(r)	13	(m)	0.03	(b)	[51]
[L-NNA; N^G-Nitro-L-arginine]	ALX-105-001-G025	25 g	IC_{50}	0.047	(h)	3.2	(h)	0.091	(h)	[50]
			K_i	0.015	(b)*	4.4	(m)*	0.16	(b)*	[6*, 3**,
								0.039	(h)***	13***]
			K_i	0.2	(r)	8.7	(r)			14]
D-NOARG	ALX-105-002-M250	250 mg	Control compound.							
[N^G-Nitro-D-arginine]	ALX-105-002-G001	1 g								
L-NIL . 2HCl	ALX-270-010-M010	10 mg	IC_{50}	4	(h)	2	(h)	7.8	(h)	[50]
[L-N^6-(1-Iminoethyl)-lysine . 2HCl]	ALX-270-010-M050	50 mg	IC_{50}	37	(h)	1.6	(h)	78	(h)	[52, 53]
			IC_{50}	4.1	(h)	0.9	(h)	10.4	(h)	[27]
			IC_{50}			1	(m)			[27]
			IC_{50}	92	(r)	3.3	(m)			[15]
			IC_{50}	7	(b)	0.5	(m)	8	(b)	[44]
			K_i	570	(b)	55	(m)			[44]
L-NIO . 2HCl	ALX-270-002-M025	25 mg	IC_{50}	0.96	(h)	1.58	(h)	1	(h)	[50]
[L-N^5-(1-Iminoethyl)-ornithine . 2HCl]	ALX-270-002-M100	100 mg	IC_{50}	0.3	(b)	0.3	(m)	0.08	(b)	[44]
			IC_{50}	3.9	(r)	2.2	(m)			[15]
			IC_{50}			3	(m)			[2]
			K_i	250	(b)	45	(m)			[44]
			K_i	1.7	(r)	3.9	(m)	3.9	(b)	[41]
7-NI	ALX-270-004-M025	25 mg	IC_{50}	0.71	(r)	5.8	(r)	0.8	(b)	[21, 29]
[7-Nitroindazole]	ALX-270-004-M100	100 mg	IC_{50}	2.5	(b)	20	(m)	0.8	(b)	[17, 18]
7-NiNa	ALX-270-200-M005	5 mg	IC_{50}	8.3	(h)	9.7	(h)	11.8	(h)	[53]
[7-Nitroindazole . Na]	ALX-270-200-M025	25 mg	K_i	0.16	(b)	1.6	(m)	0.8	(b)	[17]
	ALX-270-200-M100	100 mg								
L-NMEA . AcOH	ALX-106-010-M005	5 mg	K_i	66	(r)	81	(r)			[14]
[N^G-Monoethyl-L-arginine . AcOH]	ALX-106-010-M025	25 mg								
L-NMMHA . AcOH	ALX-106-011-M005	5 mg	No data available!							[55]
[N^G-Monomethyl-L-homoarginine . AcOH]	ALX-106-011-M025	25 mg								
L-NMMA . AcOH	ALX-106-001-M005	5 mg	IC_{50}	0.25	(h)	2.63	(h)	0.32	(h)	[50]
[N^G-Monomethyl-L-arginine . AcOH]	ALX-106-001-M025	25 mg	IC_{50}			35.7	(m)			[58]
	ALX-106-001-M100	100 mg	IC_{50}	7.1	(h)	3.3	(h)	5.3	(h)	[27]
	ALX-106-001-G001	1 g				4.2	(m)			
			K_i	0.18	(r)	6	(r)			[10]
			K_i	0.5	(r)*	2.7	(m)**	0.41	(h)***	[56*, 9**,
								0.94	(b)°	12***, 3°]
			K_i	0.7	(r)	2.5	(r)			[14]
1,3-PBIT . 2HBr; 1,3-PB-ITU . 2HBr	ALX-270-026-M010	10 mg	IC_{50}	2.3	(h)	0.5	(h)	31.6	(h)	[50]
[S,S'-(1,3-Phenylene-bis(1,2-ethanediyl))bis-isothiourea . 2HBr]	ALX-270-026-M050	50 mg	K_i	0.25	(h)	0.047	(h)	9	(h)	[13]
1,4-PBIT . 2HBr; 1,4-PB-ITU . 2HBr	ALX-270-024-M010	10 mg	IC_{50}	0.32	(h)	0.1	(h)	5.8	(h)	[50]
[S,S'-(1,4-Phenylene-bis(1,2-ethanediyl))bis-isothiourea . 2HBr]	ALX-270-024-M050	50 mg	K_i	0.016	(h)	0.007	(h)	0.36	(h)	[13]
N-ω-Propyl-L-arginine	ALX-270-203-M005	5 mg	IC_{50}	0.085	(r)	209	(m)	2	(b)	[51]
[N^5-[Imino(propylamino)methyl]-L-ornithine]	ALX-270-203-M025	25 mg	IC_{50}	4	(h)	100	(h)	8.7	(b)	[50]
			K_i	0.057	(b)	180	(m)	8.5	(b)	[40]
			K_i	0.11	(b)	80	(m)	10	(b)	[46]
SKF 525A . HCl	ALX-550-222-M100	100 mg	IC_{50}	90	(r)					[20]
	ALX-550-222-M250	250 mg								
L-Thiocitrulline . 2HCl	ALX-106-013-M010	10 mg	IC_{50}	4.6	(r)	1.7	(r)	1.9	(h)	[49]
	ALX-106-013-M050	50 mg	K_i	0.06	(r)	3.6	(r)			[10]
TRIM	ALX-270-170-M050	50 mg	IC_{50}	28.2	(m)	27	(r)	1057	(b)	[23]
[1-(2-Trifluoromethylphenyl)imidazole]			IC_{50}	32	(r)					[36]
			K_i	21.7	(m)					[28]
			K_i	47.3	(r)					[36]
Vinyl-L-NIO	ALX-270-216-M005	5 mg	IC_{50}	0.398	(h)	0.794	(h)	1.4	(h)	[50]
[L-VNIO; N^5-(1-Imino-3-butenyl)-L-ornithine]	ALX-270-216-M025	25 mg	K_i	0.1	(r)	60	(m)	12	(b)	[41]
			K_i	4.76	(h)	5.14	(h)	10.3	(h)	[57]
Zinc(II) Protoporphyrin IX	ALX-430-049-M005	5 mg	IC50	0.8	(r)	4	(m)	5	(b)	[34]
(Heme-site inhibitor)	ALX-430-049-M025	25 mg								
	ALX-430-049-M100	100 mg								

NOTE: K_i values and IC_{50} values are given only as a guide to the relative isotype selectivity of the inhibitor. These values depend upon the exact system used for measurement, i.e. assay systems, assay conditions and species. In the case of some mechanism-based inactivators (e.g. 1400W), the inhibitor binds to all isoforms, but irreversible inhibition only occurs with certain isoforms. In these cases, K_i values alone do not indicate selectivity; instead, a Selectivity Ratio must be calculated.

r=rat, m=mouse, h=human, b=bovine, p=porcine

Literature References

For further information please consult the literature references included with the catalog listing for each product.

[1] Nitric oxide synthesis in the CNS endothelium and macrophages differs in its sensitivity to inhibition by arginine analogues: L.E. Lambert, et al.; Life Sci. **48,** 69 (1991) ▪ **[2]** Identification of N-iminoethyl-L-ornithine as an irreversible inhibitor of nitric oxide synthase in phagocytic cells: T.B. McCall, et al.; Br. J. Pharmacol. **102,** 234 (1991) ▪ **[3]** Purification and characterization of particulate endothelium-derived relaxing factor synthase from cultured and native bovine aortic endothelial cells: J.S. Pollock, et al.; PNAS **88,** 10480 (1991) ▪ **[4]** Inhibition of macrophage and endothelial cell nitric oxide synthase by diphenyleneiodonium and its analogs: D.J. Stuehr, et al.; Faseb J. **5,** 98 (1991) ▪ **[5]** Accumulation of an endogenous inhibitor of nitric oxide synthesis in chronic renal failure: P. Vallance, et al.; Lancet **339,** 572 (1992) ▪ **[6]** Selective inhibition of constitutive nitric oxide synthase by L-NG-nitroarginine: E.S. Furfine, et al.; Biochemistry **32,** 8512 (1993) ▪ **[7]** Selective inhibition of the inducible nitric oxide synthase by aminoguanidine: T.P. Misko, et al.; Eur. J. Pharmacol. **233,** 119 (1993) ▪ **[8]** Induction by endotoxin of nitric oxide synthase in the rat mesentery: lack of effect on action of vasoconstrictors: J.A. Mitchell, et al.; Br. J. Pharmacol. **109,** 265 (1993) ▪ **[9]** NG-methyl-L-arginine functions as an alternate substrate and mechanism-based inhibitor of nitric oxide synthase: N.M. Olken & M.A. Marletta; Biochemistry **32,** 9677 (1993) ▪ **[10]** L-thiocitrulline. A stereospecific, heme-binding inhibitor of nitric-oxide synthases: C. Frey, et al.; J. Biol. Chem. **269,** 26083 (1994) ▪ **[11]** Potent and selective inhibition of human nitric oxide synthases. Selective inhibition of neuronal nitric oxide synthase by S-methyl-L-thiocitrulline and S-ethyl-L-thiocitrulline: E.S. Furfine, et al.; J. Biol. Chem. **269,** 26677 (1994) ▪ **[12]** Purification and characterization of the constitutive nitric oxide synthase from human placenta: E.P. Garvey, et al.; Arch. Biochem. Biophys. **311,** 235 (1994) ▪ **[13]** Potent and selective inhibition of human nitric oxide synthases. Inhibition by non-amino acid isothioureas: E.P. Garvey, et al.; J. Biol. Chem. **269,** 26669 (1994) ▪ **[14]** Inhibition of purified nitric oxide synthase from rat cerebellum and macrophage by L-arginine analogs: Y. Komori, et al.; Arch. Biochem. Biophys. **315,** 213 (1994) ▪ **[15]** L-N6-(1-iminoethyl)lysine: a selective inhibitor of inducible nitric oxide synthase: W.M. Moore, et al.; J. Med. Chem. **37,** 3886 (1994) ▪ **[16]** 2-Iminobiotin is an inhibitor of nitric oxide synthases: S.J. Sup, et al.; BBRC **204,** 962 (1994) ▪ **[17]** The inhibition of the constitutive and inducible nitric oxide synthase isoforms by indazole agents: D.J. Wolff & B.J. Gribin; Arch. Biochem. Biophys. **311,** 300 (1994) ▪ **[18]** The inhibition of the constitutive bovine endothelial nitric oxide synthase by imidazole and indazole agents: D.J. Wolff, et al.; Arch. Biochem. Biophys. **314,** 360 (1994) ▪ **[19]** alpha-Guanidinoglutaric acid, an endogenous convulsant, as a novel nitric oxide synthase inhibitor: I. Yokoi, et al.; J. Neurochem. **63,** 1565 (1994) ▪ **[20]** Subcellular localization and characterization of neuronal nitric oxide synthase: M. Hecker, et al.; J. Neurochem. **62,** 1524 (1994) ▪ **[21]** 7-Nitro indazole derivatives are potent inhibitors of brain, endothelium and inducible isoforms of nitric oxide synthase: P.A. Bland-Ward & P.K. Moore; Life Sci. **57,** PL131 (1995) ▪ **[22]** Response of cerebral blood vessels to an endogenous inhibitor of nitric oxide synthase: F.M. Faraci, et al.; Am. J. Physiol. **269,** H1522 (1995) ▪ **[23]** The antinociceptive effect of 1-(2-trifluoromethylphenyl) imidazole (TRIM), a potent inhibitor of neuronal nitric oxide synthase in vitro, in the mouse: R.L. Handy, et al.; Br. J. Pharmacol. **116,** 2349 (1995) ▪ **[24]** Novel potent and selective inhibitors of inducible nitric oxide synthase: M. Nakane, et al.; Mol. Pharmacol. **47,** 831 (1995) ▪ **[25]** S-alkyl-L-thiocitrullines. Potent stereoselective inhibitors of nitric oxide synthase with strong pressor activity in vivo: K. Narayanan, et al.; J. Biol. Chem. **270,** 11103 (1995) ▪ **[26]** Aminoguanidine is an isoform-selective, mechanism-based inactivator of nitric oxide synthase: D.J. Wolff & A. Lubeskie; Arch. Biochem. Biophys. **316,** 290 (1995) ▪ **[27]** 2-Amino-4-methylpyridine as a potent inhibitor of inducible NO synthase activity in vitro and in vivo: W.S. Faraci, et al.; Br. J. Pharmacol. **119,** 1101 (1996) ▪ **[28]** Inhibition of nitric oxide synthase by 1-(2-trifluoromethylphenyl) imidazole (TRIM) in vitro: antinociceptive and cardiovascular effects: R.L. Handy, et al.; Br. J. Pharmacol. **119,** 423 (1996) ▪ **[29]** 7-nitroindazole: an inhibitor of nitric oxide synthase: P.K. Moore & P.A. Bland-Ward; Methods Enzymol. **268,** 393 (1996) ▪ **[30]** Spontaneous rearrangement of aminoalkylisothioureas into mercaptoalkylguanidines, a novel class of nitric oxide synthase inhibitors with selectivity towards the inducible isoform: G.J. Southan, et al.; Br. J. Pharmacol. **117,** 619 (1996) ▪ **[31]** Pharmacological characterization of guanidinoethyldisulphide (GED), a novel inhibitor of nitric oxide synthase with selectivity towards the inducible isoform: C. Szabo, et al.; Br. J. Pharmacol. **118,** 1659 (1996) ▪ **[32]** Identification of the 4-amino analogue of tetrahydrobiopterin as a dihydropteridine reductase inhibitor and a potent pteridine antagonist of rat neuronal nitric oxide synthase: E.R. Werner, et al.; Biochem. J. **320 (Pt 1),** 193 (1996) ▪ **[33]** Inactivation of nitric oxide synthase isoforms by diaminoguanidine and NG-amino-L-arginine: D.J. Wolff & A. Lubeskie; Arch. Biochem. Biophys. **325,** 227 (1996) ▪ **[34]** Inhibition of nitric oxide synthase isoforms by porphyrins: D.J. Wolff, et al.; Arch. Biochem. Biophys. **333,** 27 (1996) ▪ **[35]** 1400W is a slow, tight binding, and highly selective inhibitor of inducible nitric-oxide synthase in vitro and in vivo: E.P. Garvey, et al.; J. Biol. Chem. **272,** 4959 (1997) ▪ **[36]** Mechanism of the inhibition of neuronal nitric oxide synthase by 1-(2-trifluoromethylphenyl) imidazole (TRIM): R.L. Handy & P.K. Moore; Life Sci. **60,** PL389 (1997) ▪ **[37]** Tetrahydrobiopterin binding to macrophage inducible nitric oxide synthase: heme spin shift and dimer stabilization by the potent pterin antagonist 4-amino-tetrahydrobiopterin: B. Mayer, et al.; Biochemistry **36,** 8422 (1997) ▪ **[38]** Substituted N-phenylisothioureas: potent inhibitors of human nitric oxide synthase with neuronal isoform selectivity: B.G. Shearer, et al.; J. Med. Chem. **40,** 1901 (1997) ▪ **[39]** Inactivation of nitric oxide synthase by substituted aminoguanidines and aminoisothioureas: D.J. Wolff, et al.; J. Pharmacol. Exp. Ther. **283,** 265 (1997) ▪ **[40]** Potent and selective inhibition of neuronal nitric oxide synthase by N omega-propyl-L-arginine: H.Q. Zhang, et al.; J. Med. Chem. **40,** 3869 (1997) ▪ **[41]** N5-(1-Imino-3-butenyl)-L-ornithine. A neuronal isoform selective mechanism-based inactivator of nitric oxide synthase: B.R. Babu & O.W. Griffith; J. Biol. Chem. **273,** 8882 (1998) ▪ **[42]** Aprotinin, the first competitive protein inhibitor of NOS activity: G. Venturini, et al.; BBRC **249,** 263 (1998) ▪ **[43]** Substituted 2-iminopiperidines as inhibitors of human nitric oxide synthase isoforms: R.K. Webber, et al.; J. Med. Chem. **41,** 96 (1998) ▪ **[44]** Inactivation of nitric oxide synthases and cellular nitric oxide formation by N6-iminoethyl-L-lysine and N5-iminoethyl-L-ornithine: D.J. Wolff, et al.; Eur. J. Pharmacol. **350,** 325 (1998) ▪ **[45]** HMN-1180, a small molecule inhibitor of neuronal nitric oxide synthase: M. Nishio, et al.; J. Pharmacol. Exp. Ther. **287,** 1063 (1998) ▪ **[46]** N(omega)-Nitroarginine-containing dipeptide amides. Potent and highly selective inhibitors of neuronal nitric oxide synthase: H. Huang, et al.; J. Med. Chem. **42,** 3147 (1999) ▪ **[47]** Nitric oxide synthase inhibition by dimaprit and dimaprit analogues: J.B. Paquay, et al.; Br. J. Pharmacol. **127,** 331 (1999) ▪ **[48]** Preferential inhibition of inducible nitric oxide synthase in intact cells by the 4-amino analogue of tetrahydrobiopterin: K. Schmidt, et al.; Eur. J. Biochem. **259,** 25 (1999) ▪ **[49]** Heterocyclic analogues of L-citrulline as inhibitors of the isoforms of nitric oxide synthase (NOS) and identification of N(delta)-(4,5-dihydrothiazol-2-yl)ornithine as a potent inhibitor: S. Ulhaq, et al.; Bioorg. Med. Chem. **7,** 1787 (1999) ▪ **[50]** The inhibitory potency and selectivity of arginine substrate site nitric-oxide synthase inhibitors is solely determined by their affinity toward the different isoenzymes: R. Boer, et al.; Mol. Pharmacol. **58,** 1026 (2000) ▪ **[51]** Cellular and enzymatic studies of N(omega)-propyl-l-arginine and S-ethyl-N-[4-(trifluoro methyl)phenyl]isothiourea as reversible, slowly dissociating inhibitors selective for the neuronal nitric oxide synthase isoform: G.R. Cooper, et al.; Arch. Biochem. Biophys. **375,** 183 (2000) ▪ **[52]** Inhibition of inducible nitric oxide synthase by acetamidine derivatives of hetero-substituted lysine and homolysine: R.J. Young, et al.; Bioorg. Med. Chem. Lett. **10,** 597 (2000) ▪ **[53]** Nitric oxide synthases: structure, function and inhibition: W.K. Alderton, et al.; Biochem. J. **357,** 593 (2001) ▪ **[54]** Reduced amide bond peptidomimetics. (4S)-N-(4-amino-5-[aminoakyl]aminopentyl)-N′-nitroguanidines, potent and highly selective inhibitors of neuronal nitric oxide synthase: J.M. Hah, et al.; J. Med. Chem. **44,** 2667 (2001) ▪ **[55]** Na+ – and Cl- -coupled active transport of nitric oxide synthase inhibitors via amino acid transport system B(0,+): T. Hatanaka, et al.; J. Clin. Invest. **107,** 1035 (2001) ▪ **[56]** Endogenous methylarginines regulate neuronal nitric-oxide synthase and prevent excitotoxic injury: A.J. Cardounel & J.L. Zweier; J. Biol. Chem. **277,** 33995 (2002) ▪ **[57]** Structural characterization and kinetics of nitric-oxide synthase inhibition by novel N5-(iminoalkyl)- and N5-(iminoalkenyl)-ornithines: L.E. Bretscher, et al.; J. Biol. Chem. **278,** 46789 (2003) ▪ **[58]** Staminane- and isopimarane-type diterpenes from Orthosiphon stamineus of Taiwan and their nitric oxide inhibitory activity: M.T. Nguyen, et al.; J. Nat. Prod. **67,** 654 (2004) ▪ **[59]** GW274150 and GW273629 are potent and highly selective inhibitors of inducible nitric oxide synthase in vitro and in vivo: W.K. Alderton, et al.; Br. J. Pharmacol. **145,** 301 (2005) ▪ **[60]** Inhibition of NOS-2 induction in LPS-stimulated J774.2 cells by 1, 5-isoquinolinediol, an inhibitor of PARP: R. Olszanecki, et al.; J. Physiol. Pharmacol. **57,** 109 (2006)

Selected Review Articles: NOS Inhibitors

Progress in the development of selective nitric oxide synthase (NOS) inhibitors: L. Salerno, et al.; Curr. Pharm. Des. **8,** 177 (2002) ▪ Nitric oxide synthase inhibitors. Preclinical studies of potential use for treatment of opioid withdrawal: D.B. Vaupel, et al.; Neuropsychopharmacology **13,** 315 (1995) ▪ Selective pharmacological inhibition of distinct nitric oxide synthase isoforms: G.J. Southan & C. Szabo; Biochem. Pharmacol. **51,** 383 (1996) ▪ Selective inhibitors of neuronal nitric oxide synthase--is no NOS really good NOS for the nervous system?: P.K. Moore & R.L. Handy; Trends. Pharmacol. Sci. **18,** 204 (1997) ▪ Nitric Oxide. IV. Determinants of nitric oxide protection and toxicity in liver: J. Li & T.R. Billiar; Am. J. Physiol. **276,** G1069 (1999) ▪ Nitric oxide synthases: structure, function and inhibition: W.K. Alderton, et al.; Biochem. J. **357,** 593 (2001) ▪ Nitric oxide synthase inhibition as therapy for sepsis: a decade of promise: J.P. Cobb; Surg. Infect. (Larchmt) **2,** 93 (2001) ▪ Nitric oxide synthase inhibitors for the treatment of chronic tension-type headache: M. Ashina; Expert. Opin. Pharmacother. **3,** 395 (2002) ▪ Progress in the development of selective nitric oxide synthase (NOS) inhibitors: L. Salerno, et al.; Curr. Pharm. Des. **8,** 177 (2002) ▪ Nitric oxide synthases: domain structure and alignment in enzyme function and control: D.K. Ghosh & J.C. Salerno; Front. Biosci. **8,** d193 (2003) ▪ Pharmacology of nitric oxide: molecular mechanisms and therapeutic strategies: R. Domenico; Curr. Pharm. Des. **10,** 1667 (2004) ▪ Biology and chemistry of the inhibition of nitric oxide synthases by pteridine-derivatives as therapeutic agents: H. Matter & P. Kotsonis; Med. Res. Rev. **24,** 662 (2004) ▪ Pharmacologic neuroprotection with an inhibitor of nitric oxide synthase for the treatment of glaucoma: A.H. Neufeld; Brain Res. Bull. **62,** 455 (2004) ▪ Selective neuronal nitric oxide synthase inhibitors: E.P. Erdal, et al.; Curr. Top. Med. Chem. **5,** 603 (2005) ▪ Nitric oxide synthase inhibition in sepsis? Lessons learned from large-animal studies: B. Hauser, et al.; Anesth. Analg. **101,** 488 (2005) ▪ Computational studies of competitive inhibitors of nitric oxide synthase (NOS) enzymes: towards the development of powerful and isoform-selective inhibitors: A. Tafi, et al.; Curr. Med. Chem. **13,** 1929 (2006) ▪ Computational studies of competitive inhibitors of nitric oxide synthase (NOS) enzymes: towards the development of powerful and isoform-selective inhibitors: A. Tafi, et al.; Curr. Med. Chem. **13,** 1929 (2006)

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Latest Insights

iNOS (NOS II) Regulation by Aggresome Formation

Misfolding and aggregation of proteins play an important part in the pathogenesis of several genetic and degenerative diseases. Evidence suggests that cells have evolved a pathway that involves sequestration of aggregated proteins into specialized "holding stations" called aggresomes. K.E. Kolodziejska, et al. showed that cells regulate inducible NO synthase (iNOS), an important host defense protein, through aggresome formation, whereas iNOS aggresome formation depends on a functional dynein motor and the integrity of the microtubules. The iNOS aggresome represents a "physiologic aggresome" and thus defines a new paradigm for cellular regulation of protein processing.

LIT: Regulation of inducible nitric oxide synthase by aggresome formation: K.E. Kolodziejska, et al.; PNAS **102,** 4854 (2005)

Alternatively spliced nNOS (NOS I) mediates penile erection

Nitric oxide (NO) is synthesized by three major enzymes derived from distinct genes, inducible NO synthase (iNOS/NOS II), endothelial NOS (eNOS/NOS III), and several variants of neuronal NOS (nNOS/NOS I). Penile erection is mediated by NO produced by nNOS and eNOS, the former initiating erection, and the latter providing sustained maximal erection. The relative contributions of different forms of NOS to penile erection are unclear. K.J. Hurt, et al. showed that alternatively spliced forms of nNOS mediate a major portion of penile erection.

LIT: Alternatively spliced neuronal nitric oxide synthase mediates penile erection: K.J. Hurt, et al.; PNAS **103,** 3440 (2006)

NOS Substrate

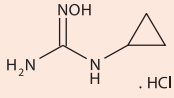
Selective nNOS (NOS I) Substrate

N-Cyclopropyl-N′-hydroxy-guanidine . HCl

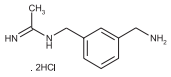
ALX-420-034-M001	1 mg
ALX-420-034-M005	5 mg

Selective substrate for nNOS (NOS I). 70% nitric oxide (NO) formation for nNOS (NOS I), 26% for eNOS (NOS III) and <0.5% for iNOS (NOS II) as compared to NO formation using N^G-hydroxy-L-arginine (NOHA, Prod. No. ALX-106-004) as a substrate.

LIT: Isoform-selective substrates of nitric oxide synthase: Q. Jia, et al.; J. Med. Chem. **46,** 2271 (2003)



Selected Key Inhibitors of NOS



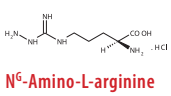
1400W . 2HCl

1400W . 2HCl

ALX-270-073-M005 5 mg
ALX-270-073-M025 25 mg

Highly selective inhibitor of iNOS (NOS II) *in vitro* and *in vivo*.

SLECTED LIT: Actions of isoform-selective and non-selective nitric oxide synthase inhibitors on endotoxin-induced vascular leakage in rat colon: F. Laszlo & B.J.R. Whittle; Eur. J. Pharmacol. **334,** 99 (1997) ▪ 1400W is a slow, tight binding, and highly selective inhibitor of inducible nitric-oxide synthase in vitro and in vivo: E.P. Garvey, et al.; J. Biol. Chem. **272,** 4959 (1997) ▪ Selective inhibition of inducible nitric oxide synthase inhibits tumor growth in vivo: studies with 1400W, a novel inhibitor: L.L. Thomsen, et al.; Cancer Res. **57,** 3300 (1997) ▪ Nitric oxide synthases: structure, function and inhibition: W.K. Alderton, et al.; Biochem. J. **357,** 593 (2001) ▪ Structural basis for the specificity of the nitric-oxide synthase inhibitors W1400 and Nomega-propyl-L-Arg for the inducible and neuronal isoforms: R. Fedorov, et al.; J. Biol. Chem. **278,** 45818 (2003) ▪ Addition of nitric oxide donor S-nitroso-N-acetylcysteine to selective iNOS inhibitor 1400W further improves contractile function in reperfused skeletal muscle: J.U. Barker, et al.; Microsurgery **25,** 338 (2005) ▪ 1400W, a potent selective inducible NOS inhibitor, improves histopathological outcome following traumatic brain injury in rats: M. Jafarian-Tehrani, et al.; Nitric Oxide **12,** 61 (2005) ▪ Effects of 1400W, a potent selective inducible NOS inhibitor, on histamine- and leukotriene D4-induced relaxation of isolated guinea pig nasal mucosa: Y. Chiba, et al.; Nitric Oxide **15,** 142 (2006) ▪ **For a comprehensive bibliography please visit our website.**



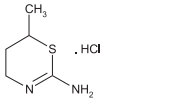
N^G-Amino-L-arginine . HCl

N^G-Amino-L-arginine . HCl

ALX-106-014-M005 5 mg

Potent inhibitor of eNOS (NOS III) and iNOS (NOS II).

SLECTED LIT: NG-amino-L-arginine: a new potent antagonist of L-arginine-mediated endothelium-dependent relaxation: J.M. Fukuto, et al.; BBRC **168,** 458 (1990) ▪ Macrophage and endothelial cell nitric oxide synthesis: cell-type selective inhibition by NG-aminoarginine, NG-nitroarginine and NG- methylarginine: S.S. Gross, et al.; BBRC **170,** 96 (1990) ▪ Comparison of the inhibitory potencies of N(G)-methyl-, N(G)-nitro- and N(G)-amino-L-arginine on EDRF function in the rat: evidence for continuous basal EDRF release: H.M. Vargas, et al.; J. Pharmacol. Exp. Ther. **257,** 1208 (1991) ▪ Inhibition of purified nitric oxide synthase from rat cerebellum and macrophage by L-arginine analogs: Y. Komori, et al.; Arch. Biochem. Biophys. **315,** 213 (1994) ▪ Inactivation of nitric oxide synthase isoforms by diaminoguanidine and NG-amino-L-arginine: D.J. Wolff & A. Lubeskie; Arch. Biochem. Biophys. **325,** 227 (1996) ▪ Studies of neuronal nitric oxide synthase inactivation by diverse suicide inhibitors: R. Bryk, et al.; Arch. Biochem. Biophys. **369,** 243 (1999) ▪ Alteration of the heme prosthetic group of neuronal nitric-oxide synthase during inactivation by N(G)-amino-L-arginine in vitro and in vivo: J.L. Vuletich, et al.; Mol. Pharmacol. **62,** 110 (2002) ▪ Metabolism of aminoguanidine, diaminoguanidine, and NG-amino-L-arginine by neuronal NO-synthase and covalent alteration of the heme prosthetic group: A.J. Lee, et al.; Chem. Res. Toxicol. **18,** 1927 (2005) ▪ **For a comprehensive bibliography please visit our website.**



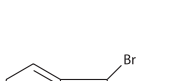
AMT . HCl

AMT . HCl

ALX-270-033-M010 10 mg
ALX-270-033-M050 50 mg

Potent and selective inhibitor of iNOS (NOS II).

SLECTED LIT: Novel potent and selective inhibitors of inducible nitric oxide synthase: M. Nakane, et al.; Mol. Pharmacol. **47,** 831 (1995) ▪ In vivo pharmacological evaluation of two novel type II (inducible) nitric oxide synthase inhibitors: W.R. Tracey, et al.; Can. J. Physiol. Pharmacol. **73,** 665 (1995) ▪ Role of inducible nitric oxide synthase in regulation of pulmonary vascular tone in the late gestation ovine fetus: R.L. Rairigh, et al.; J. Clin. Invest. **101,** 15 (1998) ▪ The inhibitory potency and selectivity of arginine substrate site nitric-oxide synthase inhibitors is solely determined by their affinity toward the different isoenzymes: R. Boer, et al.; Mol. Pharmacol. **58,** 1026 (2000) ▪ **For a comprehensive bibliography please visit our website.**



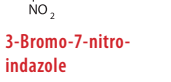
3-Bromo-7-nitroindazole

3-Bromo-7-nitroindazole

ALX-270-009-M005 5 mg
ALX-270-009-M025 25 mg
ALX-270-009-M050 50 mg

Potent inhibitor of rat cerebellar nNOS (NOS I). More potent than 7-nitroindazole (Prod. No. ALX-270-004).

SLECTED LIT: 7-Nitro indazole derivatives are potent inhibitors of brain, endothelium and inducible isoforms of nitric oxide synthase: P.A. Bland-Ward & P.K. Moore; Life Sci. **57,** PL131 (1995) ▪ Further in vivo studies on attenuating morphine withdrawal: isoform-selective nitric oxide synthase inhibitors differ in efficacy: D.B. Vaupel, et al.; Eur. J. Pharmacol. **324,** 11 (1997) ▪ 3-bromo-7-nitroindazole, a neuronal nitric oxide synthase inhibitor, impairs maternal aggression and citrulline immunoreactivity in prairie voles: S.C. Gammie, et al.; Brain Res. **870,** 80 (2000) ▪ Crystal structure of nitric oxide synthase bound to nitro indazole reveals a novel inactivation mechanism: C.S. Raman, et al.; Biochemistry **40,** 13448 (2001) ▪ Effects of selective neuronal nitric oxide synthase inhibition on sleep and wakefulness in the rat: M. Cavas & J.F. Navarro; Prog. Neuropsychopharmacol. Biol. Psychiatry **30,** 56 (2006) ▪ **For a comprehensive bibliography please visit our website.**



Diphenyleneiodonium chloride

3-Bromo-7-nitroindazole . sodium salt

ALX-270-199-M005 5 mg
ALX-270-199-M025 25 mg

More soluble salt form of 3-bromo-7-nitroindazole (Prod. No. ALX-270-009).

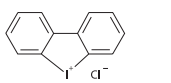
SLECTED LIT: See ALX-270-009

Diphenyleneiodonium chloride

ALX-270-003-M025 25 mg
ALX-270-003-M100 100 mg

Binds strongly to flavoproteins and is thus a powerful and specific inhibitor of several important enzymes, including nitric oxide synthase (NOS), NADPH-ubiquinone oxidoreductase, NADPH oxidases and NADPH cytochrome P450 oxidoreductase. Nitric oxide synthase (NOS) which shows significant homology with cytochrome P450 reductase, has shown to be irreversibly inhibited by this compound.

SLECTED LIT: Specific labelling of a constituent polypeptide of bovine heart mitochondrial reduced nicotinamide-adenine dinucleotide-ubiquinone reductase by the inhibitor diphenyleneiodonium: C.I. Ragan & D.P. Bloxham; Biochem. J. **163,** 605 (1977) ▪ The inhibition by diphenyleneiodonium and its analogues of superoxide generation by macrophages: J.T. Hancock & O.T.G. Jones; Biochem. J. **242,** 103 (1987) ▪ Inhibition of macrophage and endothelial cell nitric oxide synthase by diphenyleneiodonium and its analogs: D.J. Stuehr, et al.; FASEB J. **5,** 98 (1991) ▪ Inhibition of cytochrome P450 reductase by the diphenyliodonium cation. Kinetic analysis and covalent modifications: D.G. Tew; Biochemistry **32,** 10209 (1993) ▪ Diphenyleneiodonium inhibits NF-kappaB activation and iNOS expression induced by IL-1beta: involvement of reactive oxygen species: A.F. Mendes, et al.; Mediators Inflamm. **10,** 209 (2001) ▪ **For a comprehensive bibliography please visit our website.**



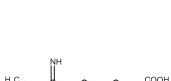
N-ω-Propyl-L-arginine

N-ω-Propyl-L-arginine

ALX-270-203-M005 5 mg
ALX-270-203-M025 25 mg

Potent and selective inhibitor of nNOS (NOS I) relative to iNOS (NOS II) (3158-fold) and eNOS (NOS III) (149-fold).

SLECTED LIT: Potent and selective inhibition of neuronal nitric oxide synthase by N-omega-propyl-L-arginine: H.Q. Zhang, et al.; J. Med. Chem. **40,** 3869 (1997) ▪ Mechanism of inactivation of neuronal nitric oxide synthase by N-allyl-L-arginine: H.Q. Zhang, et al.; JACS **119,** 10888 (1997) ▪ N(omega)-Nitroarginine-containing dipeptide amides. Potent and highly selective inhibitors of neuronal nitric oxide synthase: H. Huang, et al.; J. Med. Chem. **42,** 3147 (1999) ▪ Conformationally-restricted arginine analogues as alternative substrates and inhibitors of nitric oxide synthases: Y. Lee, et al.; Bioorg. Med. Chem. **7,** 1097 (1999) ▪ Cellular and enzymatic studies of N(omega)-propyl-l-arginine and S-ethyl-N-[4-(trifluoromethyl)phenyl]isothiourea as reversible, slowly dissociating inhibitors selective for the neuronal nitric oxide synthase isoform: G.R. Cooper, et al.; Arch. Biochem. Biophys. **375,** 183 (2000) ▪ Structural basis for the specificity of the nitric-oxide synthase inhibitors W1400 and Nomega-propyl-L-Arg for the inducible and neuronal isoforms: R. Fedorov, et al.; J. Biol. Chem. **278,** 45818 (2003) ▪ The neuronal selective nitric oxide synthase inhibitor, Nomega-propyl-L-arginine, blocks the effects of phencyclidine on prepulse inhibition and locomotor activity in mice: D. Klamer, et al.; Eur. J. Pharmacol. **503,** 103 (2004) ▪ **For a comprehensive bibliography please visit our website.**



TRIM

S-Methyl-L-thiocitrulline . 2 HCl

ALX-106-012-M010 10 mg
ALX-106-012-M050 50 mg

Inhibitor of nNOS (NOS I).

SLECTED LIT: Potent and selective inhibition of human nitric oxide synthases. Selective inhibition of neuronal nitric oxide synthase by S-methyl-L- thiocitrulline and S-ethyl-L-thiocitrulline: E.S. Furfine, et al.; J. Biol. Chem. **269,** 26677 (1994) ▪ Synthesis of L-thiocitrulline, L-homothiocitrulline, and S-methyl-L- thiocitrulline: a new class of potent nitric oxide synthase inhibitors: K. Narayanan & O.W. Griffith; J. Med. Chem. **37,** 885 (1994)

TRIM

ALX-270-170-M050 50 mg

Potent nNOS (NOS I) inhibitor.

SLECTED LIT: The antinociceptive effect of 1-(2-trifluoromethylphenyl) imidazole (TRIM), a potent inhibitor of neuronal nitric oxide synthase in vitro, in the mouse: R.L. Handy, et al.; Br. J. Pharmacol. **116,** 2349 (1995) ▪ Inhibition of nitric oxide synthase by 1-(2-trifluoromethylphenyl) imidazole (TRIM) in vitro: antinociceptive and cardiovascular effects: R.L. Handy, et al.; Br. J. Pharmacol. **119,** 423 (1996) ▪ Mechanism of the inhibition of neuronal nitric oxide synthase by 1-(2-trifluoromethylphenyl) imidazole (TRIM): R.L. Handy & P.K. Moore; Life Sci. **60,** PL389 (1997) ▪ Cerebroprotective effect of the nitric oxide synthase inhibitors, 1-(2-trifluoromethylphenyl) imidazole and 7-nitro indazole, after transient focal cerebral ischemia in the rat: K.J. Escott, et al.; J. Cereb. Blood Flow Metab. **18,** 281 (1998) ▪ Antidepressant- and anxiolytic-like effects of selective neuronal NOS inhibitor 1-(2-trifluoromethylphenyl)-imidazole in mice: V. Volke, et al.; Behav. Brain Res. **140,** 141 (2003) ▪ **For a comprehensive bibliography please visit our website.**

Vinyl-L-NIO

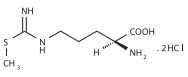
Vinyl-L-NIO

[L-VNIO]

ALX-270-216-M005 5 mg
ALX-270-216-M025 25 mg

Very selective and potent nNOS (NOS I) inhibitor.

SLECTED LIT: N5-(1-Imino-3-butenyl)-L-ornithine. A neuronal isoform selective mechanism-based inactivator of nitric oxide synthase: B. R. Babu & O.W. Griffith; J. Biol. Chem. **273,** 8882 (1998) ▪ The inhibitory potency and selectivity of arginine substrate site nitric-oxide synthase inhibitors is solely determined by their affinity toward the different isoenzymes: R. Boer, et al.; Mol. Pharmacol. **58,** 1026 (2000) ▪ Structural characterization and kinetics of nitric-oxide synthase inhibition by novel N5-(iminoalkyl)- and N5-(iminoalkenyl)-ornithines: L.E. Bretscher, et al.; J. Biol. Chem. **278,** 46789 (2003) ▪ Neuronal nitric-oxide synthase inhibition facilitates adrenergic neurotransmission in rat mesenteric resistance arteries: Y. Hatanaka, et al.; J. Pharmacol. Exp. Ther. **316,** 490 (2006) ▪ Discoordinate regulation of renal nitric oxide synthase isoforms in ovariectomized mRen2.Lewis rats: L.M. Yamaleyeva, et al.; Am. J. Physiol. Regul. Integr. Comp. Physiol. **292,** R819 (2007) ▪ **For a comprehensive bibliography please visit our website.**



S-Methyl-L-thiocitrulline . 2 HCl

Protein NOS Inhibitor

Aprotinin (bovine)

BC0-5018-1 10 mg

Isolated from bovine lung.

More Information? Please visit

www.axxora.com

NEW NOS Inhibitors – *from the leader in the field*

New Inhibitor of iNOS (NOS II)

NEW

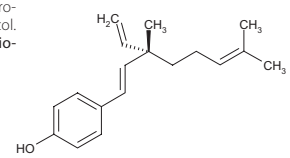
Bakuchiol

[4-(3-Ethenyl-3,7-dimethyl-1,6-octadienyl)phenol]

ALX-350-144-M001 1 mg

Inhibitor of iNOS (NOS II) expression.

LIT: Plant antimutagenic agents, 2. Flavonoids: M.E. Wall, et al.; J. Nat. Prod. **51,** 1084 (1988) ▪ DNA polymerase and topoisomerase II inhibitors from Psoralea corylifolia: N.J. Sun, et al.; J. Nat. Prod. **61,** 362 (1998) ▪ Inhibition of mitochondrial lipid peroxidation by Bakuchiol, a meroterpene from Psoralea corylifolia: H. Haraguchi, et al.; Planta Med. **66,** 569 (2000) ▪ Bakuchiol: a hepatoprotective compound of Psoralea corylifolia on tacrine-induced cytotoxicity in Hep G2 cells: H. Cho, et al.; Planta Med. **67,** 750 (2001) ▪ Bakuchiol from Psoralea corylifolia inhibits the expression of inducible nitric oxide synthase gene via the inactivation of nuclear transcription factor-kappaB in RAW 264.7 macrophages: H.O. Pae, et al.; Int. Immunopharmacol. **1,** 1849 (2001) ▪ **For a comprehensive bibliography please visit our website.**



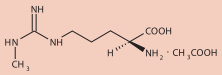
Featured Standard NOS Inhibitors

Step up to the Source™

L-NMMA . monoacetate

ALX-106-001-M005 5 mg
ALX-106-001-M025 25 mg
ALX-106-001-M100 100 mg
ALX-106-001-G001 1 g

For larger quantities – please inquire!

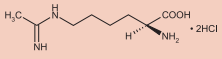


L-NMMA . monoacetate

L-NIL . 2HCl

ALX-270-010-M010 10 mg
ALX-270-010-M050 50 mg

For 1 g or larger quantities – please inquire!

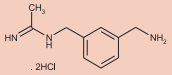


L-NIL . 2HCl

1400W . 2HCl

ALX-270-073-M005 5 mg
ALX-270-073-M025 25 mg

For 1 g or larger quantities – please inquire!



1400W . 2HCl

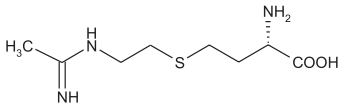
Please inquire for Bulk Quotations!

GW 274150

GW 274150

Inhibitor of iNOS (NOS II) that is potent (K_i=100nM for human iNOS/NOS II), long-acting and highly selective (over eNOS/NOS III >250-fold, over nNOS/NOS I >80-fold).

SELECTED LIT: GW274150 is a potent, long-acting, highly selective inhibitor of iNOS (NOS-2) with therapeutical potential in post-operative ileus: W. Alderton, et al.; Acta Physiol. Scand. **167 Suppl.,** O-36 (1999) ▪ Inhibition of inducible nitric oxide synthase by acetamidine derivatives of hetero-substituted lysine and homolysine: R.J. Young, et al.; Bioorg. Med. Chem. Lett. **10,** 597 (2000) ▪ Nitric oxide synthases: structure, function and inhibition: W.K. Alderton, et al.; Biochem. J. **357,** 593 (2001) ▪ Beneficial effects of GW274150, a novel, potent and selective inhibitor of iNOS activity, in a rodent model of collagen-induced arthritis: S. Cuzzocrea, et al.; Eur. J. Pharmacol. **453,** 119 (2002) ▪ A novel, potent and selective inhibitor of the activity of inducible nitric oxide synthase (GW274150) reduces the organ injury in hemorrhagic shock: M.C. McDonald, et al.; J. Physiol. Pharmacol. **53,** 555 (2002) ▪ GW274150, a potent and highly selective inhibitor of iNOS, reduces experimental renal ischemia/reperfusion injury: P.K. Chatterjee, et al.; Kidney Int. **63,** 853 (2003) ▪ GW274150 inhibits nitric oxide production by primary cultures of rat proximal tubular cells: P.K. Chatterjee, et al.; Med. Sci. Monit. **9,** BR357 (2003) ▪ Effects of GW274150, a novel and selective inhibitor of iNOS activity, in acute lung inflammation: L. Dugo, et al.; Br. J. Pharmacol. **141,** 979 (2004) ▪ GW274150 and GW273629 are potent and highly selective inhibitors of inducible nitric oxide synthase in vitro and in vivo: W.K. Alderton, et al.; Br. J. Pharmacol. **145,** 301 (2005) ▪ Beneficial effects of GW274150 treatment on the development of experimental colitis induced by dinitrobenzene sulfonic acid: R. Di Paola, et al.; Eur. J. Pharmacol. **507,** 281 (2005) ▪ GW274150, a novel and highly selective inhibitor of the inducible isoform of nitric oxide synthase (iNOS), shows analgesic effects in rat models of inflammatory and neuropathic pain: J. De Alba, et al.; Pain **120,** 170 (2006) ▪ Role of inducible nitric oxide synthase in the reduced responsiveness of the myocardium to catecholamines in a hyperdynamic, murine model of septic shock: E. Barth, et al.; Crit Care Med **34,** 307 (2006) ▪ γ(+) LAT-1 mediates transport of the potent and selective iNOS inhibitor, GW274150, in control J774 macrophages: A.R. Baydoun, et al.; Amino Acids **31,** 101 (2006)



Latest Insight

R. Olszanecki, et al. describe that the potent PARP inhibitor 1,5-isoquinolinediol is a potent inhibitor of induction of iNOS (NOS II) in LPS-treated mouse macrophages.

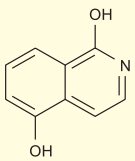
1,5-Isoquinolinediol

[5-Hydroxy-1(2H)-isoquinolinone]

ALX-480-039-M005 5 mg
ALX-480-039-M025 25 mg

Potent inhibitor of iNOS (NOS II) in mouse macrophages.

LIT: Inhibition of NOS-2 induction in LPS-stimulated J774.2 cells by 1, 5-isoquinolinediol, an inhibitor of PARP: R. Olszanecki, et al.; J. Physiol. Pharmacol. **57,** 109 (2006)



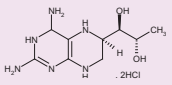
NOS Inhibitors – Latest Additions

NEW 4-Amino-(6R)-5,6,7,8-tetrahydro-L-biopterin . 2HCl

ALX-440-046-M005 5 mg

Potent, reversible inhibitor of nitric oxide synthases (NOS) at their pterin-site. Inhibits the formation of nitric oxide (NO) by nNOS (NOS I) with IC_{50}=1.1µM for arginine (Prod. No. ALX-101-004) and IC_{50}=1.3µM for N^G-hydroxy-L-arginine (Prod. No. ALX-106-004). Prolongs allograft survival *in vivo* and rescues rats from septic shock. Immunosuppressant.

SELECTED LIT: Allosteric modulation of rat brain nitric oxide synthase by the pterin-site enzyme inhibitor 4-aminotetrahydrobiopterin: S. Pfeiffer, et al.; Biochem. J. **328,** 349 (1997) ▪ Tetrahydrobiopterin binding to macrophage inducible nitric oxide synthase: heme spin shift and dimer stabilization by the potent pterin antagonist 4-amino-tetrahydrobiopterin: B. Mayer, et al.; Biochemistry **36,** 8422 (1997) ▪ Preferential inhibition of inducible nitric oxide synthase in intact cells by the 4-amino analogue of tetrahydrobiopterin: K. Schmidt, et al.; Eur. J. Biochem. **259,** 25 (1999) ▪ Protection against endotoxemia in rats by a novel tetrahydrobiopterin analogue: S. Bahrami, et al.; Shock **13,** 386 (2000) ▪ Inhibition of endotoxin-induced vascular hyporeactivity by 4-amino-tetrahydrobiopterin: H.D. Gibraeil, et al.; Br. J. Pharmacol. **131,** 1757 (2000) ▪ The 4-amino analogue of tetrahydrobiopterin efficiently prolongs murine cardiac-allograft survival: G. Brandacher, et al.; J. Heart Lung Transplant. **20,** 747 (2001) ▪ A 4-amino analogue of tetrahydrobiopterin attenuates endotoxin-induced hemodynamic alterations and organ injury in rats: F. Fitzal, et al.; Shock **18,** 158 (2002) ▪ Biopterin analogues: novel nitric oxide synthase inhibitors with immunosuppressive action: E.R. Werner and G. Werner-Felmayer; Curr. Drug Metab. **3,** 119 (2002) ▪ **For a comprehensive bibliography please visit our website.**



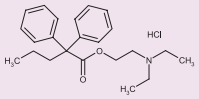
4-Amino-(6R)-5,6,7,8-tetrahydro-L-biopterin . 2HCl

NEW SKF-525A . HCl

ALX-550-222-M100 100 mg
ALX-550-222-M250 250 mg

Cell permeable inhibitor of nNOS (NOS I) (IC_{50}=90µM). Inhibitor of cytochrome P450 system. Calcium antagonist. Blocks glibenclamide-sensitive K^+ channels (IC_{50}=4.4mM). Inhibits platelet thromboxane synthesis and stimulates endothelial cell prostacyclin production. Potentiates the effects of many drugs *in vivo*.

SELECTED LIT: Effects of 2-diethylaminoethyl 2,2-diphenylpentanoate (SKF 525A) on insecticidal potency: P.S. Hewlett, et al.; Nature **192,** 1273 (1961) ▪ The effects of beta-diethylaminoethyl-diphenylpropylacetate (SKE 525-A) on biological membranes. I. SKF 525-A-induced stabilization of human erythrocytes: I.P. Lee, et al.; Biochem. Pharmacol. **17,** 1671 (1968) ▪ Heterogeneity of cytochrome P-450 in rat liver microsomes: selective interaction of metyrapone and SKF 525-A with different fractions of microsomal cytochrome P-450: H. Grasdalen, et al.; FEBS Lett. **60,** 294 (1975) ▪ Inhibition of mitochondrial oxidative metabolism by SKF-525A in intact cells and isolated mitochondria: T. Galeotti, et al.; Biochem. Pharmacol. **32,** 3285 (1983) ▪ Prostacyclin-stimulating drugs: new prospects: J.M. Boeynaems, et al.; Prostaglandins **32,** 145 (1986) ▪ Defining the active site of cytochrome P-450: the crystal and molecular structure of an inhibitor, SKF-525A: M. Rossi, et al.; Carcinogenesis **8,** 881 (1987) ▪ Stimulation of vascular prostacyclin by SKF 525-A (proadifen) and related compounds: J.M. Boeynaems, et al.; Biochem. Pharmacol. **36,** 1637 (1987) ▪ **For a comprehensive bibliography please visit our website.**



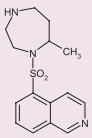
SKF-525A . HCl

NEW HMN-1180

ALX-270-457-M001 1 mg
ALX-270-457-M005 5 mg

Selective inhibitor of nNOS (NOS I) (K_i=5.4µM) due to direct binding to the L-arginine binding site of nNOS (NOS I).

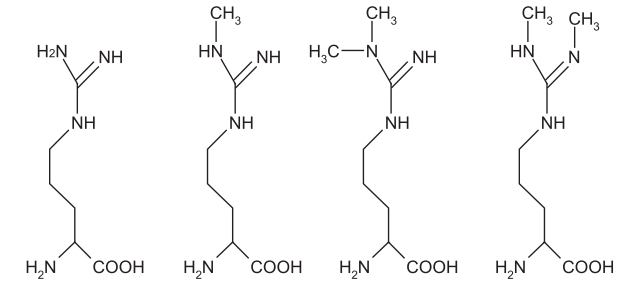
LIT: HMN-1180, a small molecule inhibitor of neuronal nitric oxide synthase: M. Nishio, et al.; J. Pharmacol. Exp. Ther. **287,** 1063 (1998)



HMN-1180

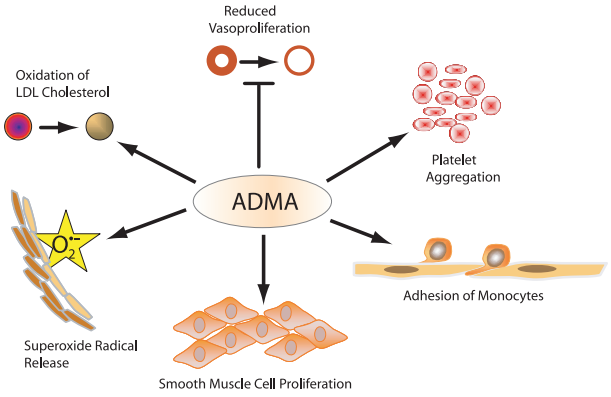
Asymmetric (ADMA) & Symmetric (SDMA) Dimethylarginine

Asymmetric dimethylarginine (ADMA) is a natural component of human plasma. It represents a post-translational modified form of L-arginine which is generated during protein turnover in all cells. In 1992 ADMA was identified as an endogenous inhibitor of nitric oxide synthases (NOS) [1]. ADMA seems to be involved in different cardiovascular pathologies and an increasing amount of clinical studies describe it as an important predictor and biomarker.



L-ARGININE L-NMMA ADMA SDMA

ADMA originates from the proteolysis of proteins methylated at arginine residues. Methylation of the terminal nitrogen atom of L-arginine within proteins is catalyzed by a family of enzymes termed protein-arginine methyltransferases (PRMTs) [2], of which two classes exist. Type I PRMTs catalyze the formation of ADMA, while type II PRMTs generate symmetric dimethylarginine (SDMA). The third arginine analog identified is produced by both types of PRMTs and known as monomethyl-L-arginine (L-NMMA). While SDMA is inactive, L-NMMA and ADMA both have been shown to inhibit nitric oxide ($NO^{\bullet}$) formation after their liberation by proteolysis [1, 3]. Free ADMA is primarily degraded by the intracellular enzyme dimethylarginine dimethylaminohydrolase (DDAH). A small part leaves the cell via cationic amino acid transporters, which are also able to import the NOS inhibitor. Liver and kidney play a key role in the clearance of ADMA from the plasma, mainly by metabolic degradation via DDAH, less by urinary excretion.



Pathophysiological effets of elevated ADMA levels, mediated by the inhibition of NOS.

ADMA is known as an endogenous inhibitor of all three NOS isoforms. Therefore, an elevated level of ADMA is considered as a risk factor, preventing the homeostatic effects of $NO^{\bullet}$ on the vascular network. Several studies have indicated an association between ADMA and endothelial dysfunction. By example, as a competitive antagonist of nitric oxide synthases, ADMA acts as an endogenous inhibitor of angiogenesis.

Recently, it has been concluded that SDMA might be a useful parameter for early stages of chronic kidney diseases and a risk marker for developing cardiovascular disease [4].

LIT: [1] Accumulation of an endogenous inhibitor of nitric oxide synthesis in chronic renal failure: P. Vallance, et al.; Lancet **339,** 572 (1992) ▪ **[2]** Protein arginine methyltransferases: guardians of the Arg?: F. O. Fackelmayer; TIBS **30,** 666 (2005) ▪ **[3]** Vascular endothelial cells synthesize nitric oxide from L-arginine: R. M. Palmer, et al.; Nature **333,** 664 (1988) ▪ **[4]** Symmetrical dimethylarginine: a new combined parameter for renal function and extent of coronary artery disease: S. M. Bode-Boger, et al.; J. Am. Soc. Nephrol. **17,** 1128 (2006)

Selected Latest Review Articles

Measurement of asymmetric dimethylarginine in plasma: methodological considerations and clinical relevance: T. Teerlink; Clin. Chem. Lab. Med. **43,** 1130 (2005) ▪ Asymmetric dimethylarginine (ADMA): a cardiovascular and renal risk factor on the move: C. Zoccali; J. Hypertens. **24,** 611 (2006) ▪ The clinical significance of asymmetric dimethylarginine: M. P. Siroen, et al.; Annu. Rev. Nutr. **26,** 203 (2006) ▪ The synthesis and metabolism of asymmetric dimethylarginine (ADMA): J. M. Leiper & P. Vallance; Eur. J. Clin. Pharmacol. **62 (Suppl 13),** 33 (2006) ▪ Asymmetric dimethylarginine (ADMA): an endogenous inhibitor of angiogenesis: J.P. Cooke; Eur. J. Clin. Pharmacol. **62 (Suppl 13),** 115 (2006)

Antibody to PRMT1

MAb to Arginine N-Methyltransferase (human) (4B12)

CVL-MAB0010-1 200 µl

CLONE: 4B12. **ISOTYPE:** Mouse IgG1. **IMMUNOGEN:** PRMT1 (arginine N-methyltransferase). **SPECIFICITY:** Recognizes human PRMT1. **APPLICATION:** IHC, WB.

ADMA & SDMA

N^G, N^G-Dimethyl-L-arginine . di(p-hydroxyazobenzene-p'-sulfonate)

[ADMA . 2HABS]

ALX-106-005-M005 5 mg
ALX-106-005-M025 25 mg

This salt form is more stable than the dihydrochloride (Prod. No. ALX-106-006).

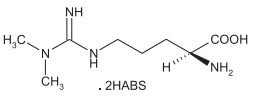
LIT: L-arginine is required for expression of the activated macrophage effector mechanism causing selective metabolic inhibition in target cells: J.B. Hibbs Jr., et al.; J. Immunol. **138,** 550 (1987) ▪ Accumulation of an endogenous inhibitor of nitric oxide synthesis in chronic renal failure: P. Vallance, et al.; Lancet **339,** 572 (1992)

N^G, N^G-Dimethyl-L-arginine . 2HCl

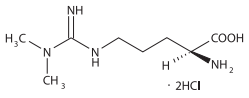
[ADMA . 2HCl]

ALX-106-006-M005 5 mg
ALX-106-006-M025 25 mg

LIT: See Prod. No. ALX-106-005 (above)



ADMA . 2HABS



ADMA . 2HCl

N^G, N'^G-Dimethyl-L-arginine . di(p-hydroxyazobenzene-p'-sulfonate)

[SDMA . 2HABS]

ALX-106-007-M005 5 mg
ALX-106-007-M025 25 mg

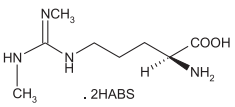
LIT: L-arginine is required for expression of the activated macrophage effector mechanism causing selective metabolic inhibition in target cells: J.B. Hibbs Jr., et al.; J. Immunol. **138,** 550 (1987) ▪ Accumulation of an endogenous inhibitor of nitric oxide synthesis in chronic renal failure: P. Vallance, et al.; Lancet **339,** 572 (1992)

N^G, N'^G-Dimethyl-L-arginine . 2HCl

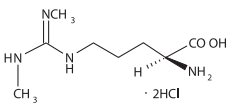
[SDMA . 2HCl]

ALX-106-008-M005 5 mg
ALX-106-008-M025 25 mg

LIT: See Prod. No. ALX-106-007 (above)



SDMA . 2HABS



SDMA . 2HCl

ADMA & SDMA Antibodies

NEW

Antibodies to ADMA & SDMA

MAb to N^G/N^G, N'^G-Mono/Di-methyl-L-arginine (7E6)

CVL-MAB0002-1 200 µl

CLONE: 7E6. **ISOTYPE:** Mouse IgG1. **IMMUNOGEN:** N^G/N^G, N'^G-mono/di-methyl-L-arginine. **SPECIFICITY:** Recognizes free and bound N^G,N'^G-dimethyl-L-arginine. Does cross-react with free or bound N^G-monomethyl-L-arginine. Does not cross-react with free or bound L-arginine. **APPLICATION:** IHC, WB.

MAb to N^G/N^G, N'^G-Mono/Di-methyl-L-arginine (Supernatant) (7E6)

CVL-MAB0003-1 1 ml

CLONE: 7E6. **ISOTYPE:** Mouse IgG1. **IMMUNOGEN:** N^G/N^G,N'^G-mono/di-methyl-L-arginine. **SPECIFICITY:** Recognizes free and bound N^G,N'^G-dimethyl-L-arginine. Does cross-react with free and bound N^G-monomethyl-L-arginine. Does not cross-react with free or bound L-arginine. **APPLICATION:** IHC, WB.

MAb to N^G, N'^G-Dimethyl-L-arginine (21C7)

CVL-MAB0004-1 200 µl

CLONE: 21C7. **ISOTYPE:** Mouse IgM. **IMMUNOGEN:** ADMA (N^G,N^G-dimethyl-L-arginine). **SPECIFICITY:** Recognizes free and bound ADMA. Does not cross-react with free or bound L-arginine or N^G-monomethyl-L-arginine. **APPLICATION:** IHC, WB.

MAb to N^G-Monomethyl-L-arginine (16B11)

CVL-MAB0005-1 200 µl

CLONE: 16B11. **ISOTYPE:** Mouse IgG2a. **IMMUNOGEN:** L-NMMA (N^G-monomethyl-L-arginine). **SPECIFICITY:** Recognizes free and bound L-NMMA. Does not cross-react with either free or bound L-arginine or N^G,N^G-dimethyl-L-arginine. **APPLICATION:** IHC, WB.

MAb to N^G-Monomethyl-L-arginine (Supernatant) (16B11)

CVL-MAB0006-1 1 ml

CLONE: 16B11. **ISOTYPE:** Mouse IgG2a. **IMMUNOGEN:** L-NMMA (N^G-monomethyl-L-arginine). **SPECIFICITY:** Recognizes free and bound L-NMMA. Does not cross-react with either free or bound L-arginine or N^G,N^G-dimethyl-L-arginine. **APPLICATION:** IHC, WB.

MAb to N^G-Monomethyl-L-arginine (5D1)

CVL-MAB0007-1 200 µl

CLONE: 5D1. **ISOTYPE:** Mouse IgG1. **IMMUNOGEN:** L-NMMA (N^G-monomethyl-L-arginine). **SPECIFICITY:** Recognizes free and bound L-NMMA. Does not cross-react with either free or bound L-arginine or N^G,N^G-dimethyl-L-arginine. **APPLICATION:** IHC, WB.

MAb to N^G-Monomethyl-L-arginine (Supernatant) (5D1)

CVL-MAB0008-1 1 ml

CLONE: 5D1. **ISOTYPE:** Mouse IgG1. **IMMUNOGEN:** L-NMMA (N^G-monomethyl-L-arginine). **SPECIFICITY:** Recognizes free and bound L-NMMA. Does not cross-react with either free or bound arginine or N^G,N^G-dimethyl-L-arginine. **APPLICATION:** IHC, WB.

Related Products

MAb to N-ε-(γ-L-Glutamyl)-L-lysine (71A3G4)

CVL-MAB0009-1 200 µl

CLONE: 71A3G4. **ISOTYPE:** Mouse IgG2a. **IMMUNOGEN:** GGEL (N-ε-(γ-L-glutamyl)-L-lysine) isopeptide. **SPECIFICITY:** Recognizes GGEL. Does cross-react with N-ε-(acetyl)-L-lysine. Does not cross-react with either free lysine or free glutamine. **APPLICATION:** IHC.

MAb to N-ε-(γ-L-Glutamyl)-L-lysine (81D1C2)

CVL-MAB0011-1 200 µl

CLONE: 81D1C2. **ISOTYPE:** Mouse IgG1. **IMMUNOGEN:** GGEL (N-ε-(γ-L-glutamyl)-L-lysine). **SPECIFICITY:** Recognizes GGEL. Does strongly cross-react with N-ε-acetyl-L-lysine. **APPLICATION:** IHC.

MAb to N-ε-(γ-L-Glutamyl)-L-lysine (81D4)

CVL-MAB0012-1 200 µl

CLONE: 81D4. **ISOTYPE:** Mouse IgM. **IMMUNOGEN:** GGEL (N-ε-(γ-L-glutamyl)-L-lysine) isopeptide. **SPECIFICITY:** Recognizes GGEL. Does cross-react in ELISA with N1,N4-bis-(γ-poly-L-glutamyl) putrescine and N1,N8-bis-(γ-poly-L-glutamyl) spermidine. Does not cross-react with protein bound N-ε acetyl lysine, protein bound N1 or N4 mono-(γ-poly-L-glutamyl) putrescine, protein bound N1 mono-(γ-poly-L-glutamyl) spermidine, protein bound N-ε-(γ-L-glutamyl) lysine having a free α amine either on its glutamyl or lysyl moieties. **APPLICATION:** IHC.

Dynein Light Chain 1 [DLC1; LC8; PIN]

The 8kDa dynein light chain 1 (DLC1; LC8) (also known as neuronal nitric oxide synthase inhibitory protein; PIN (Protein Inhibitor of nNOS)), is a component of the cytoplasmic dynein complex involved in the minus end-directed movement of organelles along microtubules. It is a highly conserved protein that is associated with the microtubular dynein intermediate chain (IC). DLC1 acts as a cargo protein and can hook many different proteins to the dynein motor complex.

In addition to playing an essential role in dynein motor function, DLC1 interacts with a number of proteins of diverse functions. PIN has been reported to be an inhibitor of nNOS (NOS I), either by decomposing nNOS dimers to monomers or another elusive mechanism [1, 2]. DLC1 also interacts and interferes with the pro-apoptotic Bcl-2 family protein BimL [3]. Interaction with Pak1 is important for the cell survival functions of both Pak1 and DLC1 [4]. DCL1 interacts with and transactivates estrogen receptor (ER), leading to increased expression of ER target genes and growth stimulation of breast cancer cells [5]. Recently, an interaction of DCL1 with KIBRA has been shown to be essential for this ER transactivation [6].

LIT: [1] PIN: an associated protein inhibitor of neuronal nitric oxide synthase: S. R. Jaffrey & S. H. Snyder; Science **274,** 774 (1996) ▪ **[2]** PIN inhibits nitric oxide and superoxide production from purified neuronal nitric oxide synthase: Y. Xia, et al.; Biochim. Biophys. Acta **1760,** 1445 (2006) ▪ **[3]** The proapoptotic activity of the Bcl-2 family member Bim is regulated by interaction with the dynein motor complex: H. Puthalakath, et al.; Mol. Cell **3,** 287 (1999) ▪ **[4]** Dynein light chain 1, a p21-activated kinase 1-interacting substrate, promotes cancerous phenotypes: R. K. Vadlamudi, et al.; Cancer Cell **5,** 575 (2004) ▪ **[5]** Functional regulation of oestrogen receptor pathway by the dynein light chain 1: S. K. Rayala, et al.; EMBO Rep. **6,** 538 (2005) ▪ **[6]** Essential role of KIBRA in co-activator function of dynein light chain 1 in mammalian cells: S. K. Rayala, et al.; J. Biol. Chem. **281,** 19092 (2006)

Antibodies to DLC1

MAb to DLC1 (10D6)

ALX-804-340-C100 100 µg

CLONE: 10D6. **ISOTYPE:** Rat IgM. **IMMUNOGEN:** Recombinant mouse DLC1 (dynein light chain 1; LC8; PIN). **SPECIFICITY:** Recognizes human and mouse DLC1. Does not cross-react with DLC2. For detection of both DLC1 and DLC2 see MAb to DLC1 and DLC2 (11F7) (Prod. No. ALX-804-341). **APPLICATION:** FC, WB.

LIT: Bmf: a proapoptotic BH3-only protein regulated by interaction with the myosin V actin motor complex, activated by anoikis: H. Puthalakath, et al.; Science **293,** 1829 (2001)

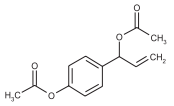
MAb to DLC1 and DLC2 (11F7)

ALX-804-341-C100 100 µg

CLONE: 11F7. **ISOTYPE:** Rat IgG2a. **IMMUNOGEN:** Recombinant mouse DLC1 (dynein light chain 1; LC8; PIN). **SPECIFICITY:** Recognizes human and mouse DLC1 and DLC2. **APPLICATION:** FC, IP, WB.

LIT: Interaction of the postsynaptic density-95/guanylate kinase domain- associated protein complex with a light chain of myosin-V and dynein: S. Naisbitt, et al.; J. Neurosci. **20,** 4524 (2000) ▪ Bmf: a proapoptotic BH3-only protein regulated by interaction with the myosin V actin motor complex, activated by anoikis: H. Puthalakath, et al.; Science **293,** 1829 (2001)

Nitric Oxide Synthase Modulators



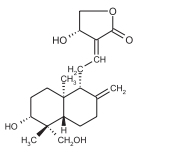
D,L-1'-Acetoxychavicol . acetate

D,L-1'-Acetoxychavicol . acetate

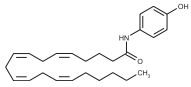
LKT-A0817-M100 100 mg
LKT-A0817-M500 500 mg
LKT-A0817-G001 1 g

Inhibitor of nitric oxide production.

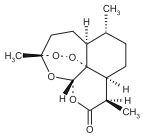
SELECTED LIT: Inhibition by 1'-acetoxychavicol acetate of lipopolysaccharide- and interferon-gamma-induced nitric oxide production through suppression of inducible nitric oxide synthase gene expression in RAW264 cells: T. Ohata, et al.; Carcinogenesis **19,** 1007 (1998) ▪ Suppression of tumor promoter-induced oxidative stress and inflammatory responses in mouse skin by a superoxide generation inhibitor 1'-acetoxychavicol acetate: Y. Nakamura, et al.; Cancer Res. **58,** 4832 (1998) ▪ Structure-activity relationships of 1'S-1'-acetoxychavicol acetate for inhibitory effect on NO production in lipopolysaccharide-activated mouse peritoneal macrophages: H. Matsuda, et al.; Bioorg. Med. Chem. Lett. **15,** 1949 (2005) ▪ 1'S-1'-Acetoxychavicol acetate as a new type inhibitor of interferon-beta production in lipopolysaccharide-activated mouse peritoneal macrophages: S. Ando, et al.; Bioorg. Med. Chem. **13,** 3289 (2005)



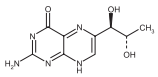
Andrographolide



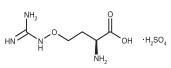
AM 404



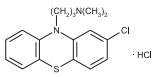
Artemisinin



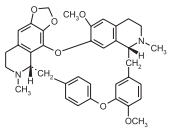
Biopterin



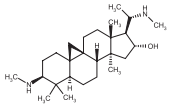
Canavanine . sulfate



Chlorpromazine . HCl



Cepharanthine (98%)



Cyclovirobuxine D

Latest Insight

News on anandamide uptake inhibitor AM404 – Regulator of nNOS (NOS I)

B. Costa, et al. recently showed that AM404 treatment affected two pathways involved in the generation and maintenance of neuropathic pain, one mediated by nitric oxide (NO) and the other by cytokines. AM404 completely prevented the overproduction of $NO^{\bullet}$ and the overexpression of nNOS, inhibited the increase in TNF-α and enhanced the production of IL-10.

LIT: AM404, an inhibitor of anandamide uptake, prevents pain behaviour and modulates cytokine and apoptotic pathways in a rat model of neuropathic pain: B. Costa, et al.; Br. J. Pharmacol. **148,** 1022 (2006)

AM 404

[N-(4-Hydroxyphenyl)arachidonoylamide]

ALX-340-032-M005 5 mg

Analog of anandamide (Prod. No. ALX-340-029). Potentiates the activity of endogeneous anandamide by blocking its re-uptake into presynaptic membranes. Activates TRPV1 (EC_{50}=0.04μM) at concentrations lower than those required to inhibit anandamide transport into the cell (neuronal (C6 glioma cells): IC_{50}=10μM; non-neuronal (rat RBL-2H3 cells): IC_{50}=11μM). Low affinity to FAAH (IC_{50}=5.9μM), to CB1 receptor (IC_{50}=1.76μM) and to CB2 receptor (IC_{50}>1μM).

Andrographolide

LKT-A5313-M001 1 mg
LKT-A5313-M005 5 mg
LKT-A5313-M010 10 mg

Isolated from *Andrographis paniculata*. Inhibitor of PAF-induced human blood platelet aggregation and of nitrite synthesis in macrophages. Protects against cell apoptosis.

SELECTED LIT: Andrographolide suppresses the expression of inducible nitric oxide synthase in macrophage and restores the vasoconstriction in rat aorta treated with lipopolysaccharide: W.F. Chiou, et al.; Br. J. Pharmacol. **125,** 327 (1998) ▪ Mechanisms of suppression of inducible nitric oxide synthase (iNOS) expression in RAW 264.7 cells by andrographolide: W.F. Chiou, et al.; Br. J. Pharmacol. **129,** 1553 (2000) ▪ Suppression of rat neutrophil reactive oxygen species production and adhesion by the diterpenoid lactone andrographolide: Y.C. Shen, et al.; Planta Med. **66,** 314 (2000) ▪ Andrographolide prevents oxygen radical production by human neutrophils: possible mechanism(s) involved in its anti-inflammatory effect: Y.C. Shen, et al.; Br. J. Pharmacol. **135,** 399 (2002) ▪ Suppression of NO production in activated macrophages in vitro and ex vivo by neoandrographolide isolated from Andrographis paniculata: J. Batkhuu, et al.; Biol. Pharm. Bull. **25,** 1169 (2002)

Artemisinin

ALX-350-219-M100 100 mg
ALX-350-219-G001 1 g

Isolated from the traditional Chinese anti-malarial herb *Artemisia annua L.* Inhibits angiogenesis by down-regulating HIF-1α and VEGF expression in mouse embryonic stem cells. Crosses the blood-brain barrier. Inhibitor of human iNOS (NOS II).

SELECTED LIT: Qinghaosu (artemisinin): an antimalarial drug from China: D.L. Klayman; Science **228,** 1049 (1985) ▪ Inhibition of angiogenesis in embryoid bodies by artemisinin: M. Wartenberg, et al.; Pflugers Arch. Eur. J. Physiol. **445,** 585 (1994) ▪ Artemisinin inhibits inducible nitric oxide synthase and nuclear factor NF-kB activation: E. Aldieri, et al.; FEBS Lett. **552,** 141 (2003)

L-Biopterin

ALX-440-008-M025 25 mg
ALX-440-008-M100 100 mg

Bradykinin

H-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH

ALX-152-006-M005 5 mg
ALX-152-006-M025 25 mg

Activator of eNOS (NOS III).

SELECTED LIT: Bradykinin and ATP stimulate L-arginine uptake and nitric oxide release in vascular endothelial cells: R.G. Bogle, et al.; BBRC **180,** 926 (1991) ▪ G proteins in aortic endothelial cells and bradykinin-induced formation of nitric oxide: J. Gil-Longo, et al.; Eur. J. Pharmacol. **247,** 119 (1993) ▪ Simultaneous measurements of Ca2+ and nitric oxide in bradykinin-stimulated vascular endothelial cells: L.A. Blatter, et al.; Circ. Res. **76,** 922 (1995) ▪ Reciprocal phosphorylation and regulation of endothelial nitric-oxide synthase in response to bradykinin stimulation: M.B. Harris, et al.; J. Biol. Chem. **276,** 16587 (2001) ▪ Bradykinin down-regulates, whereas arginine analogs up-regulates, endothelial nitric-oxide synthase expression in coronary endothelial cells: N.D. Vaziri, et al.; J. Pharmacol. Exp. Ther. **313,** 121 (2005) ▪ Bradykinin mediates phosphorylation of eNOS in odontoblasts: Y. Korkmaz, et al.; J. Dent. Res. **85,** 536 (2006)

Canavanine . H_2SO_4

ALX-350-002-M100 100 mg
ALX-350-002-M500 500 mg

The non-protein amino acid L-canavanine is an analog of L-arginine and has shown to be a selective inhibitor of iNOS (NOS II). It induces apoptotic cell death, and shows antiproliferative and immunotoxic effects.

SELECTED LIT: Inhibition of nitric oxide formation with L-canavanine attenuates endotoxin-induced vascular hyporeactivity in the rat: M. Cai, et al.; Eur. J. Pharmacol. **295,** 215 (1996) ▪ Beneficial effects of L-canavanine, a selective inhibitor of inducible nitric oxide synthase, during rodent endotoxaemia: L. Liaudet, et al.; Clin. Sci. (London) **90,** 369 (1996) ▪ Effects of L-canavanine, an inhibitor of inducible nitric oxide synthase, on endotoxin mediated shock in rats: Z. Fatehi-Hassanabad, et al.; Shock **6,** 194 (1996) ▪ Quercetin, coenzyme Q**10,** and L-canavanine as protective agents against lipid peroxidation and nitric oxide generation in endotoxin-induced shock in rat brain: H.M. Abd El-Gawad & A.E. Khalifa; Pharmacol. Res. **43,** 257 (2001) ▪ Effect of L-canavanine, an Inhibitor of inducible nitric oxide synthase, on myocardial dysfunction during septic shock: N. Suzuki, et al.; J. Nippon Med. Sch. **69,** 13 (2002) ▪ Comparative effects of vasopressin, norepinephrine, and L-canavanine, a selective inhibitor of inducible nitric oxide synthase, in endotoxic shock: B. Levy, et al.; Am. J. Physiol. Heart Circ. Physiol. **287,** H209 (2004) ▪ L-arginine analogs as alternate substrates for nitric oxide synthase: S.D. Luzzi & M.A. Marletta; Bioorg. Med. Chem. Lett. **15,** 3934 (2005)

Chlorpromazine . HCl

ALX-270-171-G001 1 g
ALX-270-171-G005 5 g

Inhibits nitric oxide synthase (NOS) in mouse brain and prevents lipopolysaccharide induction of NOS in mouse lung.

SELECTED LIT: Chlorpromazine inhibits both the constitutive nitric oxide synthase and the induction of nitric oxide synthase after LPS challenge: M. Palacios, et al.; BBRC **196,** 280 (1993) ▪ Pharmacological regulation of mitochondrial nitric oxide synthase: A. Boveris, et al.; Methods Enzymol. **359,** 328 (2002) ▪ Brain mitochondrial nitric oxide synthase: in vitro and in vivo inhibition by chlorpromazine: S. Lores-Arnaiz, et al.; Arch. Biochem. Biophys. **430,** 170 (2004)

Cepharanthine (98%)

LKT-C1718-M100 100 mg
LKT-C1718-M500 500 mg
LKT-C1718-G001 1 g

Cepharanthine is a biscoclaurine alkaloid isolated from *Stephania cepharantha* Hayata. It has anti-inflammatory, anti-allergic, immunomodulatory, and many other interesting biological activities. Suppresses NO production, which is one of the critical mediators of inflammation. Cepharanthine is an antiperoxidative agent, due to its direct radical scavenging activity.

SELECTED LIT: Inhibitory effect of bisbenzylisoquinoline alkaloids on nitric oxide production in activated macrophages: Y. Kondo, et al.; Biochem. Pharmacol. **46,** 1887 (1993) ▪ Direct radical scavenging by the bisbenzylisoquinoline alkaloid cepharanthine: K. Kogure, et al.; Biochim. Biophys. Acta **1622,** 1 (2003) ▪ Cepharanthine, an anti-inflammatory drug, suppresses mitochondrial membrane permeability transition: M. Nagano, et al.; Physiol. Chem. Phys. Med. NMR **35,** 131 (2003)

Ceruloplasmin: A Nitric Oxide Oxidase & Nitrite Synthase

Ceruloplasmin [Ferroxidase], a 132kDa plasma protein containing six copper centers, is expressed in plasma at concentrations of 1-5 μM and is known to oxidize amines in a process coupled to the reduction of molecular oxygen [1,2]. It has ferroxidase activity that is responsible for the oxidation of ferrous iron to its ferric form, which is necessary for efficient iron efflux from the cell (e.g. during hypoxia) [3,4]. Ceruloplasmin has previously been considered a target for nitric oxide (NO) [5] and to inhibit eNOS (NOS III) [6]. It can catalyze S-nitrosothiol formation in cell culture media [7]. Ceruloplasmin is a NO oxidase and nitrite synthase that determines endocrine NO homeostasis [8].

LIT: [1] Ceruloplasmin metabolism and function: N.E. Hellman & J.D. Gitlin; Annu. Rev. Nutr. **22,** 439 (2002) ▪ **[2]** Structure/function relationships in ceruloplasmin: G. Musci, et al.; Adv. Exp. Med. Biol. **448,** 175 (1999) ▪ **[3]** Aceruloplasminemia: molecular characterization of this disorder of iron metabolism: Z.L. Harris, et al.; PNAS **92,** 2539 (1995) ▪ **[4]** Role of ceruloplasmin in macrophage iron efflux during hypoxia: J. Sarkar, et al.; J. Biol. Chem. **278,** 44018 (2003) ▪ **[5]** The reactions of copper proteins with nitric oxide: J. Torres & M.T. Wilson; Biochim. Biophys. Acta **1411,** 310 (1999) ▪ **[6]** Inhibition of endothelial nitric-oxide synthase by ceruloplasmin: A. Bianchini, et al.; J. Biol. Chem. **274,** 20265 (1999) ▪ **[7]** Nitrosothiol formation catalyzed by ceruloplasmin. Implication for cytoprotective mechanism in vivo: K. Inoue, et al.; J. Biol. Chem. **274,** 27069 (1999) ▪ **[8]** Ceruloplasmin is a NO oxidase and nitrite synthase that determines endocrine NO homeostasis: S. Shiva, et al.; Nat. Chem. Biol. **2,** 486 (2006)

NEW Ceruloplasmin (human)

[Ferroxidase]

ALX-200-089-M001 1 mg

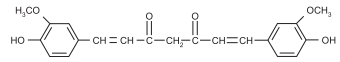
Isolated from human plasma.

Curcumin (high purity)

ALX-350-028-M010 10 mg
ALX-350-028-M050 50 mg
ALX-350-028-M250 250 mg

Isolated from turmeric *(Curcuma longa)*. Inhibits the induction of NOS in LPS-activated macrophages.

LIT: Curcumin, an anti-tumour promoter and anti-inflammatory agent, inhibits induction of nitric oxide synthase in activated macrophages: I. Brouet & H. Okshima; BBRC **206,** 533 (1995) ▪ Nitric oxide scavenging by curcuminoids: Sreejayan & M.N. Rao; J. Pharm. Pharmacol. **49,** 105 (1997) ▪ In vivo inhibition of nitric oxide synthase gene expression by curcumin, a cancer preventive natural product with anti-inflammatory properties: M.M. Chan, et al.; Biochem. Pharmacol. **55,** 1955 (1998) ▪ Effect of curcumin on the production of nitric oxide by cultured rat mammary gland: M. Onoda & H. Inano; Nitric Oxide **4,** 505 (2000) ▪ Suppression of nitric oxide oxidation to nitrite by curcumin is due to the sequestration of the reaction intermediate nitrogen dioxide, not nitric oxide: B.D. Johnston & E.G. DeMaster; Nitric Oxide **8,** 231 (2003) ▪ Curcumin, an atoxic antioxidant and natural NFkappaB, cyclooxygenase-2, lipooxygenase, and inducible nitric oxide synthase inhibitor: a shield against acute and chronic diseases: S. Bengmark; JPEN J. Parenter. Enteral. Nutr. **30,** 45 (2006) ▪ Multiple biological activities of curcumin: a short review: R.K. Maheshwari, et al.; Life Sci. **78,** 2081 (2006) ▪ Curcumin inhibits hypoxia-induced angiogenesis via down-regulation of HIF-1: M.K. Bae, et al.; Oncol. Rep. **15,** 1557 (2006) ▪ Inhibitory effect of curcumin on nitric oxide production from lipopolysaccharide-activated primary microglia: K.K. Jung, et al.; Life Sci. **79,** 2022 (2006)



Cyclovirobuxine D

LKT-C9711-M025 25 mg
LKT-C9711-M100 100 mg
LKT-C9711-M500 500 mg

Anti-atrial fibrillating agent. Induces release of eNOS (NOS III).

SELECTED LIT: Anti-atrial fibrillation effects of cyclovirobuxine-D and its electrophysiological mechanism studied on guinea pig atria: Y.X. Wang, et al.; Yao Xue Xue Bao **31,** 481 (1996) ▪ Coronary effects of cyclovirobuxine D in anesthetized pigs and in isolated porcine coronary arteries: E. Grossini, et al.; Life Sci. **65,** PL59 (1999) ▪ Regulation of Ca2+ movements by cyclovirobuxine D in ECV304 endothelial cells: E. Grossini, et al.; Pharmacol. Res. **52,** 154 (2005)

D609 . potassium salt

ALX-270-089-M001 1 mg
ALX-270-089-M005 5 mg

Selective inhibitor of phosphatidylcholine-specific phospholipase C. Inhibits induction of nitric oxide synthase (NOS). Induces apoptosis.

SELECTED LIT: DNA and RNA virus species are inhibited by xanthates, a class of antiviral compounds with unique properties: G. Sauer, et al.; PNAS **81,** 3263 (1984) ▪ Induction of nitric oxide synthase activity in phagocytic cells inhibited by tricyclodecan-9-yl-xanthogenate (D609): K. Tschaikowsky, et al.; Br. J. Pharmacol. **113,** 664 (1994) ▪ D609 inhibits ionizing radiation-induced oxidative damage by acting as a potent antioxidant: D. Zhou, et al.; J. Pharmacol. Exp. Ther. **298,** 103 (2001) ▪ Protective effect of the xanthate, D**609,** on Alzheimer's amyloid beta-peptide (1-42)-induced oxidative stress in primary neuronal cells: R. Sultana, et al.; Free Radic. Res. **38,** 449 (2004) ▪ D609 blocks cell survival and induces apoptosis in neural stem cells: N. Wang, et al.; Bioorg. Med. Chem. Lett. **16,** 4780 (2006)

Dexamethasone

ALX-370-002-M250 250 mg
ALX-370-002-G001 1 g

Inhibits the induction of nitric oxide synthase (NOS).

SELECTED LIT: Inhibition of the induction of nitric oxide synthase by glucocorticoids: yet another explanation for their anti-inflammatory effects?: S. Moncada & R.M.J. Palmer; TIPS **12,** 130 (1991) ▪ Glucocorticoids inhibit the induction of nitric oxide synthase and the related cell damage in adenocarcinoma cells: K.J. O'Connor & S. Moncada; Biochim. Biophys. Acta **1097,** 227 (1991) ▪ Dexamethasone inhibits the expression of an inducible nitric oxide synthase in infarcted rabbit myocardium: R.R. Dudek, et al.; BBRC **202,** 1120 (1994) ▪ Dexamethasone suppresses iNOS gene expression by inhibiting NF-kappaB in vascular smooth muscle cells: M. Matsumura, et al.; Life Sci. **69,** 1067 (2001) ▪ Dexamethasone inhibits inducible nitric-oxide synthase expression and nitric oxide production by destabilizing mRNA in lipopolysaccharide-treated macrophages: R. Korhonen, et al.; Mol. Pharmacol. **62,** 698 (2002) ▪ Molecular mechanisms underlying dexamethasone inhibition of iNOS expression and activity in C6 glioma cells: J. Shinoda, et al.; Glia **42,** 68 (2003) ▪ Dexamethasone prevents granulocyte-macrophage colony-stimulating factor-induced nuclear factor-kappaB activation, inducible nitric oxide synthase expression and nitric oxide production in a skin dendritic cell line: A.L. Vital, et al.; Mediators Inflamm. **12,** 71 (2003) ▪ Dexamethasone suppresses eNOS and CAT-1 and induces oxidative stress in mouse resistance arterioles: S.C. Schafer, et al.; Am. J. Physiol. Heart Circ. Physiol. **288,** H436 (2005)

2,4-Diamino-6-hydroxypyrimidine

[DAHP]

ALX-270-005-M250 250 mg
ALX-270-005-G001 1 g

Selective inhibitor of GTP cyclohydrolase I, which is the rate-limiting enzyme for de novo tetrahydrobiopterin synthesis. Thus it suppresses the activity of nitric oxide synthase (NOS).

SELECTED LIT: Biopterin. III. Purification and characterization of enzymes involved in the cerebral synthesis of 7,8-dihydrobiopterin: E.M. Gal, et al.; Neurochem. Res. **3,** 69 (1978) ▪ Tetrahydrobiopterin-dependent formation of nitrite and nitrate in murine fibroblasts: G. Werner-Felmayer, et al.; J. Exp. Med. **172,** 1599 (1990) ▪ Pteridine biosynthesis in human endothelial cells. Impact on nitric oxide-mediated formation of cyclic GMP: G. Werner-Felmayer, et al.; J. Biol. Chem. **268,** 1842 (1993) ▪ 2,4-Diamino-6-hydroxypyrimidine, an inhibitor of GTP cyclohydrolase I, suppresses nitric oxide production by chicken macrophages: Y.J. Sung, et al.; Int. J. Immunopharmacol. **16,** 101 (1994) ▪ **2,**4-Diamino-6-hydroxypyrimidine, an inhibitor of tetrahydrobiopterin synthesis, downregulates the expression of iNOS protein and mRNA in primary murine macrophages: C. Bogdan, et al.; FEBS Lett. **363,** 69 (1995) ▪ GTP cyclohydrolase I inhibition by the prototypic inhibitor 2, 4-diamino-6-hydroxypyrimidine. Mechanisms and unanticipated role of GTP cyclohydrolase I feedback regulatory protein: L. Xie, et al.; J. Biol. Chem. **273,** 21091 (1998) ▪ The mechanism of potent GTP cyclohydrolase I inhibition by 2,4-diamino-6-hydroxypyrimidine: requirement of the GTP cyclohydrolase I feedback regulatory protein: M.A. Kolinsky & S.S. Gross; J. Biol. Chem. **279,** 40677 (2004)

3*H*-1,2-Dithiole-3-thione

LKT-D0010-M025 25 mg
LKT-D0010-M100 100 mg
LKT-D0010-M500 500 mg

Potent antioxidant that shows chemopreventive properties. Targets NF-κB to block iNOS (NOS II) expression.

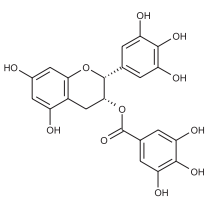
SELECTED LIT: 3H-1,2-dithiole-3-thione targets nuclear factor kappaB to block expression of inducible nitric-oxide synthase, prevents hypotension, and improves survival in endotoxemic rats: A.R. Karuri, et al.; J. Pharmacol. Exp. Ther. **317,** 61 (2006)

(-)-Epigallocatechin gallate

ALX-270-263-M010 10 mg
ALX-270-263-M050 50 mg

Isolated from green tea. Inhibits iNOS (NOS II) gene expression and enzyme activity and the peroxynitrite-mediated formation of 8-oxodeoxyguanosine and 3-nitrotyrosine.

SELECTED LIT: (-)-Epigallocatechin gallate, a polyphenolic tea antioxidant, inhibits peroxynitrite-mediated formation of 8-oxodeoxyguanosine and 3-nitrotyrosine: E.S. Fiala, et al.; Experientia **52,** 922 (1996) ▪ (-)-Epigallocatechin-3-gallate blocks the induction of nitric oxide synthase by down-regulating lipopolysaccharide-induced activity of transcription factor nuclear factor-kappaB: Y.L. Lin & J.K. Lin; Mol. Pharmacol. **52,** 465 (1997) ▪ Inhibition of inducible nitric oxide synthase gene expression and enzyme activity by epigallocatechin gallate, a natural product from green tea: M.M. Chan, et al.; Biochem. Pharmacol. **54,** 1281 (1997) ▪ A constituent of green tea, epigallocatechin-3-gallate, activates endothelial nitric oxide synthase by a phosphatidylinositol-3-OH-kinase-, cAMP-dependent protein kinase-, and Akt-dependent pathway and leads to endothelial-dependent vasorelaxation: M. Lorenz, et al.; J. Biol. Chem. **279,** 6190 (2004) ▪ Green tea polyphenols prevent toxin-induced hepatotoxicity in mice by down-regulating inducible nitric oxide-derived prooxidants: J.H. Chen, et al.; Am. J. Clin. Nutr. **80,** 742 (2004) ▪ Tea flavanols inhibit angiotensin-converting enzyme activity and increase nitric oxide production in human endothelial cells: I.A. Persson, et al.; J. Pharm. Pharmacol. **58,** 1139 (2006)



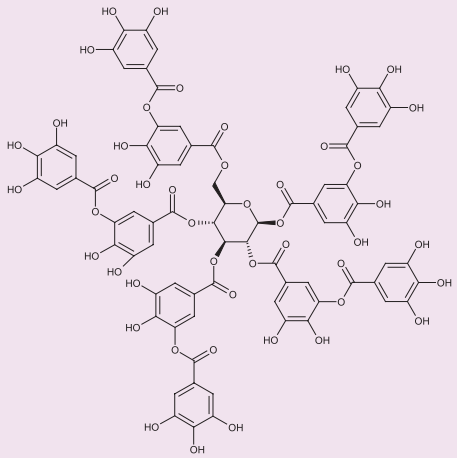
Product Highlight

Gallotannin

ALX-270-418-G001 1 g

Inhibitor of eNOS (NOS III) and weak inhibitor of iNOS (NOS II) and nNOS (NOS I). Free radical scavenger.

SELECTED LIT: Inhibition of constitutive endothelial NO-synthase activity by tannin and quercetin: M. Chiesi & R. Schwaller; Biochem. Pharmacol. **49,** 495 (1995) ▪ Green tea polyphenols and tannic acid act as potent inhibitors of phorbol ester-induced nitric oxide generation in rat hepatocytes independent of their antioxidant properties: R.C. Srivastava, et al.; Cancer Lett. **153,** 1 (2000) ▪ Prominent free radicals scavenging activity of tannic acid in lead-induced oxidative stress in experimental mice: I.H. El-Sayed, et al.; Toxicol. Ind. Health **22,** 157 (2006)



Gemfibrozil

LKT-G1749-G005 5 g
LKT-G1749-G025 25 g

Inhibits iNOS (NOS II) by inhibiting the activation of NF-κB, activator protein-1 and CCAAT/enhancer-binding protein β.

SELECTED LIT: Gemfibrozil, a lipid-lowering drug, inhibits the induction of nitric-oxide synthase in human astrocytes: K. Pahan, et al.; J. Biol. Chem. **277,** 45984 (2002) ▪ Gemfibrozil decreases atherosclerosis in experimental diabetes in association with a reduction in oxidative stress and inflammation: A.C. Calkin, et al.; Diabetologia **49,** 766 (2006)

α-MSH

LKT-M7528-M001 1 mg

Immunomodulating neuropeptide. Exerts anti-inflammatory action by inhibition of iNOS (NOS II). Regulates protein ubiquitination in T cells.

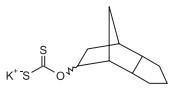
SELECTED LIT: Selective effects of alpha-MSH and MIF-1 on the blood-brain barrier: R. Sankar, et al.; Peptides **2,** 345 (1981) ▪ Alpha-MSH peptides inhibit production of nitric oxide and tumor necrosis factor-alpha by microglial cells activated with beta-amyloid and interferon gamma: D. Galimberti, et al.; BBRC **263,** 251 (1999) ▪ alpha-MSH inhibits lipopolysaccharide induced nitric oxide production in B16 mouse melanoma cells: M. Tsatmali, et al.; Ann. N. Y. Acad. Sci. **885,** 474 (1999) ▪ The immunomodulating neuropeptide alpha-melanocyte-stimulating hormone (alpha-MSH) suppresses LPS-stimulated TLR4 with IRAK-M in macrophages: A.W. Taylor; J. Neuroimmunol. **162,** 43 (2005) ▪ Alpha-MSH regulates protein ubiquitination in T cells: D.J. Biros, et al.; Cell. Mol. Biol. (Noisy-le-grand) **52,** 33 (2006)

Minocycline . HCl

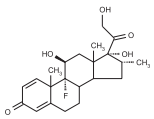
ALX-380-109-M050 50 mg

Tetracycline derivative with antimicrobial activity. Inhibitor of angiogenesis, apoptosis and PARP-1. Anti-inflammatory and neuroprotective.

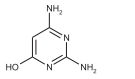
SELECTED LIT: Minocycline inhibits poly(ADP-ribose) polymerase-1 at nanomolar concentrations: C.C. Alano, et al.; PNAS **103,** 9685 (2006)



D609 . potassium salt

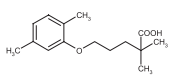


Dexamethasone

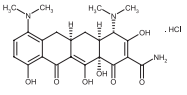


2,4-Diamino-6-hydroxypyrimidine [DAHP]

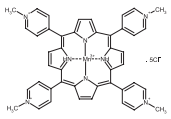
3*H*-1,2-Dithiole-3-thione



Gemfibrozil



Minocycline . HCl



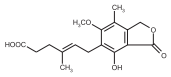
MnTMPyp . penta-chloride

MnTMPyP . pentachloride

ALX-430-070-M010 10 mg
ALX-430-070-M050 50 mg

Cell permeable SOD mimetic. Catalyzes the dismutation of O_2^- even in the presence of excess EDTA.

SELECTED LIT: Molecular actions of a Mn(III)Porphyrin superoxide dismutase mimetic and peroxynitrite scavenger: reaction with nitric oxide and direct inhibition of NO synthase and soluble guanylyl cyclase: S. Pfeiffer, et al.; Mol. Pharmacol. **53,** 795 (1998) ▪ Effects of superoxide dismutase mimetics on the activity of nitric oxide in rat aorta: A. MacKenzie, et al.; Br. J. Pharmacol. **127,** 1159 (1999) ▪ Inducible nitric oxide synthase-derived superoxide contributes to hypereactivity in small mesenteric arteries from a rat model of chronic heart failure: A.A. Miller, et al.; Br. J. Pharmacol. **131,** 29 (2000)



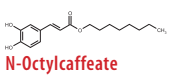
Mycophenolic acid

Mycophenolic acid

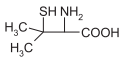
ALX-380-015-M050 50 mg
ALX-380-015-M250 250 mg
ALX-380-015-G001 1 g

Isolated from *Penicillium brevi-compactum*. Depletes tetrahydrobiopterin and decreases nitric oxide (NO) production by iNOS (NOS II) without affecting nNOS (NOS I) activity. Suppresses cytokine-induced nitric oxide (NO) production in mouse and rat vascular endothelial cells.

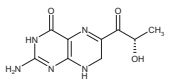
SELECTED LIT: Mycophenolic acid, an inhibitor of IMP dehydrogenase that is also an immunosuppressive agent, suppresses the cytokine-induced nitric oxide production in mouse and rat vascular endothelial cells: M. Senda, et al.; Transplantation **60,** 1143 (1995) ▪ Mycophenolate mofetil and its mechanisms of action: A.C. Allison & E.M. Eugui; Immunopharmacology **47,** 85 (2000) ▪ Mycophenolic acid downregulates inducible nitric oxide synthase induction in astrocytes: D. Miljkovic, et al.; Glia **39,** 247 (2002) ▪ Mycophenolic acid inhibits inosine 5'-monophosphate dehydrogenase and suppresses production of pro-inflammatory cytokines, nitric oxide, and LDH in macrophages: C.A. Jonsson & H. Carlsten; Cell Immunol. **216,** 93 (2002)



N-Octylcaffeate



Penicillamine



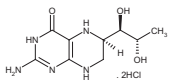
L-Sepiapterin

N-Octylcaffeate

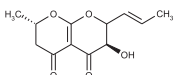
ALX-350-278-M005 5 mg
ALX-350-278-M025 25 mg

Suppressor of iNOS (NOS II).

SELECTED LIT: A novel antioxidant, octyl caffeate, suppression of LPS/IFN-gamma-induced inducible nitric oxide synthase gene expression in rat aortic smooth muscle cells: G. Hsiao, et al.; Biochem. Pharmacol. **65,** 1383 (2003) ▪ Caffeic acid derivatives: in vitro and in vivo anti-inflammatory properties: F.M. da Cunha, et al.; Free Radic. Res. **38,** 1241 (2004)



(6R)-5,6,7,8-Tetrahydro-L-biopterin . 2HCl



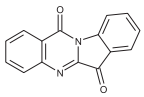
Trichodion

Penicillamine

LKT-P1753-G001 1 g
LKT-P1753-G005 5 g
LKT-P1753-G025 25 g

Exogenous nitric oxide synthase (NOS) modulator.

SELECTED LIT: Suppression of urease levels in Streptococcus salivarius by cysteine, related compounds and by sulfide: C.H. Sissons and S. Yakub; Oral Microbiol. Immunol. **15,** 317 (2000) ▪ Nitric oxide donors regulate nitric oxide synthase in bovine pulmonary artery endothelium: J.X. Chen, et al.; J. Cell. Physiol. **186,** 116 (2001) ▪ d-penicillamine reduces serum oxidative stress in Alzheimer's disease patients: R. Squitti, et al.; Eur. J. Clin. Invest. **32,** 51 (2002)



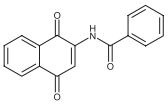
Tryptanthrin

PPM-18

ALX-270-458-M010 10 mg

Cell permeable, anti-inflammatory agent that inhibits the expression of iNOS (NOS II) (IC_{50}~ 5mM). Blocks activation of NF-κB *in vitro* and *in vivo*. Does not directly affect enzymatic activities of iNOS (NOS II) or eNOS (NOS III).

SELECTED LIT: Synthesis and antiplatelet, antiinflammatory and antiallergic activities of 2,3-disubstituted 1,4-naphthoquinones: J.C. Lien, et al.; Chem. Pharm. Bull. (Tokyo) **44,** 1181 (1996) ▪ Inhibition of nitric oxide synthase expression by PPM-18, a novel anti-inflammatory agent, in vitro and in vivo: S.M. Yu, et al.; Biochem. J. **328,** 363 (1997) ▪ Shear stress regulates endothelial nitric-oxide synthase promoter activity through nuclear factor kappaB binding: M.E. Davis, et al.; J. Biol. Chem. **279,** 163 (2004)

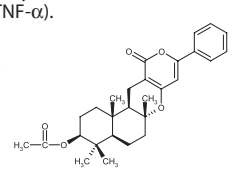


S14-95

ALX-350-299-MC05 0.5 mg

Isolated from *Penicillium sp.* Potent inhibitor of cytokine-induced activation of STAT1α leading to the inhibition of inducible expression of proinflammatory enzymes (COX-2, iNOS/NOS II) and cytokines (TNF-α).

SELECTED LIT: Sporogen, S14-95, and S-curvularin, three inhibitors of human inducible nitric-oxide synthase expression isolated from fungi: Y.Yao, et al.; Mol. Pharmacol. **63,** 383 (2003)

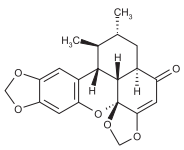


Sauchinone

ALX-350-116-M001 1 mg
ALX-350-116-M005 5 mg

Isolated from *Saururus chinensis*. Diastereomeric lignan with cytoprotective and antioxidant activities in cultured hepatocytes. Inhibitor of LPS-inducible iNOS (NOS II), expression in macrophages.

SELECTED LIT: Sauchinone, a lignan from Saururus chinensis, attenuates CCl4-induced toxicity in primary cultures of rat hepatocytes: S.H. Sung, et al.; Biol. Pharm. Bull. **23,** 666 (2000) ▪ Sauchinone, a lignan from Saururus chinensis, suppresses iNOS expression through the inhibition of transactivation activity of RelA of NF-kappaB: B.Y. Hwang, et al.; Planta Med. **69,** 1096 (2003) ▪ Inhibition of lipopolysaccharide-inducible nitric oxide synthase, TNF-alpha and COX-2 expression by sauchinone effects on I-kappaBalpha phosphorylation, C/EBP and AP-1 activation: A.K. Lee, et al.; Br. J. Pharmacol. **139,** 11 (2003)



L-Sepiapterin

ALX-440-004-M010 10 mg
ALX-440-004-5010 5x10 mg

Key intermediate in the pterin salvage pathway, tetrahydrobiopterin biosynthesis.

LIT: Biosynthesis and metabolism of tetrahydrobiopterin and molybdopterin: C. Nichol, et al.; Ann. Rev. Biochem. **54,** 729 (1985) ▪ Tetrahydrobiopterin synthesis. An absolute requirement for cytokine- induced nitric oxide generation by vascular smooth muscle: S.S. Gross & R. Levi; J. Biol. Chem. **267,** 25722 (1992)

(6R)-5,6,7,8-Tetrahydro-L-biopterin . 2HCl

[(6R)-BH_4 . 2HCl]

ALX-440-001-M005 5 mg
ALX-440-001-M025 25 mg

Cofactor of nitric oxide synthases (NOS).

LIT: Brain nitric oxide synthase is a biopterin- and flavin-containing multi-functional oxido-reductase: B. Mayer, et al.; FEBS Lett. **288,** 187 (1991) ▪ **For a comprehensive bibliography please visit our website.**

Trichodion

ALX-350-261-C100 100 µg
ALX-350-261-C500 500 µg

Isolated from *Trichosporiella sp.* Inhibits NF-κB (IC_{50}=42-84µM), AP-1 (IC_{50}=21µM) and STAT1α mediated gene expression induced by IFN-γ resulting in inhibition of cyclooxygenase-2 (COX-2) and iNOS (NOS II) expression.

SELECTED LIT: Trichodion, a new inhibitor of inflammatory signal transduction pathways from a Trichosporiella species: G. Erkel; FEBS Lett. **477,** 219 (2000) ▪ Trichodion, a new bioactive pyrone from a Trichosporiella species: G. Erkel, et al.; J. Antibiot. **53,** 1401 (2000)

Tryptanthrin

ALX-270-360-M001 1 mg
ALX-270-360-M005 5 mg

Inhibitor of iNOS (NOS II) expression in RAW 264.7 cells.

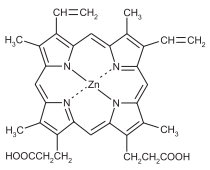
SELECTED LIT: Tryptanthrin inhibits nitric oxide and prostaglandin E(2) synthesis by murine macrophages: T. Ishihara, et al.; Eur. J. Pharmacol. **407,** 197 (2000)

Zinc(II) Protoporphyrin IX

ALX-430-049-M005 5 mg
ALX-430-049-M025 25 mg
ALX-430-049-M100 100 mg

Produces a time- and concentration-dependent inactivation of all three isoforms of nitric oxide synthase (NOS) (IC_{50}=0.8µM, 4.0µM, and 5.0µM for nNOS (NOS I), iNOS (NOS II), and eNOS (NOS III), respectively).

SELECTED LIT: Inhibition of nitric oxide synthase isoforms by porphyrins: D.J. Wolff, et al.; Arch. Biochem. Biophys. **333,** 27 (1996)

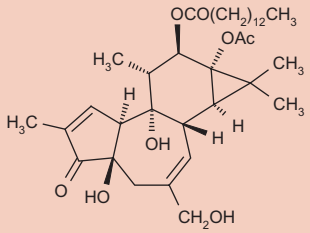


The PMA Source™

Phorbol 12-myristate 13-acetate [PMA; TPA]

Ask for* BULK *Quantities

ALX-445-004-M001 1 mg
ALX-445-004-M005 5 mg
ALX-445-004-M010 10 mg
ALX-445-004-M025 25 mg



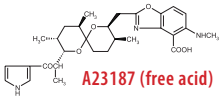
Step up to the Source™

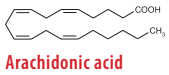
Latest Insight

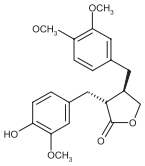
S.C. Shen, et al. recently showed that lipopolysaccharide and 12-O-tetradecanoylphorbol 13-acetate (PMA/TPA) promotes tumoral progression via activating metalloproteinase 9 enzyme activity and inducible nitric oxide synthase gene expression, in rat glioma cells C6.

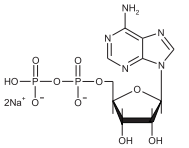
LIT: Lipopolysaccharide plus 12-O-tetradecanoylphorbol 13-acetate induction of migration and invasion of glioma cells in vitro and in vivo: Differential inhibitory effects of flavonoids: S.C. Shen, et al.; Neuroscience **140,** 477 (2006)

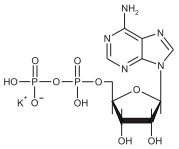
Nitric Oxide & Nitric Oxide Synthase Related Products

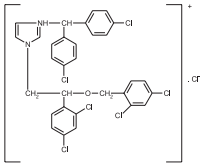

A23187 (free acid)

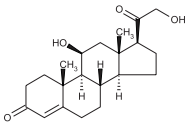

Arachidonic acid

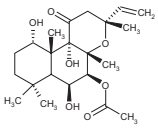

(-)-Arctigenin

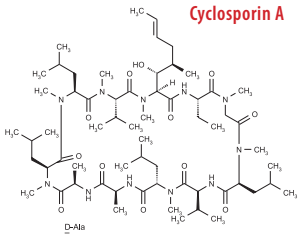

Adenosine 5′-diphosphate . disodium salt

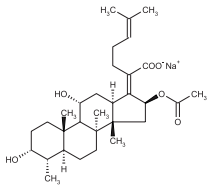

Adenosine 5′-diphosphate . potassium salt

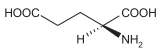

Calmidazolium chloride

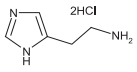

Corticosterone

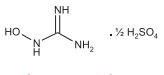

Forskolin

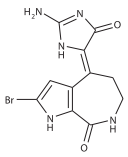

Cyclosporin A

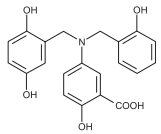

Fusidic acid . sodium salt

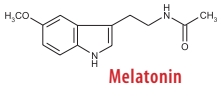

L-Glutamic acid

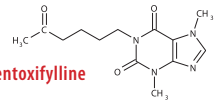

Histamine . 2HCl

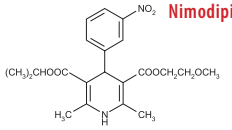

Hydroxyguanidine . hemisulfate

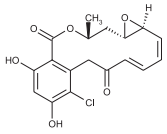

10Z-Hymenialdisine

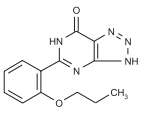

Lavendustin A

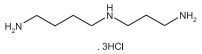

Melatonin

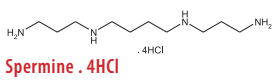

Pentoxifylline

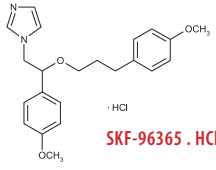

Nimodipine

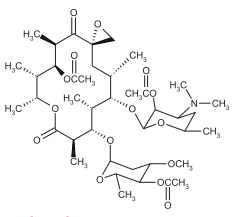

Radicicol

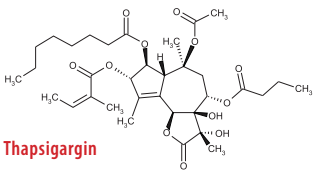

Zaprinast


Spermidine . 3HCl


Spermine . 4HCl


SKF-96365 . HCl


Troleandomycin


Thapsigargin

Product Name	Product Nr.	Size
A23187 (free acid)	ALX-450-001-M001 ALX-450-001-5001 ALX-450-001-M005 ALX-450-001-M010 ALX-450-001-M025 ALX-450-001-M050	1 mg 5 x 1 mg 5 mg 10 mg 25 mg 50 mg
Adenosine 5′-diphosphate . disodium salt	ALX-480-001-G001 ALX-480-001-G005	1 g 5 g
Adenosine 5′-diphosphate . potassium salt	ALX-480-002-G001 ALX-480-002-G005	1 g 5 g
Arachidonic acid	ALX-340-004-M100	100 mg
(-)-Arctigenin	ALX-350-312-M025	25 mg
Calcineurin (bovine brain)	ALX-202-034-U100	100 U
Calmidazolium chloride	ALX-430-026-M010 ALX-430-026-M050	10 mg 50 mg
Corticosterone	ALX-370-007-M050	50 mg
Cyclosporin A	ALX-380-002-M100 ALX-380-002-5100 ALX-380-002-G001 ALX-380-002-5001	100 mg 5 x 100 mg 1 g 5 x 1 g
Forskolin	ALX-350-001-M001 ALX-350-001-M005 ALX-350-001-M010 ALX-350-001-M025 ALX-350-001-M050	1 mg 5 mg 10 mg 25 mg 50 mg
Fusidic acid . sodium salt	ALX-380-011-M050	50 mg
Gadolinium (III) chloride . $6H_2O$	ALX-400-023-M100 ALX-400-023-M500	100 mg 500 mg
L-Glutamic acid	ALX-101-018-G001	1 g
Histamine . 2HCl	ALX-550-132-G005	5 g
Hydroxyguanidine . hemisulfate	ALX-420-001-M025	25 mg
10Z-Hymenialdisine	ALX-350-289-C500	500 µg
Lavendustin A	ALX-350-007-M001 ALX-350-007-M005	1 mg 5 mg
Melatonin	ALX-550-071-G001 ALX-550-071-G005	1 g 5 g
Nimodipine	ALX-550-277-M010 ALX-550-277-M050 ALX-550-277-M250 ALX-550-277-G001	10 mg 50 mg 250 mg 1 g
Nitrate Reductase (cytochrome)	ALX-206-003-UC05	0.5 U
Pentoxifylline	ALX-270-112-G001 ALX-270-112-G005	1 g 5 g
Radicicol	ALX-380-092-M001 ALX-380-092-M005	1 mg 5 mg
SKF-96365 . HCl	ALX-550-297-M001 ALX-550-297-M005	1 mg 5 mg
Spermidine . 3HCl	ALX-550-004-G001 ALX-550-004-G005	1 g 5 g
Spermine . 4HCl	ALX-550-003-G001 ALX-550-003-G005	1 g 5 g
Thapsigargin	ALX-350-004-M001 ALX-350-004-M005 ALX-350-004-M010 ALX-350-004-M025	1 mg 5 mg 10 mg 25 mg
Troleandomycin	ALX-380-001-G001	1 g
Zaprinast	ALX-430-020-M010 ALX-430-020-M050	10 mg 50 mg

Arginine & Arginase Related Products

Arginine is a basic amino acid that plays pivotal roles in cellular physiology. It is a substrate for protein synthesis but also modulates cellular biochemical functions via conversion to a number of biologically active compounds, such as nitric oxide, creatine phosphate, agmatine, polyamines, ornithine, and citrulline. The metabolism of arginine is determined by the expression of the arginine metabolizing enzymes inducible nitric oxide synthase (iNOS; NOS II) and two arginase isoforms (arginase I and II).

Two arginase isoforms (ARG1 and ARG2) have been identified in mammals, and they are encoded by different genes. The isoforms have 58% sequence identity at the amino-acid level, but they have distinct tissue, cellular and subcellular distributions. ARG1 (also known as liver-type ARG) is located in the cytosol of hepatocytes and is an important component of the urea cycle. ARG2 (also known as kidney-type ARG) is located in the mitochondria of various cell types, including renal cells, neurons, macrophages and enterocytes. It is constitutively expressed, and it is thought to increase L-proline synthesis in particular, because of its close proximity to ornithine aminotransferase, which is also located in mitochondria. Arginases were shown to play an important role in cellular proliferation, wound healing and they induce nitric oxide synthase (NOS) activity by competing for arginine availability in the extracellular environment.

L-Arginine is also the substrate for the family of NOS enzymes. The NOS enzymes are dimers that require the presence of regulators and cofactors (see page 7 & 8) for full activity. They catalyze the reaction between oxygen ($O_2^{\bullet -}$) and L-arginine, which produce $NO^{\bullet}$ from the oxidation of L-arginine (L-Arg) to L-citrulline through the intermediate N-Hydroxy-L-Arg.

The arginine metabolism has many effects in the body that include modulation of immune function, wound healing, hormone secretion, vascular tone, insulin sensitivity, and endothelial function.

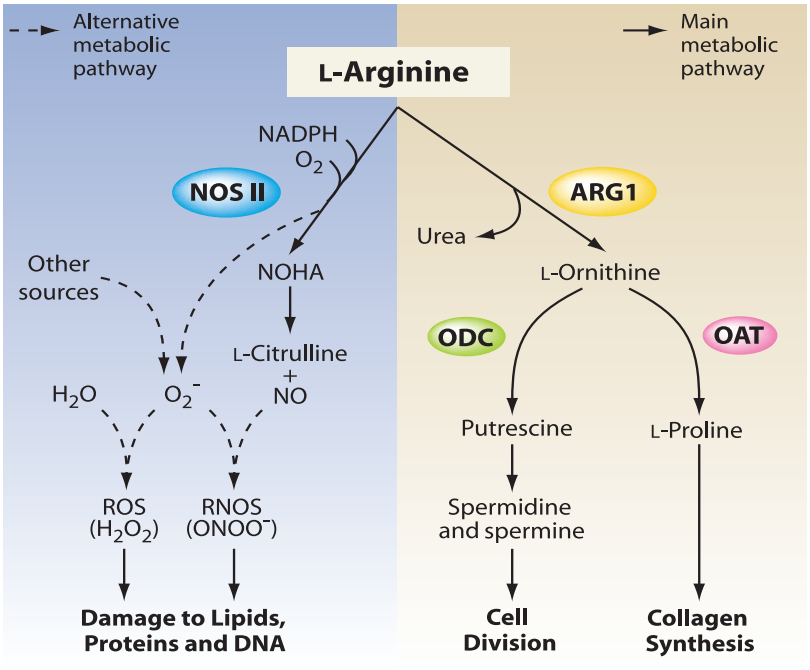


FIGURE: Schematic arginine metabolic pathway. Adpated from: *Regulation of immune responses by L-arginine metabolism:* V. Bronte & P. Zanovello; Nat. Rev. Immunol. **5,** 641 (2005)

Selected Review Articles

Arginine metabolism: nitric oxide and beyond: G. Wu & S.M. Morris, Jr.; Biochem. J. **336,** 1 (1998) ▪ Nitric oxide biosynthesis, nitric oxide synthase inhibitors and arginase competition for L-arginine utilization: J.L. Boucher, et al.; Cell. Mol. Life Sci. **55,** 1015 (1999) ▪ Arginase: a binuclear manganese metalloenzyme: D.E. Ash, et al.; Met. Ions Biol. Syst. **37,** 407 (2000) ▪ Regulation of nitric oxide production by arginine metabolic enzymes: M. Mori & T. Gotoh; BBRC **275,** 715 (2000) ▪ Arginine metabolism and the synthesis of nitric oxide in the nervous system: H. Wiesinger; Prog. Neurobiol. **64,** 365 (2001) ▪ Macrophage arginine metabolism to ornithine/urea or nitric oxide/citrulline: a life or death issue: C.D. Mills; Crit. Rev. Immunol. **21,** 399 (2001) ▪ Reduced arginine availability and nitric oxide production: M.M. Hallemeesch, et al.; Clin. Nutr. **21,** 273 (2002) ▪ Arginine availability, arginase, and the immune response: V. Bansal & J.B. Ochoa; Curr. Opin. Clin. Nutr. Metab. Care **6,** 223 (2003) ▪ Arginases I and II: do their functions overlap?: S.D. Cederbaum, et al.; Mol. Genet. Metab. **81 (Suppl 1),** S38 (2004) ▪ Structure and function of arginases: D.E. Ash; J. Nutr. **134,** 2760S (2004) ▪ Novel roles for arginase in cell survival, regeneration, and translation in the central nervous system: P.S. Lange, et al.; J. Nutr. **134,** 2812S (2004) ▪ Arginine revisited: minireview article: M.A. Grillo & S. Colombatto; Amino Acids **26,** 345 (2004) ▪ Cellular and physiological effects of arginine: B.C. Tong & A. Barbul; Mini Rev. Med. Chem. **4,** 823 (2004) ▪ Regulation of immune responses by L-arginine metabolism: V. Bronte & P. Zanovello; Nat. Rev. Immunol. **5,** 641 (2005) ▪ Arginine: beyond protein: S.M. Morris, Jr.; Am. J. Clin. Nutr. **83,** 508S (2006)

Arginine & NO Production

N^G-Hydroxy-L-arginine . monoacetate

ALX-106-004-M005 5 mg
ALX-106-004-M025 25 mg

Intermediate in the biosynthesis of nitric oxide from L-arginine by nitric oxide synthases (NOS). Inhibitor of arginases.

LIT: Macrophage oxidation of L-arginine to nitrite and nitrate: nitric oxide is an intermediate: M.A. Marletta, et al.; Biochemistry **27,** 8706 (1988) ▪ N-omega-hydroxy-L-arginine is an intermediate in the biosynthesis of nitric oxide from L-arginine: D.J. Stuehr, et al.; J. Biol. Chem. **266,** 6259 (1991) ▪ Nitric oxide and another potent vasodilator are formed from NG-hydroxy- L-arginine by cultured endothelial cells: A. Zembowicz, et al.; PNAS **88,** 11172 (1991) ▪ N-omega-hydroxy-L-arginine: a novel arginine analog capable of causing vasorelaxation in bovine intrapulmonary artery: G.C. Wallace, et al.; BBRC **176,** 528 (1991) ▪ Synthesis and bioactivity of N omega-hydroxyarginine: a possible intermediate in the biosynthesis of nitric oxide from arginine: G.C. Wallace & J.M. Fukuto; J. Med. Chem. **34,** 1746 (1991) ▪ Inhibition of rat liver arginase by an intermediate in NO biosynthesis, NG-hydroxy-L-arginine: implications for the regulation of nitric oxide biosynthesis by arginase: F. Daghigh, et al.; BBRC **202,** 174 (1994) ▪ N-omega-hydroxyl-L-arginine, an intermediate in the L-arginine to nitric oxide pathway, is a strong inhibitor of liver and macrophage arginase: J.-L. Boucher, et al.; BBRC **203,** 1614 (1994) ▪ Inhibition of purified nitric oxide synthase from rat cerebellum and macrophage by L-arginine analogs: Y. Komori, et al.; Arch. Biochem. Biophys. **315,** 213 (1994) ▪ Inhibition of arginase by NG-hydroxy-L-arginine in alveolar macrophages: implications for the utilization of L-arginine for nitric oxide synthesis: M. Hecker, et al.; FEBS Lett. **359,** 251 (1995) ▪ Arginase activity in endothelial cells: inhibition by NG-hydroxy-L-arginine during high-output NO production: G.M. Buga, et al.; Am. J. Physiol. **271,** H1988 (1996) ▪ NG-hydroxy-L-arginine and nitric oxide inhibit Caco-2 tumor cell proliferation by distinct mechanisms: G.M. Buga, et al.; Am. J. Physiol. **275,** R1256 (1998)

D-Arginine

ALX-101-005-G005 5 g

L-Arginine

ALX-101-004-G025 25 g

LIT: Vascular endothelial cells synthesize nitric oxide from L-arginine: R.M.J. Palmer, et al.; Nature **333,** 664 (1988) ▪ L-arginine is the physiological precursor for the formation of nitric oxide in endothelium-dependent relaxation: R.M.J. Palmer, et al.; BBRC **153,** 1251 (1988) ▪ Insulin secretion from pancreatic B cells caused by L-arginine-derived nitrogen oxides: H.H.H.W. Schmidt, et al.; Science **255,** 721 (1992)

L-Citrulline

ALX-106-003-G005 5 g

Essential intermediate in the biosynthesis of nitric oxide from L-arginine.

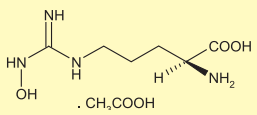
LIT: A novel citrulline-forming enzyme implicated in the formation of nitric oxide by vascular endothelial cells: R.M.J. Palmer and S. Moncada; BBRC **158,** 348 (1989) ▪ The metabolism of L-arginine and its significance for the biosynthesis of endothelium-derived relaxing factor: cultured endothelial cells recycle L-citrulline to L-arginine: M. Hecker, et al.; PNAS **87,** 8612 (1990) ▪ N-omega-hydroxy-L-arginine is an intermediate in the biosynthesis of nitric oxide from L-arginine: D.J. Stuehr, et al.; J. Biol. Chem. **266,** 6259 (1991)

D-Ornithine . HCl

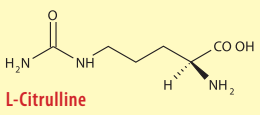
ALX-101-037-G001 1 g
ALX-101-037-G005 5 g

L-Ornithine . HCl

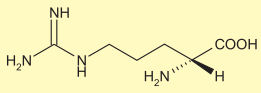
ALX-101-036-G005 5 g
ALX-101-036-G025 25 g



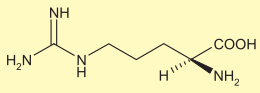
N^G-Hydroxy-L-arginine . monoacetate



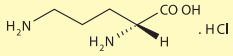
L-Citrulline



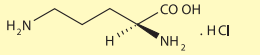
D-Arginine



L-Arginine



D-Ornithine . HCl



L-Ornithine . HCl

Arginase Specific Inhibitors

Nitric oxide synthase (NOS) utilizes L-arginine and oxygen as substrates to produce nitric oxide (NO) and citrulline. Arginase is a metalloenzyme that catalyzes the hydrolysis of L-arginine to produce L-ornithine and urea. It is proposed that arginase competes for L-arginine and reduces NOS activity in genital tissues, thus modulating sexual function. ALEXIS® Biochemicals offers two specific inhibitors of arginase: ABH and BEC. At pH 7.5, both ABH and BEC are classical, competitive inhibitors of human type II arginase with Ki values of 0.25 and 0.31µM, respectively. However, at pH 9.5, ABH and BEC are slow-binding inhibitors of the enzyme with Ki values of 8.5 and 30 nM, respectively.

LIT: Classical and slow-binding inhibitors of human type II arginase: D.M. Colleluori & D.E. Ash; Biochemistry **40,** 9356 (2001) ▪ Role of arginase in the male and female sexual arousal response: N.N. Kim, et al.; J. Nutr. **134,** 2873S (2004) (Review)

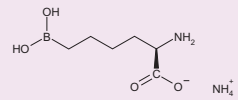
NEW ABH . ammonium salt

[2(S)-Amino-6-boronohexanoic acid . NH_4]

ALX-270-420-M001	1 mg
ALX-270-420-M005	5 mg

Potent and specific inhibitor of arginase. Does not inhibit nitric oxide synthases (NOS).

LIT: Biochemical and functional profile of a newly developed potent and isozyme-selective arginase inhibitor: R. Baggio, et al.; J. Pharmacol. Exp. Ther. **290,** 1409 (1999)



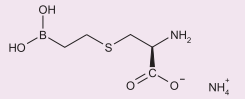
BEC . ammonium salt

[S-(2-Boronoethyl)-L-cysteine . NH_4]

ALX-270-345-M001	1 mg
ALX-270-345-M005	5 mg

Slow-binding competitive inhibitor of arginase.

LIT: Probing erectile function: S-(2-boronoethyl)-L-cysteine binds to arginase as a transition state analogue and enhances smooth muscle relaxation in human penile corpus cavernosum: N.N. Kim, et al.; Biochemistry **40,** 2678 (2001)



Arginase & Related Products

Arginase I (human) (rec.) (purified)

ALX-201-081-C020	20 µg

Full length human arginase I expressed in *E. coli.* **PURITY:** ≥90% (SDS-PAGE). **SPECIFIC ACTIVITY:** ~0.7U/µg (see J. Biol. Chem. **238,** 1012 (1962)).

Arginase (bovine liver)

ALX-202-037-UT50	5 KU
ALX-202-037-5T50	5x5 KU
ALX-202-037-UZ25	25 KU

Isolated from bovine liver. **SPECIFIC ACTIVITY:** ~150 units/mg. One unit hydrolyzes 1.0µmol of L-arginine per min. at 37°C, pH 9.5.

Inhibitors

(2S)-(+)-Amino-6-iodoacetamidohexanoic acid [AIAH]

ALX-270-008-M005	5 mg
ALX-270-008-M025	25 mg

Irreversible inhibitor of arginase.

LIT: Uterine arginase inhibition affect the rat embryonic development: J.D. Méndez, et al.; Contraception **33,** 597 (1986) ▪ J.G. Trujillo, et al.; Synth. Commun. **21,** 683 (1991) ▪ Effect of nitric oxide synthase substrate analog inhibitors on rat liver arginase: C.A. Robertson, et al.; BBRC **197,** 523 (1993) ▪ Comparison of substrate and inhibitor specificity of arginase and nitric oxide (NO) synthase for arginine analogues and related compounds in murine and rat macrophages: A. Hrabák, et al.; BBRC **198,** 206 (1994)

(2S)-(+)-Amino-5-iodoacetamidopentanoic acid [AIAP]

ALX-270-007-M005	5 mg
ALX-270-007-M025	25 mg

Irreversible inhibitor of arginase.

LIT: See Prod. No. ALX-270-008.

nor-NOHA . 2HCl

ALX-270-284-M001	1 mg
ALX-270-284-M005	5 mg

Potent and specific arginase inhibitor (K_i ~0.5µM).

LIT: The new alpha-amino acid N-omega-hydroxy-nor-L-arginine: a high-affinity inhibitor of arginase well adapted to bind to its manganese cluster: J. Custot, et al.; JACS **119,** 4086 (1997) ▪ Substrate specificity of NO synthases: detailed comparison of L-arginine, homo-L-arginine, their N-omega-hydroxy derivatives, and N-omega-hydroxynor-L-arginine: C. Moali, et al.; Biochemistry **37,** 10453 (1998) ▪ L-Arginine availability modulates local nitric oxide production and parasite killing in experimental trypanosomiasis: A.P. Gobert, et al.; Infect. Immun. **68,** 4653 (2000) ▪ **For a comprehensive bibliography please visit our website.**

Ornithine Decarboxylase (ODC) Inhibitor

DL-α-Difluoromethylornithine . HCl . H_2O

[DFMO; Eflornithine; RMI-71782]

ALX-270-283-M010	10 mg
ALX-270-283-M050	50 mg

Specific, irreversible inhibitor of ornithine decarboxylase (ODC), the rate-limiting enzyme in polyamine biosynthesis. Potent chemopreventive agent. Induces apoptosis. Arginase activity inhibitor.

LIT: Effect of alpha-difluoromethylornithine, an enzyme-activated irreversible inhibitor of ornithine decarboxylase, on L1210 leukemia in mice: N.J. Prakash, et al.; Cancer Res. **38,** 3059 (1978) ▪ The enzyme-activated irreversible inhibitor of ornithine decarboxylase, DL-alpha-difluoromethylornithine: a chemopreventive agent: A.K. Verma; Prev. Med. **18,** 646 (1989) ▪ Alpha-difluoromethylornithine (DFMO) as a potent arginase activity inhibitor in human colon carcinoma cells: M. Selamnia, et al.; Biochem. Pharmacol. **55,** 1241 (1998) ▪ Development of difluoromethylornithine (DFMO) as a chemoprevention agent: F.L. Meyskens, Jr. & E.W. Gerner; Clin. Cancer Res. **5,** 945 (1999), (Review) ▪ alpha-difluoromethylornithine induces apoptosis as well as anti-angiogenesis in the inhibition of tumor growth and metastasis in a human gastric cancer model: Y. Takahashi, et al.; Int. J. Cancer **85,** 243 (2000)

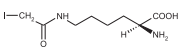
Related Product

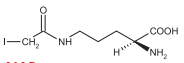
Agmatine . sulfate

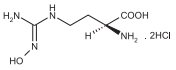
ALX-550-001-M100	100 mg
ALX-550-001-M500	500 mg

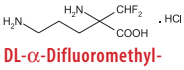
Decarboxylation product of L-arginine. Endogenous clonidine-displacing substance in the brain. Putative endogenous neurotransmitter at imidazoline receptors. Antagonist of NMDA receptor. Endogenous inhibitor of nitric oxide synthase (NOS). Antiproliferative by its suppresive effect on polyamine.

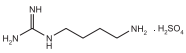
Selective inhibition of inducible nitric oxide synthase by agmatine: M. Auguet, et al.; Jpn. J. Pharmacol. **69,** 285 (1995) ▪ Inhibition of mammalian nitric oxide synthases by agmatine, an endogenous polyamine formed by decarboxylation of arginine: E. Galea, et al.; Biochem. J. **316,** 247 (1996) ▪ Agmatine suppresses nitric oxide production in microglia: K. Abe, et al.; Brain Res. **872,** 141 (2000) ▪ Agmatine enhances the NADPH oxidase activity of neuronal NO synthase and leads to oxidative inactivation of the enzyme: D.R. Demady, et al.; Mol. Pharmacol. **59,** 24 (2001) ▪ Regulation of inducible nitric oxide synthase and agmatine synthesis in macrophages and astrocytes: S. Regunathan & J.E. Piletz; Ann. N. Y. Acad. Sci. **1009,** 20 (2003) ▪ Agmatine: at the crossroads of the arginine pathways: J. Satriano; Ann. N. Y. Acad. Sci. **1009**, 34 (2003), (Review) ▪ Agmatine signaling: odds and threads: R. Berkels, et al.; Cardiovasc. Drug Rev. **22,** 7 (2004) (Review)


AIAH


AIAP


nor-NOHA . 2HCl


DL-α-Difluoromethylornithine . HCl . H_2O


Agmatine . sulfate

Highlight

Nitric Oxide (NO) in Plants

As in animals, nitric oxide in plants has been observed to play an important role as a signalling molecule. It influences multiple physiological functions such as germination, development, maturation and senescence, biotic and abiotic stress response, as well as hormone response. NO in plants originates from nitrite and arginine. While the nitrate reductase enzyme was already well known as a generator of NO, people were speculating about the existence of an enzyme producing NO from arginine, similar to the mammalian nitric oxide synthase (NOS). Although some inhibitors of mammalian NOS were known to inhibit NO generation also in plants, biologists searched for a long time without success for a plant NOS. Recently, a gene has been identified in the mustard plant *Arabidopsis thaliana* encoding the protein AtNOS1, which is capable of generating NO from L-Arg [1]. Synthesis of NO by AtNOS is needed for hormonal signalling, defense response, and flowering [1-3].

Selected Review Articles

New insights into nitric oxide metabolism and regulatory functions: N. M. Crawford & F. Q. Guo; Trends Plant Sci. **10,** 195 (2005)

Reactive oxygen species and reactive nitrogen species in peroxisomes. Production, scavenging, and role in cell signaling: L. A. del Rio, et al.; Plant Physiol. **141,** 330 (2006)

Mechanisms for nitric oxide synthesis in plants: N. M. Crawford; J. Exp. Bot. **57,** 471 (2006)

LIT: [1] Identification of a plant nitric oxide synthase gene involved in hormonal signaling: F. Q. Guo, et al.; Science **302,** 100 (2003) ▪ **[2]** Nitric oxide represses the Arabidopsis floral transition: Y. He, et al.; Science **305,** 1968 (2004) ▪ **[3]** Innate immunity in Arabidopsis thaliana: lipopolysaccharides activate nitric oxide synthase (NOS) and induce defense genes: D. Zeidler, et al.; PNAS **101,** 15811 (2004)

Nitric Oxide [NO] Donors – *The Widest Range*

Table: Half-lifes ($t_{½}$) of NO Donors

Prod. No.	Product	$t_{½}$ (pH 7.4) 24°C	37°C	Moles NO[1]	Lit.
ALX-430-126	Angeli's Salt	17-25min	2min	0.54	[1]
ALX-430-034	DEA NONOate	16min	2min	1.5	[1]
ALX-430-014	DETA NONOate	57hr	20hr	2.0	[1]
ALX-430-066	DPTA NONOate	5hr	3hr	2.0	[1]
ALX-420-028	Fructose-SNAP-1[6]				[13]
ALX-420-016	Glyco-SNAP-1	30.2hr			[8]
ALX-420-017	Glyco-SNAP-2	27.2hr			[8]
AXL-420-002	GSNO				[4]
ALX-430-015	MAHMA NONOate	3min	1min	2.0	[1]
ALX-430-003	Molsidomine		1-2hr[2]		[2, 3, 14]
ALX-430-017	NOC-5		25min	2.0	[9]
ALX-430-018	NOC-7		5min	2.0	[9]
ALX-430-019	NOC-12		100min	2.0	[9]
ALX-430-009	NOR-1		1.8min		[10]
ALX-430-010	NOR-2		28min		[10]
ALX-430-011	NOR-3 [FK 409]		40min		[10, 11]
ALX-430-012	NOR-4 [FR 144420]		60min		[11]
ALX-430-125	NOR-5 [FR 146801]		20hr		[12]
ALX-430-016	PAPA NONOate	77min	15min	2.0	[1]
ALX-430-068	PROLI NONOate		1.8s	2.0	[6]
ALX-430-002	SIN-1 . HCl		1-2hr[2]		[3]
ALX-420-003	SNAP		6hr[3]		[4, 5]
ALX-430-013	Spermine NONOate	230min	39min	2.0	[1]
ALX-430-067	Sulfo-NONOate	24min	7min	0[4]	[1]
ALX-430-075	V-PYRRO/NO		3s[5]		[7]

NOTES:
1. Moles of NO released per mole of NO donor.
2. Molsidomine is metabolised by the liver to SIN-1; $t_{½}$ values are for plasma
3. At pH 7.0; $t_{½}$ depends upon buffer and is enhanced by metals, thiols & light.
4. Does not produce NO.
5. $t_{½}$ following hepatic metabolism.
6. In the absence of Cu^{2+} releases NO mildly and continued at a constant rate, while in the presence of Cu^{2+} NO concentration reaches maximum in 30s.
7. Varies depending on nature of buffers; enhanced by thiols, metals, and light.

[1] NONOates (1-substituted diazen-1-ium-1,2-diolates) as nitric oxide donors: convenient nitric oxide dosage forms: L.K. Keefer, et al.; Meth. Enzymol. **268,** 281 (1996) ▪ **[2]** On the mechanism of NO release from sydnonimines: M. Feelisch, et al.; J. Cardiovas. Pharmacol. **14 (Suppl 11),** S13 (1989) [3] Clinical pharmacokinetics of molsidomine: B. Rosenkranz, et al.; Clin. Pharmacokinet. **30,** 372 (1996) ▪ **[4]** Donors of Nitrogen Oxides, Chapter 7: M. Feelisch & J.S. Stamler; Methods in Nitric Oxide Research, **71,** eds. M. Feelisch & J.S. Stamler (John Wiley & Sons, Inc., 1996) ▪ **[5]** New thionitrites: Synthesis, stability, and nitric oxide generation: B. Roy, et al.; JOC **59,** 7019 (1994) ▪ **[6]** Localizing antithrombotic and vasodilatory activity with a novel, ultrafast nitric oxide donor: J.E. Saavedra, et al.; J. Med. Chem. **39,** 4361 (1996) ▪ **[7]** Targeting nitric oxide (NO) delivery in vivo. Design of a liver-selective NO donor prodrug that blocks TNF-alpha-induced apoptosis and toxicity in the liver: J.E. Saavedra, et al.; J. Med. Chem. **40,** 1947 (1997) ▪ **[8]** Glyco-S-nitrosothiols, a novel class of NO donor compounds: J. Ramirez, et al.; Bioorg. Med. Chem. Lett. **6,** 2575 (1996)/Glyco-S-nitrosothiols: sugar-SNAP, a new type of nitric oxide donor: Y. Hou, et al.; Meth. Enzymol. **301,** 242 (1999) ▪ **[9]** New NO-releasing zwitterions derived from poly-amines: J.A. Hrabie & J.R. Klose; JOC **58,** 1472 (1993) ▪ **[10]** Spontaneous nitric oxide release accounts for the potent pharmacological actions of FK409: Y. Kita, et al.; Eur. J. Pharmacol. **257,** 123 (1994) ▪ **[11]** FR144420, a novel, slow, nitric oxide-releasing agent: Y. Kita, et al.; Eur. J. Pharmacol. **275,** 125 (1995) ▪ **[12]** Oral biological activities of spontaneous nitric oxide releasers are accounted for by their nitric oxide-releasing rates and oral absorption manners: Y. Kita, et al.; J. Pharmacol. Exp. Ther. **276,** 421 (1996) ▪ **[13]** The synthesis and cytotoxicity of fructose-1-SNAP, a novel fructose conjugated S-nitroso nitric oxide donor: Tetrahedron Lett. **42,** 825 (2001) ▪ **[14]** Molsidomine: alternative approaches to treat myocardial ischemia: R.E. Nitz & V.B. Fiedler; Pharmacotherapy **7,** 28 (1987)

Selected Latest Review Articles

Nitric oxide donors: T. Yamamoto & R.J. Bing; Proc. Soc. Exp. Biol. Med. **225,** 200 (2000) ▪ Nitric oxide donors: chemical activities and biological applications: P.G. Wang, et al.; Chem. Rev. **102,** 1091 (2002) ▪ Current status and future possibilities of nitric oxide-donor drugs: focus on S-nitrosothiols: H.H. Al-Sa'doni & A. Ferro; Mini Rev. Med. Chem. **5,** 247 (2005) ▪ Focus on recent approaches for the development of new NO-donors: A. Gasco, et al.; Mini Rev. Med. Chem. **5,** 217 (2005) ▪ Nitric oxide (NO)- and nitroxyl (HNO)-generating diazeniumdiolates (NONOates): emerging commercial opportunities: L.K. Keefer; Curr. Top. Med. Chem. **5,** 625 (2005) ▪ N-hydroxyurea and acyl nitroso compounds as nitroxyl (HNO) and nitric oxide (NO) donors: S.B. King; Curr. Top. Med. Chem. **5,** 665 (2005) ▪ Nitric oxide donor drugs: an update on pathophysiology and therapeutic potential: R. Scatena, et al.; Expert Opin. Investig. Drugs **14,** 835 (2005) ▪ An introduction to NO-related therapeutic agents: G.R. Thatcher; Curr. Top. Med. Chem. **5,** 597 (2005) ▪ NO-releasing hybrids of cardiovascular drugs: A. Martelli, et al.; Curr. Med. Chem. **13,** 609 (2006) ▪ Nitric oxide and cell proliferation: A. Villalobo; FEBS J. **273,** 2329 (2006)

Storage & Handling of NO Donors

The following information relates mainly to the 1-substituted diazen-1-ium-1,2-diolates (NONOates), although it is applicable to other NO• donors. For further information, refer to the excellent reviews: "NONOates" (1-substituted diazen-1-ium-1,2-diolates) as nitric oxide donors: convenient nitric oxide dosage forms: L.K. Keefer, et al.; Meth. Enzymol. **268,** 281 (1996) **[1]**. Guide for the use of nitric oxide (NO) donors as probes of the chemistry of NO• and related redox species in biological systems: D.D. Thomas, et al.; Methods Enzymol. **359,** 84 (2002). Quantum mechanical determinations of reaction mechanisms, acid base, and redox properties of nitrogen oxides and their donors: A.S. Dutton, et al.; Methods Enzymol. **396,** 26 (2005) (Review)

Storage

All NO• donors should be stored dry. Whilst many are perfectly stable at +4°C, storage at -20°C is recommended; storage at lower temperatures is generally not necessary. Ensure that the vial contents have reached room temperature before opening. As an added precaution, flush the vial with dry nitrogen or argon before closing and returning to the freezer.

Quality Control

Whilst all ALEXIS® Biochemicals products are tested and supplied with detailed analytical data sheets, it is sometimes necessary to check a compound, e.g. after prolonged storage or before an important series of experiments. The purity of a solid sample can be determined spectrophotometrically and the amount of NO• generated on decomposition can be measured by a chemiluminescence method (see [1] above for details).

Preparation of Solutions

NO• donors are generally all water soluble and are more stable at higher pH. This is the basis of the two-stage method of preparing dosing solutions.

A concentrated stock solution should be prepared in 10mM NaOH (i.e. pH ~12). The stock solution should be kept on ice and used as soon as possible; in any case, stock solutions should be prepared freshly each day.

An aliquot of the concentrated stock solution is then added to a large excess of the buffer used in the experiment (e.g. saline, culture medium, etc.). In this way, the small quantity of NaOH does not change the pH of the buffer. It may be necessary to perform the dilution in two steps; to prepare a 10µM solution for i.v. injection, first dilute a 100mM stock solution with 100 volumes of 10mM NaOH (to keep the pH high), then dilute the resulting solution with 100 volumes of injection buffer.

In some cases it is not desirable to prepare a concentrated alkaline stock solution (e.g. if a highly concentrated solution of NO• donor is needed, or if a solution of low ionic strength must be administered). If so, it is recommended that the temperature is kept as low as possible and, by working quickly, the time between disolving and administering is kept very short.

There is, of course, greater latitude when preparing solutions of NO• donors with longer $t_{½}$, as they are inherently more stable in aqueous solution.

Control Experiments

All NO• donors produce by-products which must be accounted for in the experimental design; for example, the nitrite ion (the autoxidation product of NO• in aqueous solution) is bioactive in some systems. One approach is to allow the NO• donor to decompose through many $t_{½}$ before adding it to the medium as control (the "spent" compound will contain all the ultimate by-products of the decomposition). Alternatively, the purified by-products may be used in a control experiment.

Sulfo-NONOate (Prod. No. ALX-430-067) may be used as a control compound as it decomposes to sulfate and nitrous oxide; it does not release nitric oxide at physiological pH.

NONOates from the Leading Manufacturer

Step up to the Source™

ALEXIS® BIOCHEMICALS

Selected Literature on NONOates

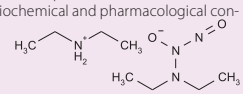
Diazeniumdiolates: pro- and antioxidant applications of the „NONOates": A.L. Fitzhugh & L.K. Keefer; Free. Radic. Biol. Med. **28,** 1463 (2000) ▪ Chemistry of the diazeniumdiolates. 3. Photoreactivity: A. Srinivasan, et al.; J. Am. Chem. Soc. **123,** 5465 (2001) ▪ Chemistry of the diazeniumdiolates. 2. Kinetics and mechanism of dissociation to nitric oxide in aqueous solution: K.M. Davies, et al.; JACS **123,** 5473 (2001) ▪ Chemistry of the diazeniumdiolates. I. Structural and spectral characteristics of the [N(O)NO]- functional group: L.K. Keefer, et al.; Nitric Oxide **5,** 377 (2001) ▪ Chemistry of the diazeniumdiolates: Z right harpoon over left harpoon E isomerism: Y.N. Wang, et al.; JACS **127,** 5388 (2005) ▪ NO-donors, part 9 : diazeniumdiolates inhibit human platelet aggregation and induce a transient vasodilatation of porcine pulmonary arteries in accordance with the NO-releasing rates: D. Abuo-Rahma Gel, et al.; Eur. J. Med. Chem. **40,** 281 (2005)

DEA NONOate

ALX-430-034-M010	10 mg
ALX-430-034-5005	5 x 5 mg
ALX-430-034-M050	50 mg

Nitric oxide (NO) donor.

LIT: The Reaction of Nitrogen(II) Oxide with Diethylamine: R.S. Drago & F.E. Paulik; JACS **82,** 96 (1960) ▪ The Reaction of Nitrogen(II) Oxide with Various Primary and Secondary Amines: R.S. Drago & B.R. Karstetter; JACS **83,** 1819 (1961) ▪ Complexes of .NO with nucleophiles as agents for the controlled biological release of nitric oxide. Vasorelaxant effects: C.M. Maragos, et al.; J. Med. Chem. **34,** 3242 (1991) ▪ New nitric oxide-releasing zwitterions derived from polyamines : J.A. Hrabie, et al.; JOC **58,** 1472 (1993) ▪ A potential role for extracellular nitric oxide generation in cGMP-independent inhibition of human platelet aggregation: biochemical and pharmacological considerations: M.S. Crane, et al.; Br. J. Pharmacol. **144,** 849 (2005)

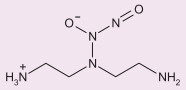


DETA NONOate

ALX-430-014-M005	5 mg
ALX-430-014-5005	5 x 5 mg
ALX-430-014-M025	25 mg

Nitric oxide (NO) donor. Induces apoptosis in macrophages.

LIT: New nitric oxide-releasing zwitterions derived from polyamines: J.A. Hrabie, et al.; J. Org. Chem. **58,** 1472 (1993) ▪ NOC, a nitric-oxide-releasing compound, induces dose dependent apoptosis in macrophages: M. Shimaoka, et al.; BBRC **209,** 519 (1995) ▪ In vitro cytotoxicity of glyco-S-nitrosothiols. a novel class of nitric oxide donors: H. Babich & H.L. Zuckerbraun; Toxicol. In Vitro **15,** 181 (2001) ▪ Superoxide-dependent consumption of nitric oxide in biological media may confound in vitro experiments: R.G. Keynes, et al.; Biochem. J. **369,** 399 (2003) ▪ The selective pulmonary vasodilatory effect of inhaled DETA/NO, a novel nitric oxide donor, in ARDS-a pilot human trial: C.F. Lam, et al.; J. Crit. Care **19,** 48 (2004) ▪ The nitric oxide donor DETA-NONOate decreases matrix metalloproteinase-9 expression and activity in rat aortic smooth muscle and abdominal aortic explants: I. Sinha, et al.; Ann. Vasc. Surg. **20,** 92 (2006)

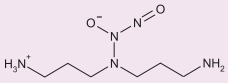


DPTA NONOate

ALX-430-066-M005	5 mg
ALX-430-066-5005	5 x 5 mg
ALX-430-066-M025	25 mg

Nitric oxide (NO) donor.

LIT: New Nitric Oxide-Releasing Zwitterions Derived from Polyamines: J.A. Hrabie, et al.; J. Org. Chem. **58,** 1472 (1993) ▪ In vitro cytotoxicity of the nitric oxide donor, S-nitroso-N-acetylpenicillamine, towards cells from human oral tissue: H. Babich, et al.; Pharmacol. Toxicol. **84,** 218 (1999) ▪ In vitro cytotoxicity of glyco-S-nitrosothiols. a novel class of nitric oxide donors: H. Babich & H.L. Zuckerbraun; Toxicol. In Vitro **15,** 181 (2001) ▪ S-nitrosylation of Bcl-2 inhibits its ubiquitin-proteasomal degradation. A novel antiapoptotic mechanism that suppresses apoptosis: N. Azad, et al.; J. Biol. Chem. **281,** 34124 (2006)

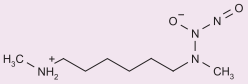


MAHMA NONOate

ALX-430-015-M005	5 mg
ALX-430-015-5005	5 x 5 mg
ALX-430-015-M025	25 mg

Nitric oxide (NO) donor.

LIT: New nitric oxide-releasing zwitterions derived from polyamines: J.A. Hrabie, et al.; JOC **58,** 1472 (1993) ▪ Inhibition of rat platelet aggregation by the diazeniumdiolate nitric oxide donor MAHMA NONOate: K.L. Homer & J.C. Wanstall; Br. J. Pharmacol. **137,** 1071 (2002) ▪ Platelet inhibitory effects of the nitric oxide donor drug MAHMA NONOate in vivo in rats: K.L. Homer & J.C. Wanstall; Eur. J. Pharmacol. **482,** 265 (2003) ▪ Nitric oxide donors inhibit 5-hydroxytryptamine (5-HT) uptake by the human 5-HT transporter (SERT): L.J. Bryan-Lluka, et al.; Br. J. Pharmacol. **143,** 63 (2004)

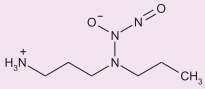


PAPA NONOate

ALX-430-016-M005	5 mg
ALX-430-016-5005	5 x 5 mg
ALX-430-016-M025	25 mg

Nitric oxide (NO) donor.

LIT: New nitric oxide-releasing zwitterions derived from polyamines: J.A. Hrabie, et al.; JOC **58,** 1472 (1993) ▪ Effects of redox-related congeners of NO on apoptosis and caspase-3 activity: J. Haendeler, et al.; Nitric Oxide **1,** 282 (1997) ▪ Role of nitric oxide-induced mtDNA damage in mitochondrial dysfunction and apoptosis: L.I. Rachek, et al.; Free Radic. Biol. Med. **40,** 754 (2006)

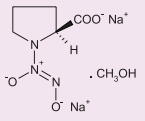


PROLI NONOate

ALX-430-068-M005	5 mg
ALX-430-068-5005	5 x 5 mg
ALX-430-068-M025	25 mg

Nitric oxide (NO) donor. The product dissociates with extreme rapidity to proline (1mol) and NO (2mol). This behavior allows the generation of highly localized anti-platelet and vasodilatory effects.

LIT: Localizing antithrombotic and vasodilatory activity with a novel, ultrafast nitric oxide donor: J.E. Saavedra, et al.; J. Med. Chem. **39,** 4361 (1996) ▪ Nitric oxide activation of guanylyl cyclase in cells revisited: B. Roy & J. Garthwaite; PNAS **103,** 12185 (2006)

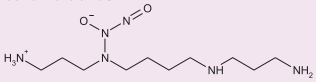


Spermine NONOate

ALX-430-013-M005	5 mg
ALX-430-013-5005	5 x 5 mg
ALX-430-013-M025	25 mg

Nitric oxide (NO) donor. A convenient reagent for preparing aqueous solutions of NO.

LIT: Complexes of .NO with nucleophiles as agents for the controlled biological release of nitric oxide. Vasorelaxant effects: C.M. Maragos, et al.; J. Med. Chem. **34,** 3242 (1991) ▪ Extracellular nitric oxide release mediates soluble guanylate cyclase-independent vasodilator action of spermine NONOate: comparison with other nitric oxide donors in isolated rat femoral arteries: M.R. Miller, et al.; J. Cardiovasc. Pharmacol. **43,** 440 (2004) ▪ Effects of antioxidant and nitric oxide on chemokine production in TNF-alpha-stimulated human dermal microvascular endothelial cells: M.Z. Jiang, et al.; Free Radic. Res. **38,** 473 (2004)

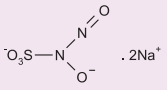


Sulfo-NONOate . disodium salt

ALX-430-067-M005	5 mg
ALX-430-067-5005	5 x 5 mg
ALX-430-067-M025	25 mg

Sulfo-NONOate dissociates to sulfate and nitrous oxide in a pH-dependent manner following first order kinetics. The decomposition is also catalyzed by the borate anion. Unlike other NONOates, Sulfo-NONOate produces nitrous oxide but not nitric oxide (NO) at physiological pH. Therefore, Sulfo-NONOate may be used as a negative control in experiments using other NO releasing NONOates.

LIT: Kinetics of the boric acid catalyzed decomposition of the N-nitrosohydroxylamine-N-sulfonate ion: E.G. Switkes, et al.; Inorg. Chem. **12,** 1120 (1973) ▪ Complexes of .NO with nucleophiles as agents for the controlled biological release of nitric oxide. Vasorelaxant effects: C.M. Maragos, et al.; J. Med. Chem. **34,** 3242 (1991)

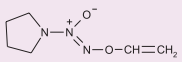


V-PYRRO/NO

ALX-430-075-M005	5 mg
ALX-430-075-5005	5 x 5 mg

Liver-selective nitric oxide (NO) donor that blocks TNF-α-induced apoptosis and toxicity.

LIT: Targeting nitric oxide (NO) delivery in vivo. Design of a liver-selective NO donor prodrug that blocks tumor necrosis factor-a-induced apoptosis and toxicity in the liver: J.E. Saavedra, et al.; J. Med. Chem. **40,** 1947 (1997) ▪ The nitric oxide prodrug, V-PYRRO/NO, protects against cadmium toxicity and apoptosis at the cellular level: W. Qu, et al.; Nitric Oxide **12,** 114 (2004) ▪ Nitric oxide and chemically induced hepatotoxicity: beneficial effects of the liver-selective nitric oxide donor, V-PYRRO/NO: J. Liu & M.P. Waalkes; Toxicology **208,** 289 (2005) ▪ Metabolism of a liver-selective nitric oxide-releasing agent, V-PYRRO/NO, by human microsomal cytochromes P450: K. Inami, et al.; Nitric Oxide **14,** 309 (2006) ▪ Hemodynamic and antifibrotic effects of a selective liver nitric oxide donor V-PYRRO/NO in bile duct ligated rats: F. Moal, et al.; World J. Gastroenterol. **12,** 6639 (2006)



Technical Note

NO functions ubiquitously as a biological messenger but has also been implicated in various pathologies, a role supported by many reports that exogenous or endogenous NO can kill cells in tissue culture. In the course of experiments aimed at examining the toxicity of exogenous NO towards cultured cells, it was found that most of the NO delivered using a NONOate (diazeniumdiolate) donor was removed by reaction with the tissue-culture medium. Two NO-consuming ingredients were identified: Hepes buffer and, under laboratory lighting, the vitamin riboflavin.

The inadvertent production of ONOO- and other reactive species in biological media, or the associated loss of NO, may contribute to the adverse effects, or otherwise, of NO *in vitro*.

Efficient Compound To Deliver Nitric Oxide (NO) Inside Cells

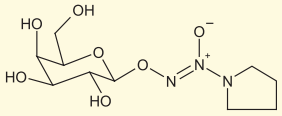
NEW

β-Gal NONOate

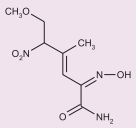
ALX-430-138-M001	1 mg
ALX-430-138-M005	5 mg

Nitric oxide (NO) donor. Glycosylated, cell permeable *in vivo* nitric oxide releasing compound.

LIT: Glycosylated diazeniumdiolates: a novel class of enzyme-activated nitric oxide donors: X. Wu et al.; Tetrahedron Lett. **42,** 3779 (2001) ▪ Delivery of Nitric Oxide Released from beta-Gal-NONOate Activation by beta-Galactosidase and Its Activity against Escherichia coli: C. Chen, et al.; Biol. Pharm. Bull. **29,** 1239 (2006) ▪ A glycosylated nitric oxide donor, beta-Gal-NONOate, and its site-specific antitumor activity: C. Chen, et al.; Arch. Pharm. (Weinheim) **339,** 366 (2006)



NOR Compounds



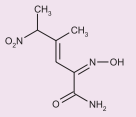
NOR-1

NOR-1

ALX-430-009-M005 5 mg
ALX-430-009-M010 10 mg

Nitric oxide (NO) donor. Has a short half-life time and is therefore a promising reagent to make NO standard solutions by adding exactly diluted NOR-1/DMSO solution to the buffer solution.

LIT: Spontaneous nitric oxide release accounts for the potent pharmacological actions of FK409: Y. Kita, et al.; Eur. J. Pharmacol. **257,** 123 (1994) ▪ Kinetic characterization of the nitric oxide toxicity for PC12 cells: effect of half-life time of NO release: T. Yamamoto, et al.; Eur. J. Pharmacol. **397,** 25 (2000)



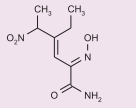
NOR-2

NOR-2

ALX-430-010-M005 5 mg
ALX-430-010-M010 10 mg

Nitric oxide (NO) donor.

LIT: See ALX-430-009



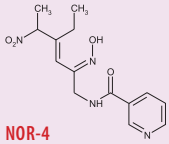
NOR-3

NOR-3

ALX-430-011-M005 5 mg
ALX-430-011-M010 10 mg

Nitric oxide (NO) donor. NOR-3 shows a variety of biological and pharmacological effects. Strong vasodilatory effects in rat aorta, rabbit aorta and dog coronary artery; potent inhibitory activities against antiplatelet aggregation and thrombus formation; effective aggregation inhibition of human platelet raising by ADP; cardioprotective effects in the ischemia/reperfusion system; antianginal effect.

LIT: FK409, a novel vasodilator isolated from the acid-treated fermentation broth of Streptomyces griseosporeus. I. Taxonomy, fermentation, isolation, and physico-chemical and biological characteristics: N. Hino, et al.; J. Antibiot. (Tokyo) **42,** 1578 (1989) ▪ Spontaneous nitric oxide release accounts for the potent pharmacological actions of FK409: Y. Kita, et al.; Eur. J. Pharmacol. **257,** 123 (1994) ▪ FR144420, a novel, slow, nitric oxide-releasing agent: Y. Kita, et al.; Eur. J. Pharmacol. **275,** 125 (1995) ▪ Comparison of antiplatelet effects of two nitric oxide-donating agents, FR146801 and FK409: Y. Hirasawa, et al.; Thromb. Haemost. **79,** 620 (1998) ▪ Effects of pre- and post-ischemic treatments with FK409, a nitric oxide donor, on ischemia/reperfusion-induced renal injury and endothelin-1 production in rats: A. Nakajima, et al.; Biol. Pharm. Bull. **29,** 577 (2006)



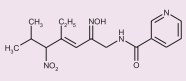
NOR-4

NOR-4

ALX-430-012-M005 5 mg
ALX-430-012-M010 10 mg

Nitric oxide (NO) donor.

LIT: FR144420, a novel, slow, nitric oxide-releasing agent: Y. Kita, et al.; Eur. J. Pharmacol. **275,** 125 (1995) ▪ Antianginal effects of FR144420, a novel slow nitric oxide-releasing agent: Y. Hirasawa, et al.; Eur. J. Pharmacol. **303,** 55 (1996) ▪ Kinetic characterization of the nitric oxide toxicity for PC12 cells: effect of half-life time of NO release: T. Yamamoto, et al.; Eur. J. Pharmacol. **397,** 25 (2000)



NOR-5

NOR-5

ALX-430-125-M005 5 mg
ALX-430-125-M010 10 mg

Nitric oxide (NO) donor with a half-life time of 20 hours (0.5mM NOR-5 in 0.1M PBS, pH 7.0, at 37°C).

LIT: Oral biological activities of spontaneous nitric oxide releasers are accounted for by their nitric oxide-releasing rates and oral absorption manners: Y. Kita, et al.; J. Pharmacol. Exp. Ther. **276,** 421 (1996) ▪ Comparison of antiplatelet effects of two nitric oxide-donating agents, FR146801 and FK409: Y. Hirasawa, et al.; Thromb. Haemost. **79,** 620 (1998)

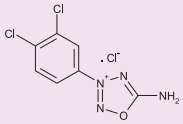
GEA Compounds

GEA 3162

ALX-430-004-M010 10 mg
ALX-430-004-M050 50 mg

Water soluble nitric oxide (NO) donor and potent inhibitor of ADP-induced platelet aggregation in platelet rich plasma (PRP). Stimulates cGMP production in platelets, granulocytes and polymorphonuclear leukocytes. Vasodepressant.

LIT: Scavenging of superoxide anions by nitric oxide donors: J. Robak, et al.; Pharmacol. Res. **25 S2,** 355 (1992) ▪ Inhibition by nitric oxide-donors of human polymorphonuclear leucocyte functions: E. Moilanen, et al.; Br. J. Pharmacol. **109,** 852 (1993) ▪ Mesoionic oxatriazole derivatives-a new group of NO-donors: G. Karup, et al.; Pol. J. Pharmacol. **46,** 541 (1994) ▪ Nitric oxide donor GEA 3162 inhibits endothelial cell-mediated oxidation of low density lipoprotein: U. Malo-Ranta, et al.; FEBS Lett. **337,** 179 (1994) ▪ Pharmacology of mesoionic oxatriazole derivatives in blood, cardiovascular and respiratory systems: T. Corell, et al.; Pol. J. Pharmacol. **46,** 553 (1994) ▪ GEA 3162 decomposes to co-generate nitric oxide and superoxide and induces apoptosis in human neutrophils via a peroxynitrite-dependent mechanism: E.L. Taylor, et al.; Br. J. Pharmacol. **143,** 179 (2004) ▪ GEA3162, a nitric oxide-releasing agent, activates non-store-operated Ca2+ entry and inhibits store-operated Ca2+ entry pathways in neutrophils through thiol oxidation: M.F. Hsu, et al.; Eur. J. Pharmacol. **535,** 43 (2006)

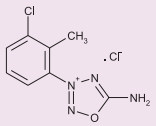


GEA 5024

ALX-430-005-M010 10 mg
ALX-430-005-M050 50 mg

Water soluble and stable nitric oxide (NO) donor.

LIT: Inhibition by nitric oxide-donors of human polymorphonuclear leucocyte functions: E. Moilanen, et al.; Br. J. Pharmacol. **109,** 852 (1993)

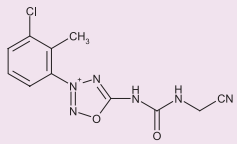


GEA 5583

ALX-430-006-M010 10 mg
ALX-430-006-M050 50 mg

Stable nitric oxide (NO) releasing compound that is orally absorbed in rats.

LIT: Scavenging of superoxide anions by nitric oxide donors: J. Robak, et al.; Pharmacol. Res. **25 S2,** 355 (1992) ▪ Mesoionic oxatriazole derivatives--a new group of NO-donors: G. Karup, et al.; Pol. J. Pharmacol. **46,** 541 (1994) ▪ Pharmacology of mesoionic oxatriazole derivatives in blood, cardiovascular and respiratory systems: T. Corell, et al.; Pol. J. Pharmacol. **46,** 553 (1994) ▪ Cytokine- or chemically derived nitric oxide alters the expression of proteins detected by two-dimensional gel electrophoresis in neonatal rat islets of Langerhans: N.E. John, et al.; Diabetes **49,** 1819 (2000)



The Supplier of Glyco-NO Donors

Step up to the Source™

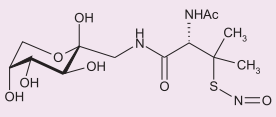
ALEXIS® BIOCHEMICALS

Fructose-SNAP-1

ALX-420-028-M005 5 mg
ALX-420-028-5001 5 x 1 mg

Nitric oxide (NO) donor with increased cytotoxicity compared to SNAP (Prod. No. ALX-420-003). See also Glyco-SNAP-1 (Prod. No. ALX-420-016) and Glyco-SNAP-2 (Prod. No. ALX-420-017).

LIT: Targeting nitric oxide to cancer cells: cytotoxicity studies of glyco-S-nitrosothiols: Y. Hou, et al.; Bioorg. Med. Chem. Lett. **9,** 2255 (1999) ▪ In vitro cytotoxicity of glyco-S-nitrosothiols. a novel class of nitric oxide donors: H. Babich & H.L. Zuckerbraun; Toxicol. In Vitro **15,** 181 (2001)

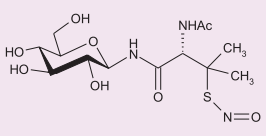


Glyco-SNAP-1

ALX-420-016-M005 5 mg

Highly water soluble nitric oxide (NO) donor, which is relatively stable at high pH (pH 8-9). See also SNAP (Prod. No. ALX-420-003).

LIT: Glyco-S-Nitrosothiols, A Novel Class of NO Donor Compounds: J. Ramirez, et al.; Bioorg. Med. Chem. Lett. **6,** 2575 (1996) ▪ IFN-gamma inhibits AP-1 binding activity in human brain-derived cells through a nitric oxide dependent mechanism: K. Conant, et al.; J. Neuroimmunol. **88,** 39 (1998) ▪ Structural and kinetic modifications of aldose reductase by S-nitrosothiols: S. Srivastava, et al.; Biochem. J. **358,** 111 (2001) ▪ In vitro cytotoxicity of glyco-S-nitrosothiols. a novel class of nitric oxide donors: H. Babich & H.L. Zuckerbraun; Toxicol. In Vitro **15,** 181 (2001)

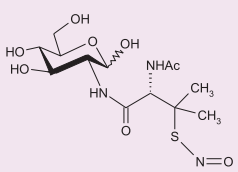


Glyco-SNAP-2

ALX-420-017-M005 5 mg

Highly water soluble nitric oxide (NO) donor which is relatively stable at high pH (pH 8-9). See also SNAP (Prod. No. ALX-420-003).

LIT: Glyco-S-Nitrosothiols, A Novel Class of NO Donor Compounds: J. Ramirez, et al.; Bioorg. Med. Chem. Lett. **6,** 2575 (1996) ▪ In vitro cytotoxicity of glyco-S-nitrosothiols. a novel class of nitric oxide donors: H. Babich & H.L. Zuckerbraun; Toxicol. In Vitro **15,** 181 (2001) ▪ Nitric oxide regulates cGMP-dependent cAMP-responsive element binding protein phosphorylation and Bcl-2 expression in cerebellar neurons: implication for a survival role of nitric oxide: E. Ciani, et al.; J. Neurochem. **82,** 1282 (2002) ▪ Characterization of a novel type of endogenous activator of soluble guanylyl cyclase: N. Balashova, et al.; J. Biol. Chem. **280,** 2186 (2005)



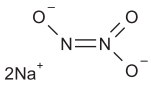
Other NO Donors

Angeli's Salt

ALX-430-126-M010 10 mg

Nitric oxide (NO) donor. The stoichiometry of NO release is 0.54 mole per mole of Angeli's Salt. The EC_{50} for relaxation of norepinephrine-constricted rabbit aorta is 0.59µM.

LIT: Complexes of .NO with nucleophiles as agents for the controlled biological release of nitric oxide. Vasorelaxant effects: C.M. Maragos, et al.; J. Med. Chem. **34,** 3242 (1991) ▪ Conversion of nitroxyl (HNO) to nitric oxide (NO) in biological systems: the role of physiological oxidants and relevance to the biological activity of HNO: J.M. Fukuto, et al.; BBRC **196,** 707 (1993) ▪ Comparison of the NO and HNO donating properties of diazeniumdiolates: primary amine adducts release HNO in Vivo: K.M. Miranda, et al.; J. Med. Chem. **48,** 8220 (2005)

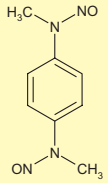


BNN3

ALX-430-148-M001 1 mg

Cell permeable, photolabile nitric oxide (NO) donor (λem (max): ~300nm). Produces two nitric oxide (NO) molecules per molecule of BNN3 upon irradiation (300-360nm) with a xenon lamp or laser flashlight. Causes photo-induced vasorelaxation in rat aortic strips.

LIT: Bis-N-nitroso-caged nitric oxides: photochemistry and biological performance test by rat aorta vasorelaxation: S. Namiki, et al.; Bioorg. Med. Chem. **7,** 1695 (1999)

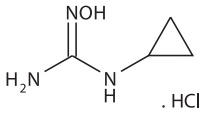


N-Cyclopropyl-N'-hydroxyguanidine . HCl

ALX-420-034-M001 1 mg
ALX-420-034-M005 5 mg

Selective substrate for nNOS (NOS I). 70% nitric oxide (NO) formation for nNOS (NOS I), 26% for eNOS (NOS III) and <0.5% for iNOS (NOS II) as compared to NO formation using N^G-Hydroxy-L-arginine (NOHA, Prod No. ALX-106-004) as a substrate.

LIT: Isoform-selective substrates of nitric oxide synthase: Q. Jia, et al.; J. Med. Chem. **46,** 2271 (2003)

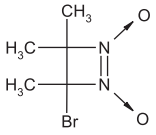


DD1

ALX-430-086-M010 10 mg
ALX-430-086-M050 50 mg

Guanylyl cyclase activator. Cell permeable thiol-induced nitric oxide (NO) donor with strong vasodilator effects.

LIT: Kinetics of nitric oxide liberation by 3,4-dihydro-1,2-diazete 1,2- dioxides and their vasodilatory properties in vitro and in vivo: D.I. Utepbergenov, et al.; BBRC **214,** 1023 (1995) ▪ Thiol-induced nitric oxide release from 3-halogeno-3,4-dihydrodiazete 1,2-dioxides: I.A. Kirilyuk, et al.; J. Med. Chem. **41,** 1027 (1998)

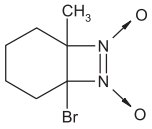


DD2

ALX-430-087-M010 10 mg
ALX-430-087-M050 50 mg

Guanylyl cyclase activator. Cell permeable thiol-induced nitric oxide (NO) donor with strong vasodilator effects.

LIT: Kinetics of nitric oxide liberation by 3,4-dihydro-1,2-diazete 1,2- dioxides and their vasodilatory properties in vitro and in vivo: D.I. Utepbergenov, et al.; BBRC **214,** 1023 (1995) ▪ Thiol-induced nitric oxide release from 3-halogeno-3,4-dihydrodiazete 1,2-dioxides: I.A. Kirilyuk, et al.; J. Med. Chem. **41,** 1027 (1998)

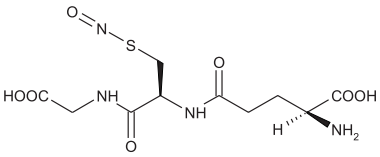


GSNO

ALX-420-002-M025 25 mg
ALX-420-002-M100 100 mg

Carrier of nitric oxide (NO), relaxing smooth muscle and inhibiting platelet activation.

LIT: An investigation of some S-nitrosothiols, and of hydroxyarginine, on the mouse anococcygeus: A. Gibson, et al.; Br. J. Pharmacol. **107,** 715 (1992) ▪ S-nitroso-glutathione inhibits platelet activation in vitro and in vivo: M.W. Radomski, et al.; Br. J. Pharmacol. **107,** 745 (1992) ▪ Endothelial heme oxygenase-1 induction by hypoxia. Modulation by inducible nitric-oxide synthase and S-nitrosothiols: R. Motterlini, et al.; J. Biol. Chem. **275,** 13613 (2000) ▪ S-Nitrosoglutathione reduces inflammation and protects brain against focal cerebral ischemia in a rat model of experimental stroke: M. Khan, et. al.; J. Cereb. Blood Flow Metab. **25,** 177 (2005) ▪ Redox regulation of PTEN by S-nitrosothiols: C.X. Yu, et al.; Mol. Pharmacol. **68,** 847 (2005) ▪ Mechanisms of cystic fibrosis transmembrane conductance regulator activation by S-nitrosoglutathione: L. Chen, et al.; J. Biol. Chem. **281,** 9190 (2006) ▪ Modulation of glucose uptake in adipose tissue by nitric oxide-generating compounds: D. McGrowder, et al.; J. Biosci. **31,** 347 (2006) ▪ GSNO attenuates EAE disease by S-nitrosylation-mediated modulation of endothelial-monocyte interactions: R. Prasad, et al.; Glia **55,** 65 (2007)

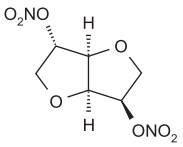


Isosorbide dinitrate

ALX-400-008-M100 100 mg
ALX-400-008-M500 500 mg

Nitric oxide (NO) donor.

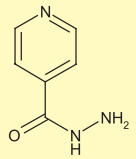
LIT: Isosorbide dinitrate pharmacokinetics: T. Taylor, et al.; Arzneimittelforschung **32,** 1329 (1982) ▪ Inhibition of platelet aggregation by isosorbide dinitrate: J. Ahlner, et al.; Am. J. Cardiol. **58,** 665 (1986) ▪ Inactivation of human aldehyde dehydrogenase by isosorbide dinitrate: N. Mukerjee & R. Pietruszko; J. Biol. Chem. **269,** 21664 (1994) ▪ Effects of nitric oxide donor, isosorbide dinitrate, on energy metabolism of rat reticulocytes: S. D. Maletic, et al.; Physiol. Res. **48,** 417 (1999) ▪ Effect of isosorbide dinitrate on nitric oxide synthase under hypoxia: H.B. Jiang, et al.; Pharmacology **62,** 10 (2001)



Isoniazid

LKT-I7341 5 g
LKT-I7341 50 g
LKT-I7341 100 g

A front-line antituberculosis agent. Generates nitric oxide (NO) when activated by KatG. Strong inhibitor of DPH metabolism.

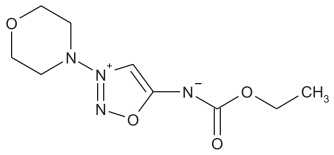


Molsidomine

ALX-430-003-M050 50 mg
ALX-430-003-M250 250 mg

Long acting antianginal drug that is enzymatically converted in the liver to yield the active metabolite SIN-1 (Prod. No. ALX-430-002).

LIT: Heilmittelchemische Studien In Der Heterocyclischen Reihe 35. Mitteilung. Über Sydnonimine. I. Herstellung und Eigenschaften von Sydnonimin-Salzen: H.U. Daeniker & J. Druey; Helv. Chim. Acta **45,** 2426 (1962) ▪ Molsidomine: alternative approaches to treat myocardial ischemia: R.-E. Nitz & V.B. Fiedler; Pharmacotherapy **7,** 28 (1987) ▪ Intrarenal administration of molsidomine, a molecule releasing nitric oxide, reduces renal ischemia-reperfusion injury in rats: A. Rodriguez-Pena, et al.; Am. J. Transplant. **4,** 1605 (2004) ▪ Molsidomine, a nitric oxide donor and L-arginine protects against rhabdomyolysis-induced myoglobinuric acute renal failure: V. Chander & K. Chopra; Biochim. Biophys. Acta **1723,** 208 (2005) ▪ Long-term treatment with the NO-donor molsidomine reduces circulating ICAM-1 levels in patients with stable angina: C. Van Hove, et al.; Atherosclerosis **180,** 399 (2005) ▪ Nitric oxide donor molsidomine attenuates psychotomimetic effects of the NMDA receptor antagonist MK-801: N. Pitsikas, et al.; J. Neurosci. Res. **84,** 299 (2006)

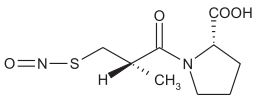


S-Nitrosocaptopril

ALX-270-213-M010 10 mg
ALX-270-213-M050 50 mg

Angiotensin-converting enzyme (ACE) inhibitor. Inhibitor of platelet aggregation. Its activity may depend on the homolytic cleavage of the S-N bond under physiological conditions, yielding nitric oxide (NO) and the parent compound, captopril (Prod. No. ALX-270-212).

LIT: S-nitrosocaptopril. I. Molecular characterization and effects on the vasculature and on platelets: J. Loscalzo, et al.; J. Pharmacol. Exp. Ther. **249,** 726 (1989) ▪ S-nitrosocaptopril. II. Effects on vascular reactivity: J.P. Cooke, et al.; J. Pharmacol. Exp. Ther. **249,** 730 (1989) ▪ The hemodynamic effects of S-nitrosocaptopril in anesthetized dogs: J.E. Shaffer, et al.; J. Pharmacol. Exp. Ther. **256,** 704 (1991) ▪ Dual role of S-nitrosocaptopril as an inhibitor of angiotensin-converting enzyme and a nitroso group carrier: J.W. Park; BBRC **189,** 206 (1992) ▪ Physicochemistry, pharmacokinetics, and pharmacodynamics of S-nitrosocaptopril crystals, a new nitric oxide donor: L. Jia, et al.; J. Pharm. Sci. **88,** 981 (1999) ▪ Antiangiogenic effects of S-nitrosocaptopril crystals as a nitric oxide donor: L. Jia, et al.; Eur. J. Pharmacol. **391,** 137 (2000) ▪ In vitro and in vivo assessment of cellular permeability and pharmacodynamics of S-nitrosylated captopril, a nitric oxide donor: L. Jia & H. Wong; Br. J. Pharmacol. **134,** 1697 (2001) ▪ S-nitrosocaptopril: acute in-vivo pulmonary vasodepressor effects in pulmonary hypertensive rats: D.Y. Tsui, et al.; J. Pharm. Pharmacol. **55,** 1121 (2003)



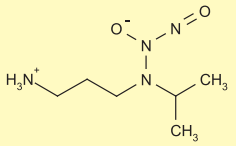
Other NO Donors *continued*

NOC-5

ALX-430-017-M005 5 mg
ALX-430-017-M010 10 mg

Nitric oxide (NO) donor. The half-life time of 0.1mM NOC-5 is 25 min. at 37°C in 0.1M PBS, pH 7.4.

LIT: New nitric oxide-releasing zwitterions derived from polyamines: J.A. Hrabie, et al.; J. Org. Chem. **58,** 1472 (1993) ▪ Characterization of the influence of nitric oxide donors on intestinal absorption of macromolecules: K. Takahashi, et al.; Int. J. Pharm. **286,** 89 (2004) ▪ Excellent absorption enhancing characteristics of NO donors for improving the intestinal absorption of poorly absorbable compound compared with conventional absorption enhancers: G. Fetih, et al.; Drug Metab. Pharmacokinet. **21,** 222 (2006)

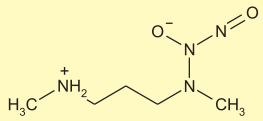


NOC-7

ALX-430-018-M005 5 mg
ALX-430-018-M010 10 mg

Nitric oxide (NO) donor. The half-life time of 0.1mM NOC-7 is 5 min. at 37°C in 0.1M PBS, pH 7.4.

LIT: New nitric oxide-releasing zwitterions derived from polyamines: J.A. Hrabie, et al.; J. Org. Chem. **58,** 1472 (1993) ▪ Characterization of the influence of nitric oxide donors on intestinal absorption of macromolecules: K. Takahashi, et al.; Int. J. Pharm. **286,** 89 (2004)

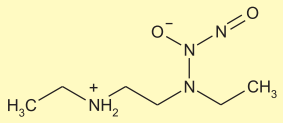


NOC-12

ALX-430-019-M005 5 mg
ALX-430-019-M010 10 mg

Nitric oxide (NO) donor. The half-life time of 0.1mM NOC-12 is 100 min. at 37°C in 0.1M PBS, pH 7.4.

LIT: New nitric oxide-releasing zwitterions derived from polyamines: J.A. Hrabie, et al.; J. Org. Chem. **58,** 1472 (1993) ▪ Characterization of the influence of nitric oxide donors on intestinal absorption of macromolecules: K. Takahashi, et al.; Int. J. Pharm. **286,** 89 (2004) ▪ Excellent absorption enhancing characteristics of NO donors for improving the intestinal absorption of poorly absorbable compound compared with conventional absorption enhancers: G. Fetih, et al.; Drug Metab. Pharmacokinet. **21,** 222 (2006)

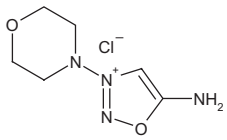


SIN-1 chloride

ALX-430-002-M010 10 mg
ALX-430-002-M050 50 mg

SIN-1 is a metabolite of the vasodilator molsidomine (Prod. No. ALX-430-003) considered to be a peroxynitrite releasing compound. Using molecular oxygen it generates superoxide and nitric oxide (NO) that together spontaneously form peroxinitrite. SIN-1 might therefore be a useful tool to study the effect of NO, produced by other NO releasing compounds, and peroxynitrite. For a physiologically active NO releasing agent see SIN-1A/γCD complex (Prod. No. ALX-400-009).

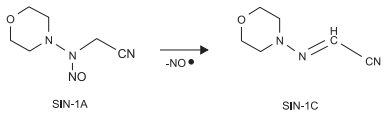
LIT: Regulation of cyclic GMP formation by soluble guanylate cyclase: stimulation by NO-containing compounds: E. Böhme, et al.; Adv. Cyclic Nucl. & Prot. Phos. **17,** 259 (1984) ▪ Effect of nitric oxide production on the redox modulatory site of the NMDA receptor-channel complex: S.Z. Lei, et al.; Neuron **8,** 1087 (1992) ▪ Nitric oxide regulates cardiac Ca2+ current. Involvement of cGMP- inhibited and cGMP-stimulated phosphodiesterases through guanylyl cyclase activation: P.-F. Méry, et al.; J. Biol. Chem. **268,** 26286 (1993) ▪ In vitro cytotoxicity of glyco-S-nitrosothiols. a novel class of nitric oxide donors: H. Babich & H.L. Zuckerbraun; Toxicol. In Vitro **15,** 181 (2001) ▪ New glycosidase activated nitric oxide donors: glycose and 3-morphorlinosydnonimine conjugates: T.B. Cai, et al.; J. Org. Chem. **70,** 3518 (2005)



SIN-1A/γCD Complex

ALX-400-009-M005 5 mg
ALX-400-009-M025 25 mg

Physiologically active nitric oxide (NO) releasing agent. Releasing one equivalent of NO the half life time at room temperature is ~40 min. mostly independent from the pH.

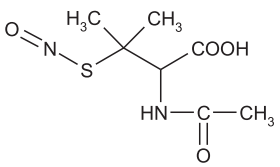


SNAP

ALX-420-003-M005 5 mg
ALX-420-003-M025 25 mg
ALX-420-003-M100 100 mg

Stable analog of endogenous S-nitroso compounds. A source of nitric oxide (NO) *in vivo.* See also Glyco-SNAP-1 (Prod. No. ALX-420-016) and Glyco-SNAP-2 (Prod. No. ALX-420-017).

LIT: Differential hemodynamic effects and tolerance properties of nitroglycerin and an S-nitrosothiol in experimental heart failure: J.A. Bauer & H.-L. Fung; J. Pharmacol. Exp. Ther. **256,** 249 (1991) ▪ Lack of tolerance to a 24-hour infusion of S-nitroso N- acetylpenicillamine (SNAP) in conscious rabbits: J.E. Shaffer, et al.; J. Pharmacol. Exp. Ther. **260,** 286 (1992) ▪ Nitric oxide donor SNAP induces apoptosis in smooth muscle cells through cGMP-independent mechanism: E. Nishio, et al.; BBRC **221,** 163 (1996) ▪ In vitro cytotoxicity of glyco-S-nitrosothiols. a novel class of nitric oxide donors: H. Babich & H.L. Zuckerbraun; Toxicol. In Vitro **15,** 181 (2001) ▪ SNAP, a NO donor, induces cellular protection only when cortical neurons are submitted to some aggression process: S. Figueroa, et al.; Brain Res. **1034,** 25 (2005) ▪ The reaction of S-nitroso-N-acetyl-D,L-penicillamine (SNAP) with the angiotensin converting enzyme inhibitor, captopril--mechanism of transnitrosation: D.V. Aquart & T.P. Dasgupta; Org. Biomol. Chem. **3,** 1640 (2005) ▪ SNAP, a NO donor, induces cortical neuron death by a mechanism in which the caspase pathway is implicated: S. Figueroa, et al.; Brain Res. **1047,** 168 (2005)

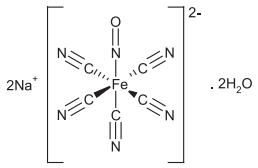


Sodium nitroprusside . dihydrate

ALX-400-001-G005 5 g
ALX-400-001-G025 25 g

Nitric oxide (NO) donor.

LIT: Sodium nitroprusside protects adult rat cardiac myocytes from cellular injury induced by simulated ischemia: role for a non-cGMP-dependent mechanism of nitric oxide protection: A.M. Garreffa, et al.; J. Cardiovasc. Pharmacol. **47,** 1 (2006) ▪ Effect of NO donor sodium nitroprusside on lipopolysaccharide induced acute lung injury in rats: Z.Y. Xia, et al.; Injury **38,** 53 (2007)

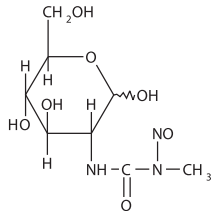


Streptozotocin

ALX-380-010-M100 100 mg
ALX-380-010-5100 5 x 100 mg
ALX-380-010-G001 1 g

Widely used diabetes inducer in rodents. Inhibition of β-cell O-GlcNAcase by streptozotocin is the mechanism that accounts for its diabetogenic toxicity. N-nitroso-containing antibiotic, acting as a nitric oxide (NO) donor. Potent methylating agent for DNA.

LIT: Studies on the diabetogenic action of streptozotocin: N. Rakieten, et al.; Cancer Chemother. Rep. **29,** 91 (1963) ▪ The structure of streptozotocin: R.R. Herr, et al.; JACS **89,** 4808 (1967) ▪ Streptozotocin: a nitric oxide carrying molecule and its effect on vasodilation: G. Thomas & P. Ramwell; Eur. J. Pharmacol. **161,** 279 (1989) ▪ Biochemical evidence for nitric oxide formation from streptozotocin in isolated pancreatic islets: J. Turk, et al.; BBRC **197,** 1458 (1993) ▪ Nitric oxide generation from streptozotocin: N.S. Kwon, et al.; FASEB J. **8,** 529 (1994) ▪ Nitric oxide generation during cellular metabolization of the diabetogenic N-methyl-N-nitroso-urea streptozotozin contributes to islet cell DNA damage: K.-D. Kroncke, et al.; Biol. Chem. Hoppe Seyler **376,** 179 (1995)

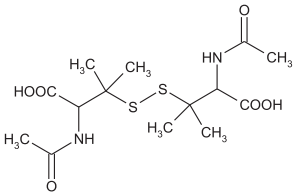


N-Acetyl-D,L-penicillamine disulfide

ALX-420-019-M001 1 mg
ALX-420-019-M005 5 mg

Degradation product of S-nitroso-N-acetyl-D,L-penicillamine (SNAP) (Prod. No. ALX-420-003).

LIT: The nitric oxide-cyclic GMP pathway and synaptic depression in rat hippocampal slices: C.L. Boulton, et al.; Eur. J. Neurosci. **6,** 1528 (1994) ▪ Neurotoxicity in conscious rats following intraventricular SNAP, a nitric oxide donor: P.M. Gross, et al.; Neuropharmacology **33,** 915 (1994)



Product Highlight

Nitric Oxide and Viral Signalling

Hepatitis C virus (HCV) infection has been shown to cause double-stranded DNA breaks and to enhance the mutation frequency of immunoglobulin genes and protooncogenes [1]. Such damages are thought to be mediated by nitric oxide (NO). Accordingly, it has been shown that HCV infections stimulate the production of NO. The stimulation is dependent on the activation of the gene for iNOS (NOS II) by the viral core and NS3 proteins [2].

LIT: [1] Hepatitis C virus induces a mutator phenotype: enhanced mutations of immunoglobulin and protooncogenes: K. Machida, et al.; PNAS **101,** 4262 (2004) ▪ **[2]** Hepatitis C virus infection activates the immunologic (type II) isoform of nitric oxide synthase and thereby enhances DNA damage and mutations of cellular genes: K. Machida, et al.; J. Virol. **78,** 8835 (2004)

Proteins

NEW NS3-NS4A (HCV) (rec.) (His)

ALX-201-247-C100 100 µg

Produced in *E. coli*. Recombinant hepatitis C virus (HCV) NS3-NS4A serine protease complex is fused to a His-tag. **MW:** 68.7kDa (NS3 subunit); 6.2kDa (NS4A subunit).

Antibodies

NEW MAb to NS3 (HCV) (1B6)

ALX-803-059-R100 100 µl

CLONE: 1B6. **ISOTYPE:** Mouse IgG1. **IMMUNOGEN:** Recombinant HCV NS3 serine protease domain. **SPECIFICITY:** HCV NS3 (epitope mapped to NS3 amino acids 160-193), recognizes NS3 alone or the NS3-4A complex. **APPLICATION:** ICC, WB.

LIT: Subcellular localization, stability, and trans-cleavage competence of the hepatitis C virus NS3-NS4A complex expressed in tetracycline-regulated cell lines: B. Wolk, et al.; J. Virol. **74,** 2293 (2000) ▪ Cardif is an adaptor protein in the RIG-I antiviral pathway and is targeted by hepatitis C virus: E. Meylan, et al.; Nature **437,** 1167 (2005)

NEW MAb to NS5B (HCV) (5B-3B1)

ALX-803-060-R100 100 µl

CLONE: 5B-3B1. **ISOTYPE:** Mouse IgG2b. **IMMUNOGEN:** Recombinant HCV NS5B. **SPECIFICITY:** HCV NS5B (epitope mapped to NS5B amino acids 372-382). **APPLICATION:** WB.

LIT: Functional properties of a monoclonal antibody inhibiting the hepatitis C virus RNA-dependent RNA polymerase: D. Moradpour, et al.; J. Biol. Chem. **277,** 593 (2002)

NEW MAb to NS5B (HCV) (5B-1-2B7)

ALX-803-061-R100 100 µl

CLONE: 5B-12B7. **ISOTYPE:** Mouse IgG2a. **IMMUNOGEN:** Recombinant HCV NS5B. **SPECIFICITY:** HCV NS5B (epitope mapped to NS5B amino acids 139-392). **APPLICATION:** ICC, IP

LIT: See Prod. No ALX-803-060 (above).

Novel Function of RANKL – eNOS (NOS III) Activator

Receptor activator of nuclear factor (NF)-κB ligand (RANKL; OPGL; TRANCE; ODF; TNFSF11)) is emerging as an important regulator of vascular pathophysiology. J.-K. Min, et al. demonstrated a novel role of RANKL as a vascular permeability factor and a critical role of eNOS (NOS III) in RANKL-induced endothelial function. RANKL increased the vascular permeability and leukocyte infiltration *in vivo* and it increased endothelial permeability and reduced VE-cadherin-facilitated endothelial cell-cell junctions in a NO-dependent manner *in vitro*. RANKL led to the activation of Akt and eNOS (NOS III) and to nitric oxide (NO) production in endothelial cells (ECs). Therefore, RANKL promotes vascular permeability and angiogenesis by stimulating eNOS (NOS III) in a TRAF6-PI3K-Akt-dependent mechanism.

LIT: Receptor activator of nuclear factor (NF)-kappaB ligand (RANKL) increases vascular permeability: impaired permeability and angiogenesis in eNOS-deficient mice: J.K. Min, et al.; Blood **109,** 1495 (2007)

Protein

RANKL, Soluble (human) (rec.)

ALX-522-012-C010 10 µg

Produced in HEK 293 cells. The extracellular domain of human RANKL (aa 151-316) is fused at the N-terminus to a linker peptide (6aa) and a FLAG®-tag. **SPECIFICITY:** Binds to human and mouse RANK. **BIOLOGICAL ACTIVITY:** Supports the survival of dendritic cells and osteoclasts.

Many citations!

LIT: For a comprehensive bibliography please visit our website.

Antibodies

PAb to RANKL (human)

ALX-210-396-C100 100 µg

From rabbit. **IMMUNOGEN:** Recombinant human RANKL. **SPECIFICITY:** Recognizes human RANKL. Detects a band of ~35kDa band by Western blot. **APPLICATION:** ELISA, FC, WB.

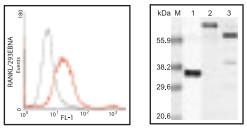


FIGURE: (left) Human RANKL transfected HEK 293 cells were stained significantly by anti-RANKL (human) PAb (Prod. No. ALX-210-396). (right) Western blot analysis of rhsRANKL with anti-RANKL (human) PAb (Prod. No. ALX-210-396) at 1:5'000 dilution. 1. rhRANKL pET fusion protein. 2. rhRANKL GST fusion protein. 3. Con-A activated human T lymphocytes lysate.

MAb to RANKL (12A668)

ALX-804-243-C100 100 µg

CLONE: 12A668. **ISOTYPE:** Mouse IgG. **IMMUNOGEN:** Recombinant mouse RANKL (aa 1-317). **SPECIFICITY:** Recognizes human and mouse RANKL. Detects a band of ~35-40kDa by Western blot. **APPLICATION:** IHC (PS), WB.

LIT: For a comprehensive bibliography please visit our website.

MAb to RANKL (12A380)

ALX-804-244-C100 100 µg

CLONE: 12A380. **ISOTYPE:** Mouse IgG1. **IMMUNOGEN:** Recombinant mouse RANKL (aa 1-317). **SPECIFICITY:** Recognizes human and mouse RANKL. Detects a band of ~40kDa by Western blot. **APPLICATION:** FC, IHC (FS), WB.

LIT: Functional expression of receptor activator of nuclear factor kappaB in Hodgkin disease cell lines: P. Fiumara, et al.; Blood **98,** 2784 (2001) ▪ Estrogen deficiency accelerates murine autoimmune arthritis associated with receptor activator of nuclear factor-kappa B ligand-mediated osteoclastogenesis: T. Yoneda, et al.; Endocrinology **145,** 2384 (2004)

NEW MAb to RANKL (human) (Ranky-1)

ALX-804-830-C100 100 µg

CLONE: Ranky-1. **ISOTYPE:** Rat IgG1. **IMMUNOGEN:** Recombinant human soluble RANKL (aa 151-316) (Prod. No. ALX-522-012). The extracellular domain of human RANKL (aa 151-316) is fused at the N-terminus to a linker peptide (6aa) and a FLAG®-tag. **SPECIFICITY:** Recognizes human RANKL. **APPLICATION:** ELISA (capture), WB.

NOSTRIN & NOSIP

Complex formation of nitric oxide synthase (NOS) with various partners regulates NOS activity and thus local NO• production. In unstimulated endothelial cells eNOS (NOS III) is bound to caveolin at the plasma membrane and thereby kept in its inactive state. Localization of eNOS in close proximity to upstream activators and downstream targets allows for efficient signal transduction. For instance, stimulation of cells with bradykinin activates the cognate receptor (B_2R) leading to Ca^{2+} transients and activation of eNOS via Ca^{2+}-calmodulin dependent displacement from caveolin. Similarly, stimulation of the NMDA receptor elicits Ca^{2+} influx in neuronal cells effecting Ca^{2+}-calmodulin mediated activation of neuronal NOS (nNOS; NOS I). The early Ca^{2+}-dependent activation of eNOS is followed by the late phosphorylation-dependent activation mediated by Akt (PKB), PKA and HSP90. HSP90 also binds to the ubiquitous NO• receptor, i.e. soluble guanylyl cyclase (sGC), thereby physically coupling NO• synthesis and cGMP production.

NOSIP and NOSTRIN are mediators of eNOS (NOS III) redistribution. NOSTRIN induces trafficking of eNOS away from the plasma membrane to intracellular vesicular structures, concomitant with a decrease in eNOS activity. Thereby, NOSTRIN interacts with the large GTPase dynamin and the Arp2/3 (actin-related protein 2/3 complex) activating protein N-WASP (neuronal Wiskott –Aldrich syndrome protein). NOSTRIN is enriched at caveolar membranes and forms a ternary complex of eNOS, caveolin-1 and NOSTRIN. It may be critical for caveolar transport of eNOS both in internalization and to the plasma membrane. Unlike NOSTRIN, the eNOS-interacting protein NOSIP competes with the caveolin scaffolding domain for binding of eNOS. Overexpression of NOSIP results in the dislocation of eNOS from the plasma membrane and inhibition of NO• release. NOSIP predominantly localize to the nucleus, but is enriched in the cytoplasm during G2 phase of the cell cycle. Cytoplasmic accumulation, corresponding to NOSIP overexpression, mediates cytoskeletal targeting and inhibition of eNOS. NOSIP also has inhibitory potential towards nNOS (NOS I).

Selected Articles

NOSIP, a novel modulator of endothelial nitric oxide synthase activity: J. Dedio, et al.; FASEB J. **15,** 79 (2001) ▪ NOSTRIN: a protein modulating nitric oxide release and subcellular distribution of endothelial nitric oxide synthase: K. Zimmermann, et al.; PNAS **99,** 17167 (2002) ▪ There's NO binding like NOS binding: protein-protein interactions in NO/cGMP signaling: P.I. Nedvetsky, et al.; PNAS **99,** 16510 (2002) ▪ Subcellular targeting and trafficking of nitric oxide synthases: S. Oess, et al.; Biochem. J. **396,** 401 (2006)

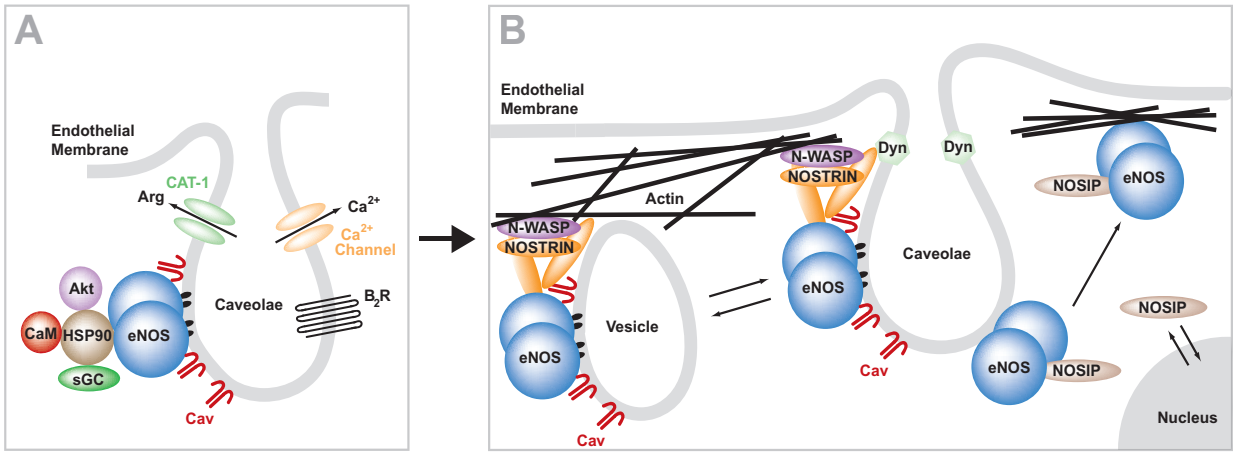


FIGURE: (A) Association of eNOS (NOS III) with the endothelial membrane. (B) Potential roles of the eNOS-associated proteins NOSIP and NOSTRIN in the redistribution of eNOS from the plasma membrane. NOSIP may target eNOS from its caveolar localization towards the actin cytoskeleton. In contrast, NOSTRIN may have a role in vesicular internalization of eNOS, probably involving a caveolar mechanism. Akt (protein kinase B); B_2R (bradykinin B_2 receptor); CaM (Ca^{2+}/calmodulin); CAT (cationic amino acid transporter); Cav (caveolin-1/-3); Dyn (dynamin); NST (NOSTRIN); N-WASP (neuronal Wiskott-Aldrich syndrome protein). *Adapted from: Subcellular targeting and trafficking of nitric oxide synthases: S. Oess, et al.; Biochem. J.* ***396,*** *401 (2006)*

Product Highlight

MAb to NOSTRIN [eNOS (NOS III) Trafficking Inducer] (human) (NG6)

ALX-804-646-R100 100 µl

CLONE: NG6. **ISOTYPE:** Mouse IgG1. **IMMUNOGEN:** Human recombinant NOSTRIN (eNOS (NOS III) trafficking inducer) (aa 242-506). **SPECIFICITY:** Recognizes human NOSTRIN. **APPLICATION:** ICC, IP, WB.

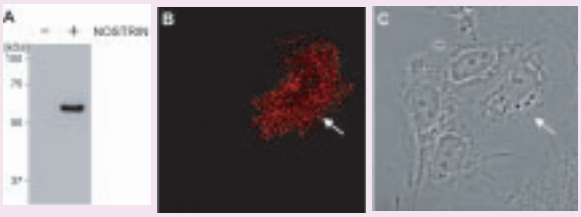


FIGURE: MAb to NOSTRIN in Western Blot and immunofluorescence. (A) Western blot of whole cell lysates from CHO cells (-) and CHO cells exogenously expressing human NOSTRIN (+). (B) Immunofluorescence labeling for NOSTRIN in CHO cells exogenously expressing human NOSTRIN (arrow). Untransfected cells do not show any background staining. (C) Phase contrast image.

Related Products

PAb to NOSIP [eNOS (NOS III) Interacting Protein]

ALX-210-869-R200 200 µl

From rabbit. **IMMUNOGEN:** Mouse recombinant NOSIP (eNOS (NOS III) interacting protein) (aa 1-301). **SPECIFICITY:** Recognizes human, mouse and hamster NOSIP. **APPLICATION:** ICC, IP, WB.

LIT: NOSIP, a novel modulator of endothelial nitric oxide synthase activity: J. Dedio, et al.; FASEB J. **15,** 79 (2001) ▪ Distribution of the novel eNOS-interacting protein NOSIP in the liver, pancreas, and gastrointestinal tract of the rat: P. Konig, et al.; Gastroenterology **123,** 314 (2002)

PAb to NOSTRIN [eNOS (NOS III) Trafficking Inducer] (human)

ALX-210-871-R200 200 µl

From rabbit. **IMMUNOGEN:** Human recombinant NOSTRIN (eNOS (NOS III) trafficking inducer) (aa 242-506). **SPECIFICITY:** Recognizes human NOSTRIN. **APPLICATION:** ICC, IP, WB.

LIT: NOSTRIN: a protein modulating nitric oxide release and subcellular distribution of endothelial nitric oxide synthase: K. Zimmermann, et al.; PNAS **99,** 17167 (2002)

Recombinant eNOS (NOS III)

eNOS [NOS III] (human) (rec.)

ALX-201-070-R100 100 µl

Produced in Sf9 cells. **MW:** ~130kDa/subunit; homodimer. **PURITY:** 50,000 x g supernatant. **SPECIFIC ACTIVITY:** ≥0.1nmol/mg/min at pH 7.0, 37°C, 1mM L-arginine, 1mM NADPH, 10µM (6R)-tetrahydrobiopterin, 5µM FAD, 5µM FMN, 50nM calmodulin, 1mM $CaCl_2$ and 7mM GSH.

LIT: Endothelial nitric-oxide synthase (type III) is activated and becomes calcium independent upon phosphorylation by cyclic nucleotide-dependent protein kinases: E. Butt, et al.; J. Biol. Chem. **275,** 5179 (2000)

eNOS [NOS III] (bovine) (rec.)

ALX-201-127-U010 10 U

Produced in Sf9 cells. **MW:** ~135kDa/subunit; homodimer. **PURITY:** 100,000 x g supernatant. **SPECIFIC ACTIVITY:** ≥1 U/ mg protein. One unit is defined as the amount of enzyme that produces 1nmol nitric oxide per min. at 37°C in 50mM HEPES, pH 7.4, containing 5µM oxyhemoglobin, 1mM $CaCl_2$, 20µg/ml calmodulin, 0.1mM NADPH, 50µM arginine, 12µM tetrahydrobio-pterin and 170µM DTT.

Bradykinin & Bradykinin Receptors

Kinins such as bradykinin, kallidin (lysyl-bradykinin), and desArg^{10}-kallidin are proinflammatory peptides that mediate numerous vascular responses to tissue injury. In humans kinins activate two types of kinin receptors, i.e. the inducible B_1 receptor (desArg^{10}-kallidin) and the constitutively expressed kinin B_2 receptor (bradykinin, kallidin). Both B_1R and B_2R are G protein-coupled receptors that signal primarily, though not exclusively, through Gαq and the phospholipase C/IP_3/Ca^{2+} pathway. Biological effects evoked by the kinins are vasodilation, increase in vascular permeability and activation of sensory neurons, mediated by their major downstream effectors such as prostaglandins, leukotrienes and nitric oxide.

Bradykinin
[H-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH]

ALX-152-006-M005 5 mg
ALX-152-006-M025 25 mg

NEW MAb to Bradykinin (MBK3)
ALX-804-647-R100 100 µl

CLONE: MBK3. **ISOTYPE:** Mouse IgG1. **IMMUNOGEN:** Synthetic full-length Bradykinin (R^{1}PPGF-SPFR^9). **SPECIFICITY:** Recognizes human, mouse, rat and other mammalian species expressing the canonical bradykinin sequence. **APPLICATION:** WB.

LIT: Anti-idiotypic antibodies bearing the internal image of a bradykinin epitope: production, characterization, and interaction with the kinin receptor; M. Haasemann, et al.; J. Immunol. **147,** 3882 (1991)

NEW Bradykinin Receptor B_1 (human) (MRP*grade*™)
BXT-C103220-4 4x50 Units

BHK or CHO cells. **QUANTITY:** 4x50Units. One unit is defined as the amount of protein sufficient to obtain at least 2000 specific dpms for a concentration of radioligand close to 10-fold the theoretical Kd.

NEW Bradykinin Receptor B_2 (human) (MRP*grade*™)
BXT-C103320-4 4x50 Units

Adherent CHO cells. **QUANTITY:** 4x50Units. One unit is defined as the amount of protein sufficient to obtain at least 2000 specific dpms for a concentration of radioligand close to 10-fold the theoretical Kd.

PAb to Bradykinin B_2 Receptor [B_2R]
ALX-210-872-R200 200 µl

From rabbit. **IMMUNOGEN:** Synthetic peptide corresponding to aa 356-391 of human bradykinin B_2 receptor (B_2R). **SPECIFICITY:** Recognizes human, mouse and rat B_2R. **APPLICATION:** IP, WB.

LIT: Ligand-induced phosphorylation/dephosphorylation of the endogenous bradykinin B2 receptor from human fibroblasts: A. Blaukat, et al.; J. Biol. Chem. **271,** 32366 (1996) ▪ Overexpression and functional characterization of kinin receptors reveal subtype-specific phosphorylation: A. Blaukat, et al.; Biochemistry **38,** 1300 (1999) ▪ Bradykinin-induced internalization of the human B2 receptor requires phosphorylation of three serine and two threonine residues at its carboxyl tail: A. Pizard, et al.; J. Biol. Chem. **274,** 13738 (1999) ▪ Determination of bradykinin B2 receptor in vivo phosphorylation sites and their role in receptor function: A. Blaukat, et al.; J. Biol. Chem. **276,** 40431 (2001) ▪ Functional specialization of calreticulin domains: K. Nakamura, et al.; J. Cell Biol. **154,** 961 (2001)

More Information? Please visit

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Antibodies to Caveolins & Related Products

Caveolae are specialized domains of the plasma membrane that are implicated in the sequestration of a variety of lipid and protein molecules. It has been suggested that these important cellular organelles have a pivotal role in such diverse biochemical processes as lipid metabolism, growth regulation, signal transduction, and apoptosis.

PAb to Caveolin-1
ALX-210-347-C100 100 µg

From rabbit. **IMMUNOGEN:** Synthetic peptide corresponding to aa 1-17 of human caveolin-1. **SPECIFICITY:** Recognizes human, mouse, rat, dog and hamster caveolin-1. **APPLICATION:** IP, WB. **BP:** Prod. No. ALX-153-054.

PAb to Caveolin-2 (rat)
ALX-210-240-C100 100 µg

From rabbit. **IMMUNOGEN:** Synthetic peptide corresponding to aa 1-19 of human caveolin-2. This sequence is completely conserved in mouse and rat caveolin-1. **SPECIFICITY:** Recognizes rat caveolin-2. Does not react with caveolin-1 or -3. **APPLICATION:** IP, WB. **BP:** Prod. No. ALX-153-029.

PAb to Caveolin-2 (mouse) (phosphorylated) (pTyr^{19})
ALX-210-888-C100 100 µg

From rabbit. **IMMUNOGEN:** Synthetic peptide corresponding to aa 14-25 of mouse caveolin-2 phosphorylated at Tyr^{19}. **SPECIFICITY:** Recognizes mouse phosphorylated caveolin-2. **APPLICATION:** ICC, WB. **BP:** Prod. No. ALX-153-060.

PAb to Caveolin-2 (human)
PTS-22-3460-C050 50 µg

From rabbit. **IMMUNOGEN:** Synthetic peptide corresponding to aa 18-31 of human caveolin-2. **SPECIFICITY:** Recognizes phosphorylated and non-phosphorylated human caveolin-2. **APPLICATION:** WB.

LIT: The phosphorylation of caveolin-2 on serines 23 and 36 modulates caveolin-1-dependent caveolae formation: G. Sowa, et al.; PNAS **100,** 6511 (2003)

PAb to Caveolin-2 (human) (phosphorylated) (pSer^{23})
PTS-22-3461-C050 50 µg

From rabbit. **IMMUNOGEN:** Synthetic peptide corresponding to aa 18-31 of human caveolin-2 phosphorylated at Ser^{23}. **SPECIFICITY:** Recognizes human caveolin-2 phosphorylated at Ser^{23}. Does not cross-react with non-phosphorylated human caveolin-2. **APPLICATION:** WB.

LIT: The phosphorylation of caveolin-2 on serines 23 and 36 modulates caveolin-1-dependent caveolae formation: G. Sowa, et al.; PNAS **100,** 6511 (2003)

PAb to Caveolin-2 (human) (phosphorylated) (pSer^{36})
PTS-22-3462-C050 50 µg

From rabbit. **IMMUNOGEN:** Synthetic peptide corresponding to aa 32-45 of human caveolin-2 phosphorylated at Ser^{36}. **SPECIFICITY:** Recognizes human caveolin phosphorylated at Ser^{36}. Does not cross-react with non-phosphorylated caveolin-2. **APPLICATION:** WB.

LIT: The phosphorylation of caveolin-2 on serines 23 and 36 modulates caveolin-1-dependent caveolae formation: G. Sowa, et al.; PNAS **100,** 6511 (2003)

PAb to Caveolin-3 (rat)
ALX-210-241-C100 100 µg

From rabbit. **IMMUNOGEN:** Synthetic peptide corresponding to aa 1-18 of human caveolin-3. This sequence is completely conserved in mouse caveolin-3. **SPECIFICITY:** Recognizes rat caveolin-3. Does not react with caveolin-1 or -2. **APPLICATION:** IP, WB. **BP:** Prod. No. ALX-153-030.

NEW Caveolin-1 Scaffolding Domain Peptide [Cavtratin]
[H-Arg-Gln-Ile-Lys-Ile-Trp-Phe-Gln-Asn-Arg-Arg-Met-Lys-Trp-Lys-Lys-Asp-Gly-Ile-Trp-Lys-Ala-Ser-Phe-Thr-Thr-Phe-Thr-Val-Thr-Lys-Tyr-Trp-Phe-Tyr-Arg-OH]

ALX-153-064-M001 1 mg
ALX-153-064-M005 5 mg

Reported to block eNOS (NOS III) activity and cellular nitric oxide (NO) release *in vitro* and reduce inflammation and tumorigenesis *in vivo*. Caveolin-1 interacts with several lipid-modified signalling ligands, such as EGFR, eNOS, G-protein α-subunits, PKCα, H-Ras, and Src, via the C1-SD82-101 sequence.

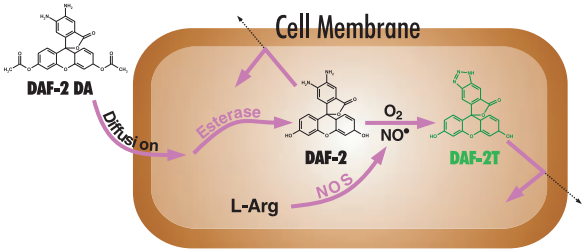
LIT: Caveolins, a family of scaffolding proteins for organizing „preassembled signaling complexes" at the plasma membrane: T. Okamoto, et al.; J. Biol. Chem. **273,** 5419 (1998) ▪ In vivo delivery of the caveolin-1 scaffolding domain inhibits nitric oxide synthesis and reduces inflammation: M. Bucci, et al.; Nat. Med. **6,** 1362 (2000) ▪ Selective inhibition of tumor microvascular permeability by cavtratin blocks tumor progression in mice: J.P. Gratton, et al.; Cancer Cell **4,** 31 (2003) ▪ Caveolin-1 gene disruption promotes mammary tumorigenesis and dramatically enhances lung metastasis in vivo. Role of Cav-1 in cell invasiveness and matrix metalloproteinase (MMP-2/9) secretion: T.M. Williams, et al.; J. Biol. Chem. **279,** 51630 (2004) ▪ Dissecting the molecular control of endothelial NO synthase by caveolin-1 using cell-permeable peptides: P.N. Bernatchez, et al.; PNAS **102,** 761 (2005)

Nitric Oxide Detection Kits & Reagents

Fluorescent Probes for the Detection of Nitric Oxide (NO) in Cells

DAF-2/DAF-2 DA

Nitric oxide (NO) is involved in various physiological and pathological processes in the cell. However, the extremely short half-life of $NO^{\bullet}$ limits the study of its physiological effects *in vivo*. The fluorescent probe, 4,5-diaminofluorescein (DAF-2), has been described for use in real-time imaging of NO. The cell permeable diacetate derivative, DAF-2 DA, is used to load cells. Subsequent hydrolysis by cytosolic esterases releases DAF-2, which is relatively non-fluorescent at physiological pH. However, in the presence of $NO^{\bullet}$ and $O_2^{\bullet -}$, DAF-2 is converted to the fluorescent triazole derivative, DAF-2T (Ex. 492nm; Em. 513nm), thereby increasing the quantum yield of fluorescence >180-fold. The excitation wavelength (visible region) is less damaging to cells than other probes.



The flow cytometry application of DAF-2 DA has been described in J. Navarro-Antolin, et al.; FASEB J. **15,** 1291 (2001).

LIT: Direct evidence of nitric oxide production from bovine aortic endothelial cells using new fluorescence indicators: diaminofluoresceins: N. Nakatsubo, et al.; FEBS Lett. **427,** 263 (1998) ▪ Direct evidence of NO production in rat hippocampus and cortex using a new fluorescent indicator: DAF-2 DA: H. Kojima, et al.; Neuroreport **9,** 3345 (1998) ▪ Development of a fluorescent indicator for nitric oxide based on the fluorescein chromophore: H. Kojima, et al.; Chem. Pharm. Bull. **46,** 373 (1998) ▪ Detection and imaging of nitric oxide with novel fluorescent indicators: diaminofluoresceins: H. Kojima, et al.; Anal. Chem. **70,** 2446 (1998) ▪ Hypotonic stress-induced NO production in endothelium depends on endogenous ATP: C. Kimura, et al.; BBRC **274,** 736 (2000) ▪ Anti-NO action of carvedilol in cell-free system and in vascular endothelial cells: T. Yoshioka, et al.; Br. J. Pharmacol. **129,** 1530 (2000) ▪ Formation of peroxynitrite in vascular endothelial cells exposed to cyclosporine A: J. Navarro-Antolin, et al.; FASEB J. **15,** 1291 (2001) ▪ Impairment of endothelial NO production by acute glucose overload: C. Kimura, et al.; Am. J. Physiol. Endocrinol. Metab. **280,** E171 (2001) ▪ Photoactivation and calcium sensitivity of the fluorescent NO indicator 4,5-diaminofluorescein (DAF-2): implications for cellular NO imaging: M. Broillet, et al.; FEBS Lett. **491,** 227 (2001)

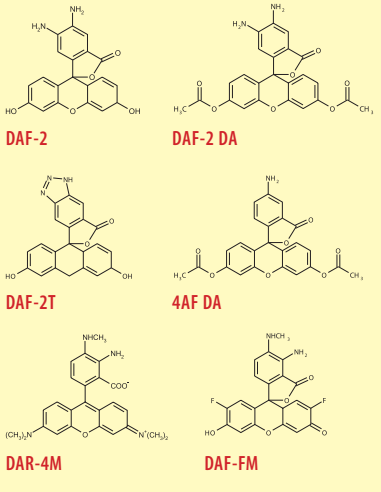
DAF-2 Compounds

DAF-2
[4,5-Diaminofluorescein]
ALX-620-052-M001 1 mg

DAF-2 DA (cell permeable)
[4,5-Diaminofluorescein diacetate]
ALX-620-056-M001 1 mg

4AF DA
[4-Aminofluorescein diacetate]
ALX-620-054-M001 1 mg
Negative control for Prod. No. ALX-620-052 & ALX-620-056.

DAF-2T
[Diaminofluorescein-2T]
ALX-620-060-C100 100 µg
Positive control for Prod. No. ALX-620-052 & ALX-620-056.



Probe	Fluorescent Triazole Derivative	Ex. Max	Em. Max	pH Range
DAF-2	DAF-2 T	492	513	7-12
DAF-FM	DAF-FM T	495	515	5.5-12
DAR-4M	DAR-4 T	554	572	4-12

More Fluorescent Probes

ALEXIS® Biochemicals introduces more fluorescent probes for the detection of nitric oxide (NO). Now you can choose the probe that is best suited to your particular experimental conditions.

DAF-FM Compounds

LIT: Fluorescent indicators for nitric oxide: H. Kojima & T. Nagano; Adv. Mater. **12,** 763 (2000) ▪ Determination and bioimaging method for nitric oxide in biological specimens by diaminofluorescein fluorometry: Y. Ito, et al.; Anal. Biochem. **287,** 203 (2000) ▪ Bioimaging of nitric oxide with fluorescent indicators based on the rhodamine chromophore: H. Kojima, et al.; Anal. Chem. **73,** 1967 (2001) ▪ Visualization of oxygen concentration-dependent production of nitric oxide in rat hippocampal slices during aglycemia: H. Kojima, et al.; J. Neurochem. **76,** 1404 (2001)

DAF-FM
[Diaminofluorescein-FM]
ALX-620-070-M001 1 mg

DAF-FM DA (cell permeable)
[Diaminofluorescein-FM diacetate]
ALX-620-071-M001 1 mg

Features of DAF-FM Compounds:
- **Excitation range ~495nm**
- **Measurement at 515nm**
- **Stable in a pH range of 5.5-12**
- **High yield (quantum efficiency)**
- **Detection limit 3nM**

DAR Compounds

LIT: Fluorescent indicators for nitric oxide: H. Kojima & T. Nagano; Adv. Mater. **12,** 763 (2000) ▪ Bioimaging of nitric oxide with fluorescent indicators based on the rhodamine chromophore: H. Kojima, et al.; Anal. Chem. **73,** 1967 (2001)

DAR-4M
[Diaminorhodamine-4M]
ALX-620-067-M001 1 mg

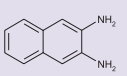
DAR-4M AM (cell permeable)
[Diaminorhodamine-4M AM]
ALX-620-069-M001 1 mg

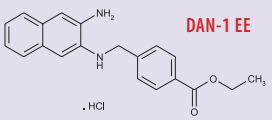
Features of DAR Compounds:
- **Excitation range ~554nm**
- **Measurement at 575nm**
- **Stable in a pH range of 4-12**
- **Low decrease in fluorescence intensity over time**

DAN-related Probes for the Detection of Nitric Oxide (NO)

2,3-Diaminonaphthalene
ALX-610-001-M100 100 mg
Inhibitor of nitric oxide (NO) formation. The relatively nonfluorescent DAN reacts rapidly with the NO-derived N-nitrosating agent(s) to yield the highly fluorescent product 2,3-naphthotriazole (NAT).

LIT: A fluorometric assay for the measurement of nitrite in biological samples: T.P. Misko, et al.; Anal. Biochem. **214,** 11 (1993) ▪ Improved nitric oxide detection using 2,3-diaminonaphthalene and its application to the evaluation of novel nitric oxide synthase inhibitors: N. Nakatsubo, et al.; Biol. Pharm. Bull. **21,** 1247 (1998)





DAN-1 EE . HCl
ALX-610-023-M005 5 mg
Fluorescent indicator for the bioimaging of nitric oxide (NO). Upon entry into the cell, DAN-1 EE is transformed by cytosolic esterases into the less cell permeable DAN-1. Intracellular formation of NO can be monitored (Ex(max): 360-380nm; Em(max): 420-450nm).

LIT: Development of a fluorescent indicator for the bioimaging of nitric oxide: H. Kojima, et al.; Biol. Pharm. Bull. **20,** 1229 (1997)

Nitric Oxide Detection Kits

New and Improved Assay Kits for Nitric Oxide Research

The amount of nitric oxide (NO) produced in different biological systems can vary over several orders of magnitude. ALEXIS® Biochemicals provides two types of kit for the measurement of total nitrate/nitrite concentrations. The well established and recently improved Colorimetric Assay Kit accurately measures combined nitrate/nitrite down to about 1.0µM. A new version of this kit uses the LDH method to destroy excess NADPH and is well suited to measurement of NOS activity. Previously undetectable concentrations of nitrate/nitrite can be accurately measured using the Fluorometric Assay Kit as it extends the lower limit of detection 50 fold.

NOS*detect*™ Assay Kit (Stratagene)

ALX-850-006-KI01 1 Kit

APPLICATION: For the measurement of nitric oxide synthase (NOS).

Measuring nitric oxide synthase (NOS) activity by monitoring the conversion of L-arginine to L-citrulline is currently the standard assay for NOS activity in both crude and purified enzyme preparations. Advantages of the NOS*detect*™ Assay Kit (Stratagene) include the use of radioactive substrates ([^{3}H]-arginine or [^{14}C]arginine) that enable sensitivity to the picomole level, as well as the specificity of the assay for the NOS pathway due to the direct enzymatic conversion of L-arginine to L-citrulline in eukaryotic cells. Additionally, the easy separation of neutrally charged L-citrulline from positively charged L-arginine allows hundreds of assays to be performed in less than an hour.

For routine assays, radioactive L-arginine is added to intact tissues or protein extracts. After incubation, the reactions are stopped with a buffer containing ethylenediaminetetraacetic acid (EDTA), which chelates the calcium required by NOS and, consequently, inactivates the NOS. The sample reactions are then applied to spin cups containing equilibrated resin to which the L-arginine binds. The radioactive L-citrulline, being ionically neutral at pH 5.5, flows through the column completely. The NOS activity is then quantitated by counting the radioactivity in the eluate.

- **The assay is extremely sensitive (pM levels)**
- **Rapid results (1 hour)**
- **Easy to perform (simple to follow protocol)**
- **Measures enzyme activity directly**
- **Useful for the measurement of inducible NOS (NOS II) and other isoforms**
- **Detailed Technical Manual is included in each shipment**

Nitrate/Nitrite Colorimetric Assay Kit

ALX-850-001-KI01 1 Kit

QUANTITY: 2x96 wells (approx. 160 tests). **APPLICATION:** For the measurement of total nitric oxide (NO) production. Validated in urine, culture media and plasma. The kit includes sufficient reagents for assay of total NO (nitrate + nitrite) in 2x96 wells, together with a third 96 well plate for measurement of nitrite only.

- **Easy-to-use, simple 2-step process**
- **2x96 well format for flexibility and value-for-money**
- **Accurate & convenient method for measurement of total nitrate/nitrite in plasma, urine and culture media**

Nitrate/Nitrite Colorimetric Assay Kit (LDH Method)

ALX-850-022-KI01 1 Kit

QUANTITY: 96 wells (approx. 80 tests). **APPLICATION:** For the measurement of total nitric oxide (NO) production. Use of LDH to destroy excess NADPH (an essential cofactor of NOS) makes this kit well suited to measurement of NOS activity, particularly in high-throughput screening applications using recombinant NOS. Validated in urine, culture media and plasma. The kit includes all reagents and full instructions.

- **Allows use of excess NADPH**
- **Well suited for measurement of NOS activity**
- **High throughput screening applications using recombinant NOS can be quickly and accurately assayed**

For further information, please request a copy of the manual.

Nitrate/Nitrite Fluorometric Assay Kit

ALX-850-012-KI01 1 Kit

QUANTITY: 2x96 wells (approx. 160 tests). **APPLICATION:** For the measurement of total nitric oxide (NO) production. Validated in culture media and plasma. Includes sufficient reagents for assay of total NO (nitrate + nitrite) in 2x96 wells, together with a third 96 well plate for measurement of nitrite only.

- **Lower limit of detection ~0.02µM**

FOR A REVIEW SEE: Improved methods to measure end products of nitric oxide in biological fluids: nitrite, nitrate, and S-nitrosothiols: M. Marzinzig, et al.; Nitric Oxide **1,** 177 (1997)

Nitroso-thiol (RSNOs) Detection Kit (OXONO•N)

ALX-850-037-KI01 1 Kit

QUANTITY: 96 wells (approx. 80 tests). The assay of nitrosothiols in many biological fluids appears to provide a very useful index of nitric oxide synthase (NOS) activity. The concentration of RSNOs has been shown to increase with localized induction of NOS and to fall rapidly upon administration of NOS inhibitors. The kit provides a complete solution for the assay of high or low MW RSNOs. The assay is divided essentially into two steps. In the first step, the sample is prepared according to which subgroup of RSNO is desired. The S-N bond is then cleaved, resulting in the formation of an equivalent of nitrite, which is assayed using the Griess reaction. The intensity of the color formed is linearly related to the concentration of nitrite, and hence, to the original RSNO concentration.

Detection of S-nitrosothiols:

SELECTED REVIEW ARTICLES: Detection of S-nitrosothiols by fluorometric and colorimetric methods: D.A. Wink, et al.; Meth. Enzymol. **301,** 201 (1999) ▪ Fluorometric detection of S-nitrosothiols: P. Kostka and J.K. Park; Meth. Enzymol. **301,** 227 (1999) ▪ Mechanisms of biological S-nitrosation and its measurement: T. Akaike; Free Radic. Res. **33,** 461 (2000)

Detection of Nitrite and Nitric Oxide (NO)

Griess Reagent

ALX-400-004-L050 50 ml

Reagent for the determination of nitrite (NO_2^-) in biological media. Consists of 1 part 0.1% naphthylethylenediamine dihydrochloride in distilled water plus 1 part 1% sulfanilamide (or sulfanilic acid) in 5% concentrated H_3PO_4.

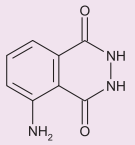
LIT: Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids: L.C. Green, et al.; Anal. Biochem. **126,** 131 (1982) ▪ Anticoagulants and other preanalytical factors interfere in plasma nitrate/nitrite quantification by the Griess method: D. Ricart-Jane, et al.; Nitric Oxide **6,** 178 (2002)

Luminol

ALX-610-002-G001 1 g
ALX-610-002-G005 5 g

Key reagent for generating luminescence. A reagent for the highly sensitive and selective detection of nitric oxide (NO) in biological samples by a luminol-H_2O_2 chemiluminescence method.

LIT: The chemiluminescence of luminol and related hydrazides: D.F. Roswell & E.H. Shite; Meth. Enzymol. **57,** 409 (1978) ▪ New method of detecting nitric oxide production: K. Kikuchi, et al.; Chem. Pharm. Bull. **40,** 2233 (1992) ▪ Detection of nitric oxide production from a perfused organ by a luminol- H2O2 system: K. Kikuchi, et al.; Anal. Chem. **65,** 1794 (1993) ▪ Real time measurement of nitric oxide produced ex vivo by luminol-H2O2 chemiluminescence method: K. Kikuchi, et al.; J. Biol. Chem. **268,** 23106 (1993)



Luminol

From the Source!

Step up to the Source™

ALEXIS® BIOCHEMICALS

Peroxynitrite

Peroxynitrite . tetramethylammonium

ALX-400-036-L001 1 ml
ALX-400-036-5001 5x1 ml

Produced from the reaction of nitrogen monoxide with tetramethylammonium superoxide according to the method of D.S. Bohle, et al. described in Meth. Enzymol. **269,** 302 (1996), and dissolved in 0.01M sodium hydroxide (NaOH).

- **Low nitrite content (~1%)**
- **No hydrogen peroxide**
- **Includes application manual**

SELECTED LITERATURE REFERENCE: Preventing nitrite contamination in tetramethylammonium peroxynitrite solutions: P. Latal, et al.; Inorg. Chem. **43,** 6519 (2004) ▪ Reactive oxygen/nitrogen species at the fulcrum of life-death decisions: a commentary on„peroxynitrite transforms nerve growth factor into an apoptotic factor for motor neurons": C.T. Chu & D.C. Hooper; Free Radic. Biol. Med. **41,** 1629 (2006) ▪ Nitric oxide and peroxynitrite in health and disease: P. Pacher, et al.; Physiol. Rev. **87,** 315 (2007)

Soluble Guanylyl Cyclase [sGC]

Introduction

Guanylyl cyclases constitute a family of enzymes [EC 4.6.1.2] catalyzing the conversion of guanosine triphosphate (GTP) to cyclic guanosine monophosphate (cGMP). cGMP is a second messenger which alters the conductance of cGMP-gated ion channels, stimulates the activity of members of the protein kinase G family, and changes the activity of cGMP-regulated phosphodiesterases. By regulating cGMP levels guanylyl cyclases are important modulators of physiological processes such as neurotransmission and smooth muscle relaxation. The guanylyl cyclase family comprises both membrane-bound and soluble isoforms that are expressed in nearly all cell types. While the membranous forms bind natriuretic peptides (GC-A and GC-B) or heat-stable enterotoxins (GC-D), soluble guanylyl cyclase (sGC) is activated by endogenous nitric oxide (NO).

sGC is a heterodimeric hemoprotein composed of an α (large) and a β (small) subunit. Two isoforms for each subunit currently exist and are termed α_1, α_2 and β_1, β_2; each transcribed by a separate gene [1-3]. Although original reports described α_3 and β_3 subunits, these are generally accepted as the human forms of rat α_1 and β_1 based on sequence homology [4]. A variant of the α_2 subunit (α_{2i}) has been identified which contains 31 additional amino acids within the catalytic domain and functions as a dominant negative protein [5]. For the formation of a catalytically active enzyme one α and one β subunit is required. While the $\alpha_1\alpha_1$ isoform is ubiquitous, the $\alpha_2\beta_1$ isoform is less broadly distributed. Evidence suggests that the β_2 subunit of sGC can form homodimers [6], although it has not been purified to date.

sGC subunits are divided in four distinct domains based on homology searches. The i) C-terminal cyclase catalytic domain is next to ii) a putative amphipathic helix, iii) a predicted Per/Arnt/Sim (PAS)-like domain, and iv) a heme-binding domain localized to the N-terminus of the β_1 subunit where the heme cofactor is ligated by histidine 105 [7, 8]. The heme binding domain is part of a conserved family of proteins in prokaryotes and non-eukaryotes called H-NOX (heme-nitric oxide and/or oxygen binding domain) [9]. Members of the H-NOX family are thought to use a homologous protein fold and identical heme cofactor to bind NO and/or O_2, where discrimination is based on the presence or absence of an hydrogen-bond donor in the distal heme pocket crucial for O_2 binding [10]. Activation of sGC has been thought to occur with a conformational change induced by NO binding to heme and the severing of the proximal histidine bond. Recently, activation and regulation has been shown to be more complex, relying on binding of non-heme NO and the presence of ATP and GTP [11-13].

LIT: [1] Guanylyl cyclases, a growing family of signal-transducing enzymes: D. Koesling, et al.; FASEB J. **5,** 2785 (1991) ▪ **[2]** Sequence homologies between guanylyl cyclases and structuralanalogies to other signal-transducing proteins: D. Koesling, et al.; FEBS Lett. **280,** 301 (1991) ▪ **[3]** Soluble guanylyl cyclase: structure and regulation: D. Koesling & A. Friebe; Rev. Physiol. Biochem. Pharmacol. **135,** 41 (1999) ▪ **[4]** Human soluble guanylate cyclase: functional expression and revised isoenzyme family: U. Zabel, et al.; Biochem. J. **335 (Pt 1),** 51 (1998) ▪ **[5]** A variant of the alpha 2 subunit of soluble guanylyl cyclase contains an insert homologous to a region within adenylyl cyclases and functions as a dominant negative protein: S. Behrends, et al.; J. Biol. Chem. **270,** 21109 (1995) ▪ **[6]** Nitric oxide activates the beta 2 subunit of soluble guanylyl cyclase in the absence of a second subunit: M. Koglin, et al.; J. Biol. Chem. **276,** 30737 (2001) ▪ **[7]** Spectroscopic characterization of the soluble guanylate cyclase-like heme domains from Vibrio cholerae and Thermoanaerobacter tengcongensis: D. S. Karow, et al.; Biochemistry **43,** 10203 (2004) ▪ **[8]** Ancient conserved domains shared by animal soluble guanylyl cyclases and bacterial signaling proteins: L. M. Iyer, et al.; BMC Genomics **4,** 5 (2003) ▪ **[9]** Characterization of functional heme domains from soluble guanylate cyclase: D. S. Karow, et al.; Biochemistry **44,** 16266 (2005) ▪ **[10]** A molecular basis for NO selectivity in soluble guanylate cyclase: E. M. Boon, et al.; Nat. Chem. Biol. **1,** 53 (2005) ▪ **[11]** Tonic and acute nitric oxide signaling through soluble guanylate cyclase is mediated by nonheme nitric oxide, ATP, and GTP: S. P. Cary, et al.; PNAS **102,** 13064 (2005) ▪ **[12]** Guanylyl cyclase is an ATP sensor coupling nitric oxide signaling to cell metabolism: I. Ruiz-Stewart, et al.; PNAS **101,** 37 (2004) ▪ **[13]** NO activation of guanylyl cyclase: M. Russwurm & D. Koesling; EMBO J. **23,** 4443 (2004)

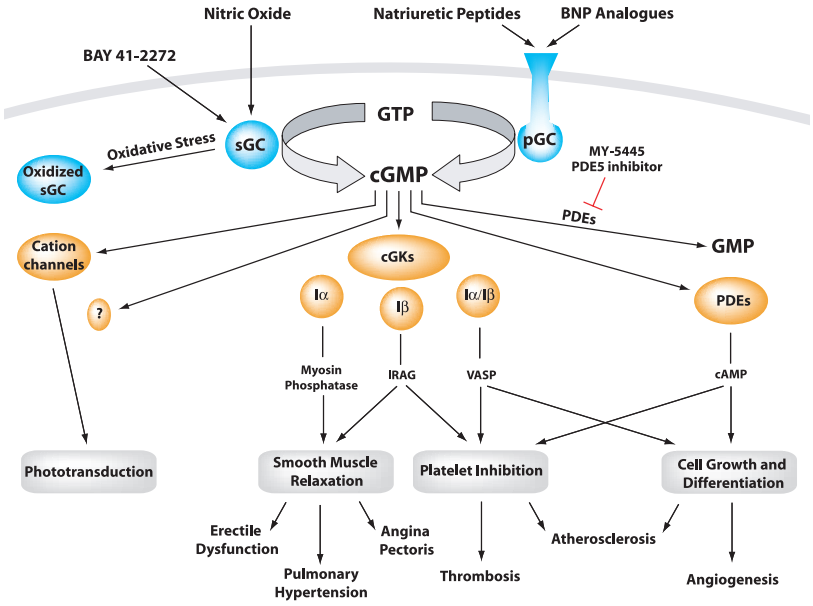


FIGURE: cGMP Signalling Concept: Adapted from: *cGMP signalling: from bench to bedside. Conference on cGMP generators, effectors and therapeutic implications:* R. Feil & B. Kemp-Harper; EMBO Rep. **7,** 149 (2006).

Subunit	MW (kDa)	Human Chromosome Location	Tissue	Function
α_1 (α_3)	77.5	4q31.3-q33	Ubiquitous	+
α_2	79.0	11q21-q22	Brain, Retina, Kidney, Placenta, Pancreas, Spleen, Uterus	+
α_{2i}	81.7	Splice Variant of α_2	Liver, Colon, Endothelium, Leukemia Cells	--
β_1 (β_3)	70.5	4q31.3-q33	Ubiquitous	+
β_2	70.3	13q14.3	Kidney, Liver	?

Selected Review Articles

Negative feedback in NO/cGMP signalling: D. Koesling, et al.; Biochem. Soc. Trans. **33,** 1119 (2005) ▪ Ligand specificity of H-NOX domains: from sGC to bacterial NO sensors: E. M. Boon & M. A. Marletta; J. Inorg. Biochem. **99,** 892 (2005) ▪ Soluble guanylyl cyclase: more secrets revealed: A. Pyriochou & A. Papapetropoulos; Cell. Signal. **17,** 407 (2005) ▪ Guanylyl cyclases across the tree of life: P. Schaap; Front. Biosci. **10,** 1485 (2005) ▪ Nitric oxide signaling: no longer simply on or off: S. P. Cary, et al.; TIBS **31,** 231 (2006) ▪ NO-cGMP signaling in development and stem cells: J.S. Krumenacker & F. Murad; Mol. Genet. Metab. **87,** 311 (2006) ▪ Purification and characterization of NO-sensitive guanylyl cyclase: M. Russwurm & D. Koesling; Methods Enzymol. **396,** 492 (2005)

Core Products

Proteins

Guanylyl Cyclase, Soluble (bovine)

ALX-202-039-C005 5 µg

SPECIFIC ACTIVITY: >10,000nmol cGMP/min/mg at 37°C under NO-stimulated conditions.

LIT: Purification of soluble guanylyl cyclase from bovine lung by a new immunoaffinity chromatographic method: P. Humbert, et al.; Eur. J. Biochem. **190,** 273 (1990)

Guanylyl Cyclase ($\alpha_1\beta_1$), Soluble (human) (rec.) (purified)

[sGCα_1/sGCβ_1 (human) (rec.) (purified)]

ALX-201-177-C010 10 µg

Human recombinant soluble guanylyl cyclase $\alpha_1\beta_1$ (sGC$\alpha_1\beta_1$) produced in Sf9 cells. **MW:** 70kDa/86kDa subunit, respectively (heterodimer). **SPECIFIC ACTIVITY:** Basal ≥0.1µmole/mg/min; 2µM BAY 41-2272 (Prod. No. ALX-420-030) ≥1.0µmole/mg/min; 100µM sodium nitroprusside (Prod. No. ALX-400-001) ≥5.0µmole/mg/min. **APPLICATION:** Suitable for biophysical and enzymatic studies and drug screening. **PURITY:** ≥90% (SDS-PAGE).

LIT: Human recombinant soluble guanylyl cyclase: expression, purification, and regulation: Y.C. Lee, et al.; PNAS **97,** 10763 (2000)

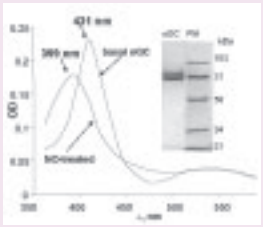


FIGURE: Spectral characteristics of basal and NO-treated purified human sGC$\alpha_1\beta_1$ (3µM). Inset – Coomassie staining of 3µg of purified sGC$\alpha_1\beta_1$. PM-protein marker.

Guanylyl Cyclase ($\alpha_1\beta_1$), Soluble (human) (rec.) (enriched)

[sGCα_1/sGCβ_1 (human) (rec.) (enriched)]

ALX-201-180-R050 50 µl

Human recombinant soluble guanylyl cyclase $\alpha_1\beta_1$ (sGC$\alpha_1\beta_1$) enriched from high-speed supernatant of Sf9 cells expressing human sGC. **SPECIFIC ACTIVITY:** Basal ≥0.5nmole/mg/min; 2µM BAY 41-2272 (Prod. No. ALX-420-030) ≥5.5nmole/mg/min; 100µM sodium nitroprusside (Prod. No. ALX-400-001) ≥150nmole/mg/min. **APPLICATION:** Suitable for enzymatic studies and drug screening.

LIT: Human recombinant soluble guanylyl cyclase: expression, purification, and regulation: Y.C. Lee, et al.; PNAS **97,** 10763 (2000)

More Information? Please visit

www.axxora.com

Antibodies

MAb to Guanylyl Cyclase (α_1), Soluble (human) (10G11)

ALX-804-648-R200 200 µl

CLONE: 10G11. **ISOTYPE:** Mouse IgG1. **IMMUNOGEN:** Recombinant human soluble guanylyl cyclase α_1 subunit (sGCα_1) (aa 1-419). **SPECIFICITY:** Recognizes human sGCα_1. **APPLICATION:** IHC, ICC, IP, WB.

PAb to Guanylyl Cyclase (α_1), Soluble (human)

ALX-210-868-R200 200 µl

From rabbit. **IMMUNOGEN:** Regulatory domain (aa 1-419) of human soluble guanylyl cyclase α_1 subunit (sGCα_1). **SPECIFICITY:** Recognizes human sGCα_1. **APPLICATION:** IP, WB. Not recommended for IHC or FUNC.

MAb to Guanylyl Cyclase (β_1), Soluble (5A5)

ALX-804-649-R200 200 µl

CLONE: 5A5. **ISOTYPE:** Mouse IgG2. **IMMUNOGEN:** Recombinant human soluble guanylyl cyclase β_1 subunit (sGCβ_1) (aa 1-348). **SPECIFICITY:** Recognizes human, mouse and rat sGCβ_1 N-terminal epitope (aa 1-60). **APPLICATION:** IHC, ICC, IP, WB.

PAb to Guanylyl Cyclase (β_1), Soluble (human)

ALX-210-870-R200 200 µl

From rabbit. **IMMUNOGEN:** Catalytic domain (aa 404-619) of human soluble guanylyl cyclase β_1 subunit (sGCβ_1). **SPECIFICITY:** Recognizes human sGCβ_1. **APPLICATION:** IP, WB. Not recommended for IHC or FUNC.

PAb to Guanylyl Cyclase (β_1), Soluble

ALX-210-867-1 1 Vial

From rabbit. **IMMUNOGEN:** Synthetic peptide corresponding to aa 188-207 of rat soluble guanylyl cyclase β_1 subunit (sGCβ_1). **SPECIFICITY:** Recognizes human, rat and bovine sGCβ_1. Does not cross-react with sGCα_1. **APPLICATION:** WB. **BLOCKING PEPTIDE:** ALX-157-023.

PAb to Guanylyl Cyclase ($\alpha_1\beta_1$), Soluble

ALX-210-786-R100 100 µl

From chicken. **IMMUNOGEN:** Purified rat lung soluble guanylyl cylase $\alpha_1\beta_1$ subunits (sGC$\alpha_1\beta_1$) (native protein). **SPECIFICITY:** Recognizes endogenous rat sGC$\alpha_1\beta_1$. Recognizes human and mouse sGCβ_1. Does not cross-react with human and mouse sGCα_1. **APPLICATION:** WB.

LIT: Soluble guanylate cyclase purified from bovine lung contains heme and copper: R. Gerzer, et al.; FEBS Lett. **132,** 71 (1981) ▪ For a comprehensive bibliography please visit our website.

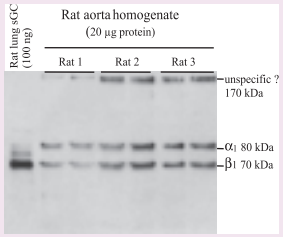


FIGURE: Detection of endogenous rat sGC$\alpha_1\beta_1$ using PAb to Guanylyl Cyclase ($\alpha_1\beta_1$), Soluble (Prod. No. ALX-210-786).

Lane 1: Purified sGC from rat lung (with sGCα_1 largely degraded) served as a positive control. Lane 2-7: Protein from rat aorta.

MAbs to Guanylyl Cyclase-Activating Proteins [GCAPs]

MAb to Guanylyl Cyclase-activating Protein 1 [GCAP-1] (G2)

ALX-804-540-C100 100 µg

CLONE: G2. **ISOTYPE:** Mouse IgG2a. **IMMUNOGEN:** Bacterial expressed full length guanylyl cyclase-activating protein 1 (GCAP-1). **SPECIFICITY:** Recognizes human, non-human primate and bovine GCAP-1. Does not cross-react with other isotypes. **APPLICATION:** WB.

MAb to Guanylyl Cyclase-activating Protein 2 [GCAP-2] (bovine) (A1)

ALX-804-541-C100 100 µg

CLONE: A1. **ISOTYPE:** Mouse IgG2a. **IMMUNOGEN:** Bacterial expressed full length guanylyl cyclase-activating protein 2 (GCAP-2). **SPECIFICITY:** Recognizes bovine GCAP-2. Does not cross-react with other isotypes. **APPLICATION:** WB.

Technical Note

Nitric Oxide Activation of Guanylyl Cyclase in Cells Revisited

Nitric oxide (NO•) elicits physiological effects in cells largely by activating guanylyl cyclase (GC)-coupled receptors, leading to cGMP accumulation. Like other receptor-coupled effector mechanisms, NO• stimulation of GC activity was previously considered to be a graded, concentration-dependent response, with deactivation following swiftly once the agonist disappeared. A new and unconventional mechanism has been proposed from experiments on purified protein by S. P. L. Cary, et al. (2005). It was concluded that GC *in vivo* will display a dual regulation by NO•: a long-lasting tonic activity due to persistent occupation by NO• of the heme binding site and phasic activity due to engagement of another unidentified, lower affinity site. However, recent findings by B. Roy & J. Garthwaite show that the new scheme for regulation of GC activity by NO• is of doubtful relevance to cells.

LIT: Tonic and acute nitric oxide signaling through soluble guanylate cyclase is mediated by nonheme nitric oxide, ATP, and GTP: S.P. Cary, et al.; PNAS **102,** 13064 (2005) ▪ Nitric oxide activation of guanylyl cyclase in cells revisited: B. Roy & J. Garthwaite; PNAS **103,** 12185 (2006)

Latest Insight

Therapeutic Potency of sGC Stimulators/Activators

The NO-sGC-cGMP pathway is crucial for the control of many physiological processes such as vascular homeostasis. Dysfunction of this signalling pathway may occur when the redox state of sGC changes and the enzyme becomes unresponsive to endogenous NO, or by a reduced availability of NO. This seems to be the case during pathophysiological conditions associated with oxidative stress. With the availability of haem-dependent sGC stimulators (including YC-1 (Prod. No. ALX-420-005) and BAY 41-2272 (Prod. No. ALX-420-030) and haem-independent sGC activators, first studies have been undertaken to evaluate the therapeutic potency of these direct sGC activators/stimulators.

LIT: NO-independent stimulators and activators of soluble guanylate cyclase: discovery and therapeutic potential: O. V. Evgenov, et al.; Nat. Rev. Drug Discov. **5,** 755 (2006) ▪ Soluble guanylate cyclase stimulation on cardiovascular remodeling in angiotensin II-induced hypertensive rats: H. Masuyama, et al.; Hypertension **48,** 972 (2006) ▪ Targeting Heme-Oxidized Soluble Guanylate Cyclase in Experimental Heart Failure: G. Boerrigter, et al.; Hypertension (in print), (2007) ▪ Targeting Heme-Oxidized Soluble Guanylate Cyclase. Solution for All Cardiorenal Problems in Heart Failure?: T. Munzel, et al.; Hypertension (in print), (2007)

Products

Activators

BAY 41-2272

ALX-420-030-M005 5 mg
ALX-420-030-M025 25 mg

DD1

ALX-430-086-M010 10 mg
ALX-430-086-M050 50 mg

DD2

ALX-430-087-M010 10 mg
ALX-430-087-M050 50 mg

Guanylin (human)

H-Pro-Gly-Thr-Cys-Glu-Ile-Cys-Ala-Tyr-Ala-Ala-Cys-Thr-Gly-Cys-OH (Disulfide bonds between Cys^4-Cys^{12} and Cys^7-Cys^{15}

ALX-157-010-MC01 0.1 mg
ALX-157-010-M001 1 mg

Endogenous intestinal guanylyl cyclase activator.

LIT: Human guanylin: cDNA isolation, structure, and activity: R.C. Wiegand, et al.; FEBS Lett. **311,** 150 (1992) ▪ Precursor structure, expression, and tissue distribution of human guanylin: F.J. de Sauvage, et al.; PNAS **89,** 9089 (1992) ▪ Guanylin: an endogenous activator of intestinal guanylate cyclase: M.G. Currie, et al.; PNAS **89,** 947 (1992)

Guanylin (rat, mouse)

H-Pro-Asn-Thr-Cys-Glu-Ile-Cys-Ala-Tyr-Ala-Ala-Cys-Thr-Gly-Cys-OH (Disulfide bonds between Cys^4-Cys^{12} and Cys^7-Cys^{15})

ALX-157-011-MC01 0.1 mg
ALX-157-011-M001 1 mg

Endogenous intestinal guanylyl cyclase activator.

LIT: Guanylin: an endogenous activator of intestinal guanylate cyclase: M.G. Currie, et al.; PNAS **89,** 947 (1992) ▪ Rat guanylin cDNA characterization of the precursor of an endogenous activator of intestinal guanylate cyclase: R.C. Wiegand, et al.; BBRC **185,** 812 (1992) ▪ Cloning and expression of guanylin. Its existence in various mammalian tissues: S. Schulz, et al.; J. Biol. Chem. **267,** 16019 (1992)

Protoporphyrin IX (free acid)

ALX-430-041-M300 300 mg
ALX-430-041-G001 1 g

Protoporphyrin IX free acid, a distinct from its zinc salt (which inhibits heme oxygenase), activates soluble guanylyl cyclase (sGC) by binding directly to the enzyme. A useful reagent in cases where the use of nitric oxide (NO) or nitric oxide donors is undesirable.

LIT: Guanylate cyclase from bovine lung. A kinetic analysis of the regulation of the purified soluble enzyme by protoporphyrin IX, heme, and nitrosyl-heme: M.S. Wolin, et al.; J. Biol. Chem. **257,** 13312 (1982)

YC-1

ALX-420-025-M001 1 mg
ALX-420-025-M005 5 mg
ALX-420-025-M025 25 mg

Nitric oxide (NO) independent, superoxide-sensitive stimulator of soluble guanylyl cyclase (sGC). Inhibits platelet adhesion to collagen. Non-specific phosphodiesterase inhibitor. YC-1 inhibits HIF-α activity in tumors resulting in blocked angiogenesis and an inhibition of tumor growth [1].

LIT: YC-1, a novel activator of platelet guanylate cyclase: F.N. Ko, et al.; Blood **84,** 4226 (1994) ▪ YC-1 inhibited human platelet aggregation through NO-independent activation of soluble guanylate cyclase: C.C. Wu, et al.; Br. J. Pharmacol **116,** 1973 (1995) ▪ Activation of soluble guanylyl cyclase by YC-1 in aortic smooth muscle but not in ventricular myocardium from rat: J.W. Wegener, et al.; Br. J. Pharmacol. **122,** 1523 (1997)

Selected Review Article

NO-independent stimulators and activators of soluble guanylate cyclase: discovery and therapeutic potential: O.V. Evgenov, et al.; Nat. Rev. Drug Discov. **5,** 755 (2006)

Inhibitors

Carnosine

ALX-153-055-G001 1 g

Antioxidant. Inhibits soluble guanylyl cyclase (sGC) by interacting with the heme iron to form a chelate complex.

LIT: Carnosine as a regulator of soluble guanylate cyclase: I.S. Severina, et al.; Biochemistry (Mosc) **65,** 783 (2000)

Isatin

LKT-I7302-G100 100 g
LKT-I7302-G500 500 g

Endogenous monoamine oxidase inhibitor. Inhibitor of alkaline phosphatase and nitric oxide (NO)-stimulated soluble guanylyl cyclase (sGC).

LY-83,583

ALX-550-002-M005 5 mg
ALX-550-002-M025 25 mg
ALX-550-002-M100 100 mg

Competitive inhibitor of soluble guanylyl cyclase (sGC) (IC_{50}=2μM) and of cGMP production.

LIT: Pharmacologic analysis of two novel inhibitors of leukotriene (slow reacting substance) release: J.H. Fleisch, et al.; J. Pharmacol. Exp. Ther. **229,** 681 (1984) ▪ LY 83583 interferes with the release of endothelium-derived relaxing factor and inhibits soluble guanylate cyclase: A. Mülsch, et al.; J. Pharmacol. Exp. Ther. **247,** 283 (1988) ▪ A comparative study of the effects of three guanylyl cyclase inhibitors on the L-type Ca2+ and muscarinic K+ currents in frog cardiac myocytes: N. Abi-Gerges, et al.; Br. J. Pharmacol. **121,** 1369 (1997)

Methylene blue . trihydrate

ALX-610-003-G005 5 g

Inhibitor of soluble guanylyl cyclase (sGC). Biological staining agent. Spin-trap which forms stable adducts with oxygen free radicals in solution.

LIT: Novel actions of methylene blue: B. Mayer, et al.; Eur. Heart J. **14 Suppl I,** 22 (1993) ▪ Inhibition of nitric oxide synthesis by methylene blue: B. Mayer, et al.; Biochem. Pharmacol. **45,** 367 (1993) ▪ Methylene blue induces cytotoxicity in human brain tumor cells: Y.S. Lee & R.D. Wurster; Cancer Lett. **88,** 141 (1995) ▪ A comparative study of the effects of three guanylyl cyclase inhibitors on the L-type Ca2+ and muscarinic K+ currents in frog cardiac myocytes: N. Abi-Gerges, et al.; Br. J. Pharmacol. **121,** 1369 (1997)

NS-2028

ALX-270-210-M001 1 mg
ALX-270-210-M005 5 mg
ALX-270-210-M025 25 mg

Potent and selective inhibitor of nitric oxide (NO)-sensitive soluble guanylyl cyclase (sGC). Inhibits also the basal, the YC-1 and the coenhanced sGC activity.

LIT: Characterization of NS 2028 as a specific inhibitor of soluble guanylyl cyclase: S.P. Olesen, et al.; Br. J. Pharmacol. **123,** 299 (1998)

ODQ

ALX-270-034-M010 10 mg
ALX-270-034-M050 50 mg

Potent and selective inhibitor of nitric oxide (NO)-sensitive guanylyl cyclase.

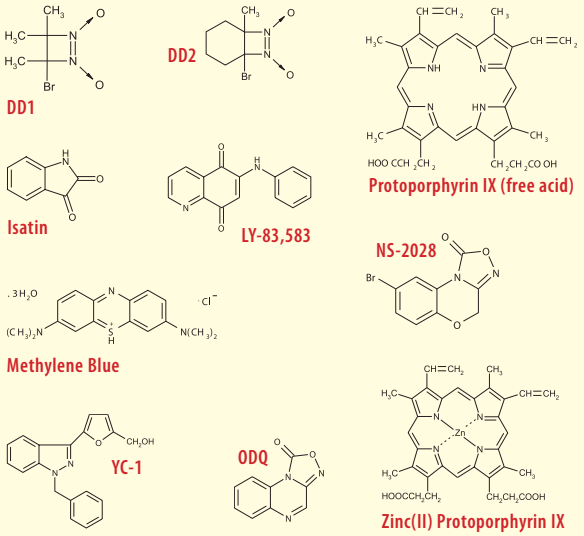
LIT: Potent and selective inhibition of nitric oxide-sensitive guanylyl cyclase by 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one: J. Garthwaite, et al.; Mol. Pharmacol. **48,** 184 (1995) ▪ Nitric oxide-dependent long-term potentiation is blocked by a specific inhibitor of soluble guanylyl cyclase: C.L. Boulton, et al.; Neuroscience **69,** 699 (1995) ▪ Novel guanylyl cyclase inhibitor, ODQ reveals role of nitric oxide, but not of cyclic GMP in endothelin-1 secretion: F. Brunner, et al.; FEBS Lett. **376,** 262 (1995)

Zinc(II) Protoporphyrin IX

ALX-430-049-M005 5 mg
ALX-430-049-M025 25 mg
ALX-430-049-M100 100 mg

Inhibits soluble guanylyl cyclase (sGC).

LIT: Effect of heme oxygenase inhibitors on soluble guanylyl cyclase activity: L. Serfass and J.N. Burstyn; Arch. Biochem. Biophys. **359,** 8 (1998)



Product Highlight

BAY 41-2272 NO-independent Activator of sGC

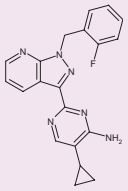
BAY 41-2272

[3-(4-Amino-5-cyclopropylpyrimidine-2-yl)-1-(2-fluorobenzyl)-1*H*-pyrazolo [3,4-b]pyridine]

ALX-420-030-M005 5 mg
ALX-420-030-M025 25 mg

The pyrazolopyridine compound BAY 41-2272 is an NO-independent activator of soluble guanylyl cyclase (sGC) [1,2]. BAY 41-2272 activates both isoforms $\alpha_1\beta_1$ and $\alpha_1\beta_2$ of sGC [3].

While the related compound YC-1 (Prod. No. ALX-420-025) also acts as a non-specific phosphodiesterase inhibitor, BAY 41-2272 is devoid of any effect on phosphodiesterases. Stimulation of sGC is not blocked by high concentrations of NO scavengers (e.g. PTIO, Prod. No. ALX-430-007) and the combination of BAY 41-2272 with the NO donor DEA NONOate (Prod. No. ALX-430-034) potentiates the activation of sGC. Although BAY 41-2272 alone is not as strong a stimulator of sGC as NO, concentrations as low as 10-100nM stimulate sGC to a level that would be expected to cause biologically significant increases in cGMP [1]. ODQ (Prod. No. ALX-270-034), a potent and selective inhibitor of sGC, completely inhibited the effect of BAY 41-2272 [1].



The activity profile of BAY 41-2272 is described in selected literature references [4-11].

LIT: [1] NO-independent regulatory site on soluble guanylate cyclase: J.P. Stasch, et al.; Nature **410,** 212 (2001) ▪ **[2]** NO-independent regulatory site of direct sGC stimulators like YC-1 and BAY 41-2272: E.M. Becker, et al.; BMC Pharmacol. **1,** 13 (2001) ▪ **[3]** BAY 41-2272 activates two isoforms of nitric oxide-sensitive guanylyl cyclase: M. Koglin, et al.; BBRC **292,** 1057 (2002) ▪ **[4]** Metabolites of orally active NO-independent pyrazolopyridine stimulators of soluble guanylate cyclase: A. Straub, et al.; Bioorg. Med. Chem. **10,** 1711 (2002) ▪ **[5]** Cardiorenal and humoral properties of a novel direct soluble guanylate cyclase stimulator BAY 41-2272 in experimental congestive heart failure: G. Boerrigter, et al.; Circulation **107,** 686 (2003) ▪ **[6]** BAY 41-2272: a stimulator of soluble guanylyl cyclase induces nitric oxide-dependent penile erection in vivo: E. Bischoff, et al.; Urology **61,** 464 (2003) ▪ **[7]** Cardiorenal and humoral properties of a novel direct soluble guanylate cyclase stimulator BAY 41-2272 in experimental congestive heart failure: G. Boerrigter, et al.; Circulation **107,** 686 (2003) ▪ **[8]** Macrophage endothelial nitric oxide synthase auto-regulates cellular activation and pro-inflammatory protein expression: L. Connelly, et al.; J. Biol. Chem. **278,** 26480 (2003) ▪ **[9]** Antiplatelet properties of a novel, non-NO-based soluble guanylate cyclase activator, BAY 41-2272: A.J. Hobbs & S. Moncada; Vascul. Pharmacol. **40,** 149 (2003) ▪ **[10]** Relaxing effects induced by the soluble guanylyl cyclase stimulator BAY 41-2272 in human and rabbit corpus cavernosum: J.S. Baracat, et al.; Eur. J. Pharmacol. **477,** 163 (2003) ▪ **[11]** Antiinflammatory activity of soluble guanylate cyclase: cGMP-dependent down-regulation of P-selectin expression and leukocyte recruitment: A. Ahluwalia, et al.; PNAS **101,** 1386 (2004)

Soluble Guanylyl Cyclase & Vasodilator Phosphoprotein [VASP]

Vasodilator-stimulated phosphoprotein (VASP) is one of the few established PKG substrates and has been characterized as an important substrate of both PKA and PKG in human platelets. VASP phosphorylation in response to cyclic nucleotide-regulating substances (e.g. soluble guanylyl cyclase (sGC) activators) correlates with inhibition of platelet aggregation and with the inhibition of fibrinogen binding to the integrin αIIβ3 of human platelets. Thus VASP phosphorylation closely correlates with sGC stimulation, platelet cGMP increase and inhibition of platelet aggregation.

MAb to VASP (IE273)

ALX-804-177-C050 50 µg

CLONE: IE273. **ISOTYPE:** Mouse IgG1. **IMMUNOGEN:** Purified human VASP (vasodilator stimulated phosphoprotein). **SPECIFICITY:** Recognizes both the 46kDa (Ser^{157} dephospho-) and 50kDa (Ser^{157} phospho-) form of human, rabbit, pig and bovine VASP. **APPLICATION:** ICC, IP, WB.

MAb to VASP (phosphorylated) (pSer^{157}) (5C6)

ALX-804-403-C100 100 µg

CLONE: 5C6. **ISOTYPE:** Mouse IgG1. **IMMUNOGEN:** Synthetic peptide corresponding to a portion of human VASP (vasodilator-stimulated phosphoprotein) phosphorylated at Ser^{157}. **SPECIFICITY:** Recognizes human and mouse Ser^{157}-phosphorylated VASP. Does not cross-react with the non-phosphorylated form of VASP. **APPLICATION:** ICC, IP, WB.

More Information? Please visit

www.axxora.com

MAb to VASP (phosphorylated) (pSer^{239}) (16C2)

ALX-804-240-C100 Purified 100 µg
ALX-804-240B-C100 Biotin 100 µg
ALX-804-240F-C100 FITC 100 µg

CLONE: 16C2. **ISOTYPE:** Mouse IgG1. **IMMUNOGEN:** Synthetic peptide corresponding to a portion of VASP (vasodilator stimulated phosphoprotein) phosphorylated at Ser^{239}. **SPECIFICITY:** Recognizes human, mouse, rat and rabbit Ser^{239}-phosphorylated VASP. Does not cross-react with the non-phosphorylated form of VASP. **APPLICATION:** FC, ICC, IP, WB. **FUNC:** Use to monitor PKG/PKA activity.

LIT: Analysis and Regulation of Vasodilator-stimulated Phosphoprotein Serine 239 Phosphorylation in Vitro and in Intact Cells Using a Phosphospecific Monoclonal Antibody: A. Smolenski, et al.; J. Biol. Chem. 273, 20029 (1998) ▪ Flow cytometry analysis of intracell. VASP phosphorylation for the assessment of activating and inhibitory signal transduct. pathways in human platelets-definition & detect. of ticlopidine/clopidogrel: U.R. Schwarz, et al.; Thromb. Haemost. 82, 1145 (1999) ▪ KT5823 inhibits cGMP-dependent protein kinase activity in vitro but not in intact human platelets and rat mesangial cells: M. Burkhardt, et al.; J. Biol. Chem. 275, 33536 (2000) ▪ Cyclic nucleotides modulate store-mediated calcium entry through the activation of protein-tyrosine phosphatases and altered actin polymerization in human platelets: J.A. Rosado, et al.; J. Biol. Chem. 276, 15666 (2001) ▪ Effects of In Vivo Nitroglycerin Treatment on Activity and Expression of the Guanylyl Cyclase and cGMP-Dependent Protein Kinase and Their Downstream Target Vasodilator-Simulated Phosphoprotein in Aorta: A. Mülsch, et al.; Circulation 103, 2188 (2001)

PAb to VASP (human)

ALX-210-898-R100 100 µl

From rabbit. **IMMUNOGEN:** Recombinant human VASP (vasodilator stimulated phosphoprotein) fused to a His-tag. **SPECIFICITY:** Recognizes human VASP. Does not cross-react with mouse or rat VASP. For the detection of mouse VASP use Prod. No. ALX-210-880. **APPLICATION:** ICC, WB.

PAb to VASP (affinity purified)

ALX-210-880-C025 25 µg

From rabbit. **SPECIFICITY:** Recognizes human, mouse and pig VASP. Detects both the 46kDa (Ser^{157} dephospho) and 50kDa (Ser^{157} phospho) forms of VASP. **APPLICATION:** ICC, IP, WB.

Latest Insight

Purine and Pyrimidine Nucleotides – Inhibitors of soluble Guanylyl Cyclase (sGC) & Adenylyl Cyclase

A. Gille, et al. showed that soluble guanylyl cyclase and adenylyl cyclase isoforms are differentially inhibited by purine and pyrimidine nucleotides.

LIT: Differential inhibition of adenylyl cyclase isoforms and soluble guanylyl cyclase by purine and pyrimidine nucleotides: A. Gille, et al.; J. Biol. Chem. **279,** 19955 (2004)

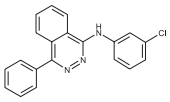
Product No	Prod. Name	Size	
BLG-M008-10	MANT-cAMP . Na	10 µmol	
BLG-M009-10	MANT-cGMP . Na	10 µmol	
BLG-M030-05	MANT-ATP . Na	5 µmol	
BLG-M032-05	MANT-GTP . Na	5 µmol	
BLG-M037-10	MANT-AMP . Na	10 µmol	
BLG-M040-05	MANT-ADP . Na	5 µmol	
BLG-M041-05	MANT-GDP . Na	5 µmol	
BLG-M042-10	MANT-GMP . Na	10 µmol	

Phosphodiesterase [PDE]

Phosphodiesterases (PDE) are enzymes that convert the intracellular second messengers cAMP and cGMP to the corresponding nucleotides AMP and GMP. PDEs have differing substrate specificities: PDE1, -2, -3, -10 and -11 act on both cAMP and cGMP, while PDE4, -7 and -8 are specific for cAMP, and PDE5, -6 and -9 are specific for cGMP.

Inhibitors of PDE5

MY-5445

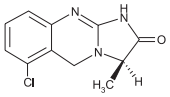


ALX-270-085-M001 1 mg
ALX-270-085-M005 5 mg

Selective inhibitor of phosphodiesterase 5 (PDE5).

LIT: Effect of 1-(3-chloroanilino)-4-phenylphthalazine (MY-5445), a specific inhibitor of cyclic GMP phosphodiesterase, on human platelet aggregation: M. Hagiwara, et al.; J. Pharmacol. Exp. Ther. **228,** 467 (1984)

Quazinone

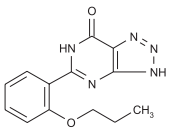


ALX-270-116-M005 5 mg
ALX-270-116-M025 25 mg

Selective inhibitor of cGMP-specific phosphodiesterase.

LIT: Studies on the mechanism of positive inotropic activity of Ro 13-6438, a structurally novel cardiotonic agent with vasodilating properties: M. Holck, et al.; J. Cardiovasc. Pharmacol. **6,** 520 (1984)

Zaprinast



ALX-430-020-M010 10 mg
ALX-430-020-M050 50 mg

Phosphodiesterase (PDE) inhibitor.

LIT: Primary sequence of cyclic nucleotide phosphodiesterase isozymes and the design of selective inhibitors: J.A. Beavo & D.H. Reifsnyder; TIPS **11,** 150 (1990)

PDE5 Protein

PDE5A (bovine) (recombinant)

ALX-201-257-1 1 Vial

Recombinant bovine PDE5A (phosphodiesterase 5A) expressed in Sf9 cells. **SPECIFIC ACTIVITY:** ~4µmol/min/mg. **QUANTITY:** sufficient for 500-1000 assays.

See our website for PDE5A activity measuring assay protocol.

Antibody to PDE5A

PAb to PDE5A

ALX-210-099-C050 50 µg

From rabbit. **IMMUNOGEN:** Synthetic peptide corresponding to aa 857-875 (CA^{857}EQQEKMLINGESGQAKRN^{875}) of human PDE5A (phosphodiesterase 5A). **SPECIFICITY:** Recognizes mouse and bovine PDE5A. **APPLICATION:** WB.

Nitric Oxide (NO) Signalling Pathways

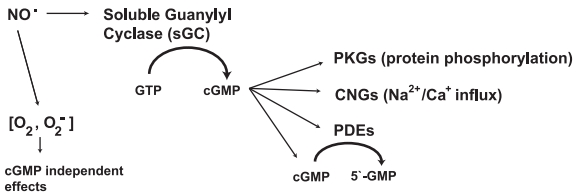
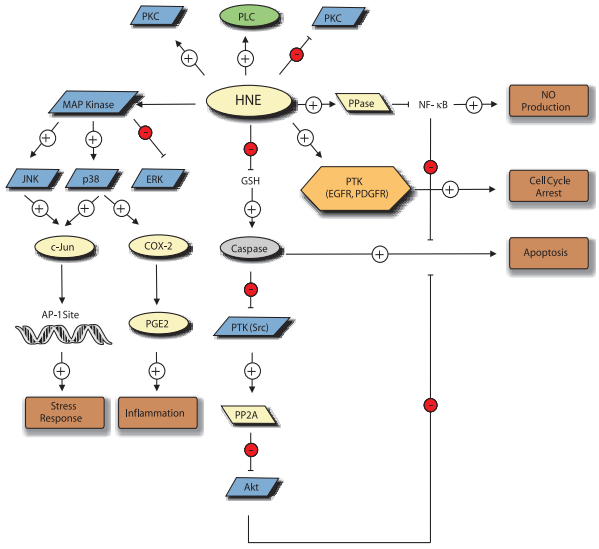


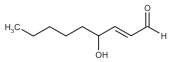
FIGURE: Signal Transduction pathways and effectors of nitric oxide.

NO, nitric oxide; O_2, oxygen; $O_2^{\bullet-}$, superoxide; GTP, guanosine triphosphate; cGMP, 3′, 5′-cyclic guanosine monophosphate; 5′-GMP, 5′-guanosine mono- phosphate; PKG, protein kinase G; CNG, cyclic nucleotide-gated channels; PDE, phosphodiesterases.

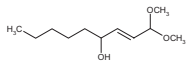
4-Hydroxynonenal [HNE] & Lipid Peroxidation

HNE – A Key Marker of Oxidative Stress-linked Pathological Events

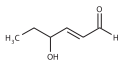




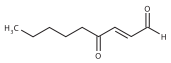
(E)-4-Hydroxynonenal [HNE]



(E)-4-Hydroxynonenal-dimethylacetal [HNE-DA]



(E)-4-Hydroxyhexenal



4-Oxo-2-nonenal [4-ONE]

Malondialdehyde [MDA]

In 1980 Prof. Esterbauer, et al. identified 4-hydroxynonenal (HNE) as a cytotoxic product originating from the peroxidation of liver microsomal lipids [1]. Since 1991, the year of publication of the seminal review of Prof. Esterbauer and colleagues [2], really a huge number of reports has been published which support a role for HNE in a variety of human disease processes. HNE started as a "toxic aldehyde product of membrane lipid peroxidation" and "toxic second messenger of free radicals". Today HNE is considered as a reliable marker of oxidative stress, a possible causative agent of several diseases (such as Alzheimer's disease), a growth modulating factor (inhibition), and a signalling molecule (e.g. inducer of apoptosis). Recent research revealed that HNE can be formed in soybean oil at frying temperature which might be important with regard to public health [3].

LIT: [1] Identification of 4-hydroxynonenal as a cytotoxic product originating from the peroxidation of liver microsomal lipids: A. Benedetti, et al.; Biochim. Biophys. Acta **620,** 281 (1980) ▪ **[2]** Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes: H. Esterbauer, et al.; Free Radic. Biol. Med. **11,** 81 (1991) ▪ **[3]** Formation of 4-hydroxynonenal, a toxic aldehyde, in soybean oil at frying temperature: C.M. Seppanen & A. Saari Csallany; J. Am. Oil Chem. Soc. **79,** 1033 (2002)

Selected Review Articles

See The Molecular Aspects of Medicine, 24 (Issue 4-5) (2003) dedicated to reviews on HNE presented at the HNE-Club Meeting 2003 ▪ Lipid peroxidation and cell cycle signaling: 4-hydroxynonenal, a key molecule in stress mediated signaling: Y. Yang, et al.; Acta Biochim. Pol. **50,** 319 (2003) ▪ Hydroxynonenal, toxic carbonyls, and Alzheimer disease: Q. Liu, et al.; Mol. Aspects Med. **24,** 305 (2003) ▪ Oxidative stress and cell signalling: G. Poli, et al.; Curr. Med. Chem. **11,** 1163 (2004) ▪ Mass spectrometry for detection of 4-hydroxy-trans-2-nonenal (HNE) adducts with peptides and proteins: M. Carini, et al.; Mass. Spectrom. Rev. **23,** 281 (2004) ▪ Signaling kinases modulated by 4-hydroxynonenal: G. Leonarduzzi, et al.; Free Radic. Biol. Med. **37,** 1694 (2004) ▪ Reactions of 4-hydroxynonenal with proteins and cellular targets: D.R. Petersen and J.A. Doorn; Free Radic. Biol. Med. **37,** 937 (2004) ▪ Potential markers of oxidative stress in stroke: A. Cherubini, et al.; Free Radic. Biol. Med. **39,** 841 (2005)

The Standard

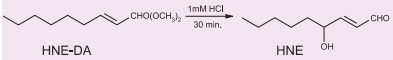
(E)-4-Hydroxynonenal [HNE]
ALX-270-245-M005 5 mg

NEW Stable Form of HNE!

(E)-4-Hydroxynonenal-dimethylacetal [HNE-DA]
ALX-270-375-1 1 Vial

- **For *in situ* production of active HNE**
- **Yields ~ 5.2mg aldehyde after hydrolysis**
- **Allows quantitative and reproducible experiments**

The Product Data Sheet includes detailed information for the *in situ* preparation of HNE before your experiment.



More Information? Please visit

www.axxora.com

Related Products

PAb to (E)-4-Hydroxynonenal [HNE]
ALX-210-767-R100 100 µl
From rabbit. IMMUNOGEN: Free (E)-4-hydroxynonenal (HNE). SPECIFICITY: Recognizes HNE-adducts. APPLICATION: ELISA, IHC, WB.
LIT: Immunochemical detection of 4-hydroxynonenal protein adducts in oxidized hepatocytes: K. Uchida, et al.; PNAS **90,** 8742 (1993) ▪ For a comprehensive bibliography please visit our website.

(E)-4-Hydroxyhexenal
ALX-270-405-M001 1 mg
ALX-270-405-M005 5 mg
ALX-270-405-M010 10 mg

4-Oxo-2-nonenal [4-ONE]
ALX-270-407-M001 1 mg
ALX-270-407-M005 5 mg

4-Hydroperoxy-2-nonenal
ALX-270-447-M001 1 mg
ALX-270-447-M005 5 mg

Malondialdehyde [MDA]
[1,1,3,3-Tetramethoxypropane]
ALX-280-018-L005 5 ml

PAb to Malondialdehyde [MDA]
ALX-210-879-R100 100 µl
From rabbit. IMMUNOGEN: Malondialdehyde (MDA) conjugated to a carrier protein. SPECIFICITY: Recognizes MDA adducts. APPLICATION: ELISA, IHC, WB.

Highlight

HNE-histidine FINE ELISA Kit

ALX-850-320-KI01 1 Kit

QUANTITY: 2 x 96 wells. For quantitative detection of HNE-histidine in human cell lysates. Indirect enzyme immunoassay (EIA) SENSITIVITY: 7,1pmol/mg to 143 pmol/mg

- **Quantitative determination of HNE-histidine in human cell lysates**
- **Indirect enzyme immunoassay (EIA)**
- **Based on a well described monoclonal antibody used in ICC, IHC, & WB**
- **Range of Detection between 7,1pmol/mg to 143 pmol/mg**
- **Human plasma spiking tested**
- **Tissue homogenates have not been tested yet**

LIT: Enzyme-linked immunosorbent assay for 4-hydroxynonenal-histidine conjugates: S. Borovic, et al.; Free Radic. Res. **40,** 809 (2006) ▪ Differential effect of 4-hydroxynonenal on normal and malignant mesenchimal cells: S. Borovic, et al.; Redox. Rep. **207,** 50 (2007)

(E)-4-hydroxy-nonenal (HNE) is one of the most important breakdown products and reliable maker of lipid peroxidation (LPO). This indirect enzyme immunoassay (EIA) allows for the first time the quantitative determination of HNE-histidine conjugates from lysates of human cells which were exposed to mild oxidative stress.

For quantitative determination of HNE-histidine conjugates exposed to strong oxidative stress please inquire for HNE-histidine STRESS ELISA Kit.

For more information visit the HNE-Club at www.kfunigraz.ac.at/hne-club/

Lipid Hydroperoxide [LPO] Assay Kits

Many investigators have tried to detect lipid hydroperoxides in biological samples to obtain direct evidence for free radical injury *in vivo* or to measure the degree of oxidative stress.

Traditionally, lipid peroxidation is quantified by measuring malondialdehyde (MDA) [1,2] and (E)-4-hydroxynonenal (HNE) (Prod. No. ALX-270-245, page 38) [1,3], the degradation products of polyunsaturated fatty acids hydroperoxides. Sensitive colorimetric assays have been developed to measure these aldehydes [1-3]. However, these assays are non-specific and often lead to an overestimation of lipid peroxidation.

There are important additional problems in using these byproducts as indicators of lipid peroxidation. The by-product formation is highly inefficient and varies according to the transition metal ion content of the sample. Only hydroperoxides derived from polyunsaturated fatty acids give rise to these byproducts. For example HNE is formed only from ω-6 polyunsaturated fatty acid hydroperoxides and is catalyzed by transition metal ions [4].

Decomposition of hydroperoxides derived from abundant cellular lipids such as cholesterol and oleic acid does not produce MDA or HNE. These factors can lead to an underestimation of lipid peroxidation.

MDA is also produced in ng/ml concentrations by the platelet enzyme thromboxane synthase during whole blood clotting and platelet activation [5]. This leads to gross overestimation of lipid peroxidation. Estimation of lipid hydroperoxide levels range from 0.3-30 µM depending on the method used. However, direct methods of estimation suggest that the concentration in normal human plasma is approximately 0.5 µM [6,7].

Given the limitations of the indirect methods, direct measurement of hydroperoxides is the obvious choice.

The Lipid Hydroperoxide Assay Kit measures the hydroperoxides directly utilizing the redox reactions with ferrous ions [8]. Hydroperoxides are highly unstable and react readily with ferrous ions to produce ferric ions. The resulting ferric ions are detected using thiocyanate ion as the chromogen:

$ROOH + Fe^{2+} \rightarrow RO^{\cdot} + Fe^{3+}$

$RO^{\cdot} + Fe^{2+} + H^{+} \rightarrow ROH + Fe^{3+}$

$Fe^{3+} + 5SCN^{-} \rightarrow Fe(SCN)^{52-}$

λmax: 500nm ε: 16,667$M^{-1}cm^{-1}$

Since this method relies on the measurement of ferric ions generated during the reaction, ferric ions present in the sample are a potential source of error. Also, many biological samples contain hydrogen peroxide which readily reacts with ferrous ions to give an over-estimation of lipid hydroperoxides. These problems are easily circumvented by performing the assay in chloroform.

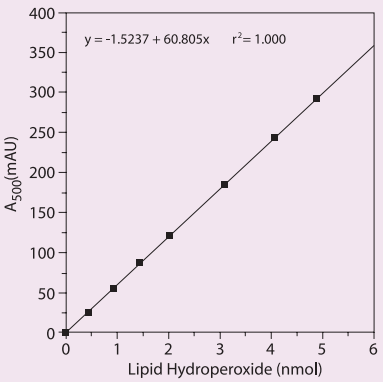
An easy to use, quantitative extraction method was developed to extract lipid hydroperoxides into chloroform and the extract is directly used in the assay. This procedure eliminates any interference caused by hydrogen peroxide or endogenous ferric ions in the sample and provides a sensitive and reliable assay for lipid peroxidation.

LIT: [1] Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes: H. Esterbauer, et al.; Free Radic. Biol. Med. **11,** 81 (1991) ▪ **[2]** Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury: D.R. Janero; Free Radic. Biol. Med. **9,** 515 (1990) ▪ **[3]** Quantitation of 4-hydroxynonenal protein adducts: K. Uchida & E.R. Stadtman; Meth. Enzymol. **233,** 371 (1994) ▪ **[4]** Suggested mechanisms for the production of 4-hydroxy-2-nonenal from the autoxidation of polyunsaturated fatty acids: W.A. Pryor & N.A. Porter; Free Radic. Biol. Med. **8,** 541 (1990) ▪ **[5]** Conversion of prostaglandin endoperoxides to C17-hydroxy acids catalyzed by human platelet thromboxane synthase: U. Diczfalusy, et al.; FEBS Lett. **84,** 271 (1977) ▪ **[6]** Pathophysiologic modulation of arachidonate metabolism: M.A. Warso & W.E. Lands; Clin. Physiol. Biochem. **2,** 70 (1984) ▪ **[7]** Detection and characterization of lipid hydroperoxides at picomole levels by high-performance liquid chromatography: Y. Yamamoto, et al.; Anal. Biochem. **160,** 7 (1987) ▪ **[8]** The reevaluation of the ferric thiocyanate assay for lipid hydroperoxides with special considerations of the mechanistic aspects of the response: B. Mihaljevic, et al.; Free Radic. Biol. Med. **21,** 53 (1996)

Lipid Hydroperoxide (LPO) Assay Kit (100 determinations)

ALX-850-026-KI01 100 det. 1 Kit

Any sample containing lipid hydroperoxide can be easily and reliably quantified spectrophotometrically using either glass or quarz 1ml cuvettes (λmax: 500nm; ε: 16,667$M^{-1}cm^{-1}$). **SENSITIVITY:** 0.25 - 5 nmol hydroperoxide per assay tube.



Lipid Hydroperoxide (LPO) Assay Kit (96 wells)

ALX-850-203-KI01 96 wells 1 Kit

Same Kit as Prod. No. ALX-850-026-KI01 but contains additionally a reusable glass 96 well plate (for use of organic solvents).

Lipid Peroxidation

Lipid peroxidation has been associated with important pathophysiological events in a variety of diseases, drug toxicities, and traumatic or ischemic injuries. Lipid peroxidation results in the formation of highly reactive and unstable hydroperoxides of both saturated and unsaturated lipids. Hydroperoxides are the primary products of lipid peroxidation.

Inhibitors

Carazostatin, *Streptomyces chromofuscus*
ALX-350-253-C100 100 µg
ALX-350-253-M001 1 mg

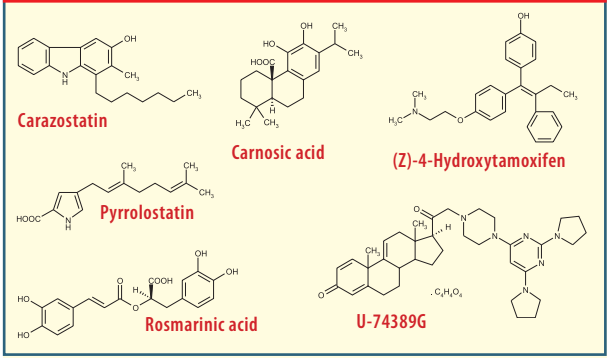
Carnosic acid
ALX-270-264-M010 10 mg
ALX-270-264-M050 50 mg

(Z)-4-Hydroxytamoxifen
ALX-550-361-M001 1 mg
ALX-550-361-M005 5 mg

Pyrrolostatin, *Spreptomyces chrestomyceticus*
ALX-350-252-C100 100 µg
ALX-350-252-M001 1 mg

Rosmarinic acid
ALX-270-253-M010 10 mg
ALX-270-253-M050 50 mg

U-74389G
ALX-270-265-M010 10 mg
ALX-270-265-M050 50 mg



Selected Review Articles on Lipid Peroxidation

Lipid peroxidation-induced membrane structural alterations: G. van Ginkel & A. Sevanian; Meth. Enzymol. **233,** 273 (1994) ▪ The isoprostanes: unique bioactive products of lipid peroxidation: J.D. Morrow & L.J. Roberts; Prog. Lipid Res. **36,** 1 (1997) ▪ The isoprostanes: unique bioactive products of lipid peroxidation. An overview: T.Z. Liu, et al.; J. Biomed. Sci. **5,** 415 (1998) ▪ Measurement of lipid peroxidation: K. Moore & L.J. Roberts 2nd.; Free Radic. Res. **28,** 659 (1998) ▪ Nitric oxide and lipid peroxidation: N. Hogg & B. Kalyanaraman; Biochim. Biophys. Acta **1411,** 378 (1999) ▪ The isoprostanes: unique prostaglandin-like products of free-radical-initiated lipid peroxidation: J.D. Morrow, et al.; Drug Metab. Rev. **31,** 117 (1999) ▪ Lipid peroxidation in diabetes mellitus: G. Davi, et al.; Antioxid. Redox. Signal. **7,** 256 (2005) ▪ Lipid peroxidation and renal cell carcinoma: further supportive evidence and new mechanistic insights: M. Gago-Dominguez and J.E. Castelao; Free Radic. Biol. Med. **40,** 721 (2006) ▪ Lipid peroxidation in aging brain and Alzheimer's disease: T.J. Montine, et al.; Free Radic. Biol. Med. **33,** 620 (2002) ▪ Lipid peroxidation: mechanisms, inhibition, and biological effects: E. Niki, et al.; BBRC **338,** 668 (2005)

Antioxidant Assay Kit

NEW Antioxidant Assay Kit
ALX-850-318-KI01 1 Kit

This Antioxidant Assay is designed to measure the overall antioxidant capacity within a given sample. The assay relies on the ability of antioxidants in the sample to inhibit the oxidation of ABTS in comparision to Trolox, a water-soluble tocopherol analog. By quantifying the cumulative effect of all antioxidants present, more relevant biological information is acquired compared with the measurement of individual components alone. The 96-well plate format can be used for the rapid measurement of antioxidant capacity in a variety of sample types, including plasma, serum, urine, saliva, and cell lysates.

High Purity & Stable Spin Traps

- **Highest purity spin traps for *in vitro* and *in vivo* applications.**
- **No additional purification is required.**
- **All products are quality tested by ESR spectroscopy.**
- **Bulk production & custom synthesis are our speciality.**

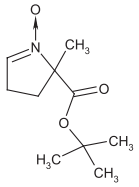
NEW BMPO (high purity)

[5-*tert*-Butoxycarbonyl-5-methyl-1-pyrroline-N-oxide (high purity)]

ALX-430-141-M010 10 mg
ALX-430-141-M050 50 mg

Nitrone spin trap for the specific *in vivo* or *in vitro* detection of short-lived superoxide, hydroxyl and thiyl radicals. Forms distinguishable adducts which can be measured by EPR spectroscopy. Unlike with DMPO (Prod. No. ALX-430-090), the superoxide adduct does not decay into a hydroxyl adduct and it has a much longer half-life ($t_{1/2}$=23min). Low paramagnetic impurities. **No further purification required.**

LIT: Synthesis and biochemical applications of a solid cyclic nitrone spin trap: a relatively superior trap for detecting superoxide anions and glutathiyl radicals: H. Zhao, et al.; Free Radic. Biol. Med. **31,** 599 (2001) ▪ Spin traps: in vitro toxicity and stability of radical adducts: N. Khan, et al.; Free Radic. Biol. Med. **34,** 1473 (2003) ▪ Detection and characterization of the product of hydroethidine and intracellular superoxide by HPLC and limitations of fluorescence: H. Zhao, et al.; PNAS **102,** 5727 (2005)



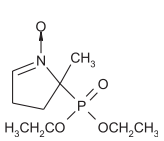
DEPMPO

[5-(Diethoxyphosphoryl)-5-methyl-1-pyrroline-N-oxide]

ALX-430-093-M050 50 mg
ALX-430-093-M500 500 mg

PURITY: ≥99%. **SPECIFICITY:** Most efficient spin trap for the *in vitro* and *in vivo* detection of O-, N-, S- and C-centered free radicals. Has a longer life-time than DMPO (Prod. No. ALX-430-090). Can distinguish between superoxide-dependent and independent mechanisms that lead to the hydroxyl radical. Less lipophilic (Kp=0.16) than DIPPMPO (Prod. No. ALX-430-119). *See Figure 1 and 2.*

LIT: 5-(Diethoxyphosphoryl)-5-methyl-1-pyrroline N-oxide: a new efficient phosphorylated nitrone for the in vitro and in vivo spin trapping of oxygen-centered radicals: C. Frejaville, et al.; J. Med. Chem. **38,** 258 (1995) ▪ Quantitative measurement of superoxide generation and oxygen consumption from leukocytes using electron paramagnetic resonance spectroscopy: V. Roubaud, et al.; Anal. Biochem. **257,** 210 (1998) ▪ Evaluation of DEPMPO as a spin trapping agent in biological systems: K.J. Liu, et al.; Free Radic. Biol. Med. **26,** 714 (1999) ▪ **For a comprehensive bibliography please visit our website.**



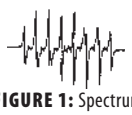


FIGURE 1: Spectrum of the (O_2^-)-DEPMPO adduct.

FIGURE 2: Spectrum of the (HO)-DEPMPO adduct.

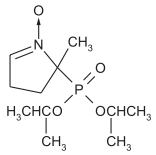
DIPPMPO

[5-(Diisopropoxyphosphoryl)-5-methyl-1-pyrroline-N-oxide; 2-Diisopropylphosphono-2-methyl-3,4-dihydro-2*H*-pyrrole-1-oxide]

ALX-430-119-M050 50 mg
ALX-430-119-M500 500 mg

PURITY: ≥99%. **SPECIFICITY:** Beside DEPMPO (Prod. No. ALX-430-093), most efficient spin trap for the *in vitro* and *in vivo* detection of O-, N-, S-, and C-centered free radicals. Has a longer life-time than DMPO (Prod. No. ALX-430-090). More lipophilic (Kp=2.1) than DEPMPO (Prod. No. ALX-430-093).

LIT: 5-(Diisopropoxyphosphoryl)-5-methyl-1-pyrroline-N-oxide, DIPPMPO, a crystalline analog of the nitrone DEPMPO: synthesis and spin trapping properties: F. Chalier & P. Tordo; J. C. S. Perkin Trans. II **2002,** 2110 ▪ The line asymmetry of electron spin resonance spectra as a tool to determine the cis:trans ratio for spin-trapping adducts of chiral pyrrolines N-oxides: the mechanism of formation of hydroxyl radical adducts of EMPO, DEPMPO, and DIPPMPO : M. Culcasi, et al.; Free Radic. Biol. Med. **40,** 1524 (2006)



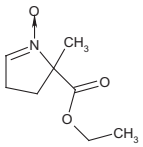
EMPO

[2-Ethoxycarbonyl-2-methyl-3,4-dihydro-2*H*-pyrrole-1-oxide]

ALX-430-098-M010 10 mg
ALX-430-098-M050 50 mg

PURITY: ≥95%. **SPECIFICITY:** Beside DEPMPO (Prod. No. ALX-430-093), most efficient spin trap for the *in vitro* and *in vivo* detection of O-, N-, S-, and C-centered free radicals. Has a longer life-time than DMPO (Prod. No. ALX-430-090). *See Figure 3.*

LIT: 2-ethoxycarbonyl-2-methyl-3,4-dihydro-2H-pyrrole-1-oxide: evaluation of the spin trapping properties: G. Olive, et al.; Free Radic. Biol. Med. **28,** 403 (2000) ▪ Detection of superoxide anion using an isotopically labeled nitrone spin trap: potential biological applications: H. Zhang, et al.; FEBS Lett. **473,** 58 (2000) ▪ Kinetic study and theoretical analysis of hydroxyl radical trapping and spin adduct decay of alkoxycarbonyl and dialkoxyphosphoryl nitrones in aqueous media: F.A. Villamena, et al.; J. Phys. Chem. (A) **107,** 4407 (2003) ▪ Spin adduct formation from lipophilic EMPO-derived spin traps with various oxygen- and carbon-centered radicals: K. Stolze, et al.; Biochem. Pharmacol. **69,** 297 (2005) ▪ **For a comprehensive bibliography please visit our website.**



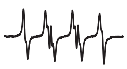


FIGURE 3: Spectrum of the (O_2^-)-EMPO adduct.

Ultrapure DMPO

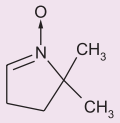
DMPO (high purity)

[5,5-Dimethyl-1-pyrroline-N-oxide]

ALX-430-090-M500 500 mg
ALX-430-090-G001 1 g

PURITY: ≥99%. Low paramagnetic impurities. **SPECIFICITY:** Cell permeable hydrophilic spin trap for both *in vivo* and *in vitro* studies of superoxide, O-, C-, S- and N- centered free radicals. **No further purification required.**

LIT: The spin trapping of superoxide and hydroxyl free radicals with DMPO (5,5-dimethylpyrroline-N-oxide): more about iron: G.R. Buettner; Free Radic. Res. Comm. **19** Suppl **1,** S79 (1993) ▪ **For a comprehensive bibliography please visit our website.**



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Step up to the Source™

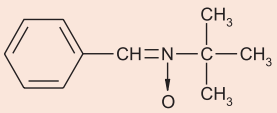
PBN [N-*t*-Butyl-α-phenylnitrone]

ALX-430-082-G001 1 g

For Bulk Quantities please inquire !

Cell permeable spin trap for both *in vivo* and *in vitro* studies.

LIT: For a comprehensive bibliography please visit our website.



Technical Note

Stability and *In Vitro* Toxicity of Radical Adducts

The radical adducts of the new spin traps DEPMPO and EMPO have significantly increased stability as compared to DMPO. This indicates that the new spin traps potentially offer increased stability of spin adducts in functioning cells. As there are some effects on cells from these spin traps, each type of *in vivo* application is likely to need preliminary studies of stability and toxicity to determine which spin trap is the most appropriate for the experimental goal.

LIT: Spin traps: in vitro toxicity and stability of radical adducts: N. Khan, et al.; Free Radic. Biol. Med. **34,** 1473 (2003) ▪ Cytotoxicity of novel derivatives of the spin trap EMPO: N. Rohr-Udilova, et al.; Bioorg. Med. Chem. Lett. **16,** 541 (2006)

Immuno-spin Trapping

a breakthrough for the sensitive detection of protein-derived and DNA radicals

Introduction

Detection of protein-derived radicals in isolated proteins has typically required the utilization of direct electron spin resonance (ESR) and/or spin trapping ESR. ESR can be applied both to understand the mechanisms of protein radical formation and for structural identification of the amino acid-derived radical involved. However, ESR measurements require significant levels of protein and of its derived radicals (greater than micromolar) and rely on rather specialized and costly equipment. Moreover, detection of protein radicals by ESR becomes a difficult task in cells and tissues.

Immuno-spin trapping is based on the fact that certain amino acid-derived radicals (most notably, but not exclusively, tyrosyl radicals) can react with the spin trap 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) to form a protein-DMPO nitroxide radical adduct, which can then be oxidized by one electron to the corresponding ESR-silent protein-DMPO nitrone adduct. Indeed, DMPO spin adducts are not stable, have a half-life on the order of minutes, and tend to decay under oxidizing conditions to the rather stable DMPO nitrone. To detect the more stable adducts a polyclonal antiserum to DMPO (Prod. No. ALX-210-530) was developed and validated in Mason's laboratory (C. D. Detweiler, L. J. Deterding, K. B. Tomer, C. F. Chignell, D. Germolec, R. P. Mason: *Immunological identification of the heart myoglobin radical formed by hydrogen peroxide;* Free Radic. Biol. Med. **33,** 364 (2002)). A series of studies have shown the utility of this antiserum to demonstrate protein radical formation in different proteins exposed to a variety of oxidants.

In summary, immuno-spin trapping is proving to be a potent, sensitive, and accessible method to detect low levels (e.g. greater than nanomolar) of protein-derived radicals produced *in vitro* and potentially, and yet to be established, *in vivo.* Moreover, it has just been established that anti-DMPO nitrone antibodies can be utilized for the detection of DNA-derived radicals in the form of DNA-DMPO nitrone adducts (D. C. Ramirez, S. E. Mejiba, R. P. Mason: *Immuno-spin trapping of DNA radicals;* Nat. Methods **3,** 127 (2006)), which broadens even more the scope of applications of this breakthrough technique in free radical biology and medicine.

Adapted from: *Immuno-spin trapping: A breakthrough for the sensitive detection of protein-derived radicals, a commentary on "Protein radical formation on thyroid peroxidase during turnover";* R. Radi; Free Radic. Biol. Med. **41,** 416 (2006).

Selected Review Article

Using anti-5,5-dimethyl-1-pyrroline N-oxide (anti-DMPO) to detect protein radicals in time and space with immuno-spin trapping: R.P. Mason; Free Radic. Biol. Med. **36,** 1214 (2004)

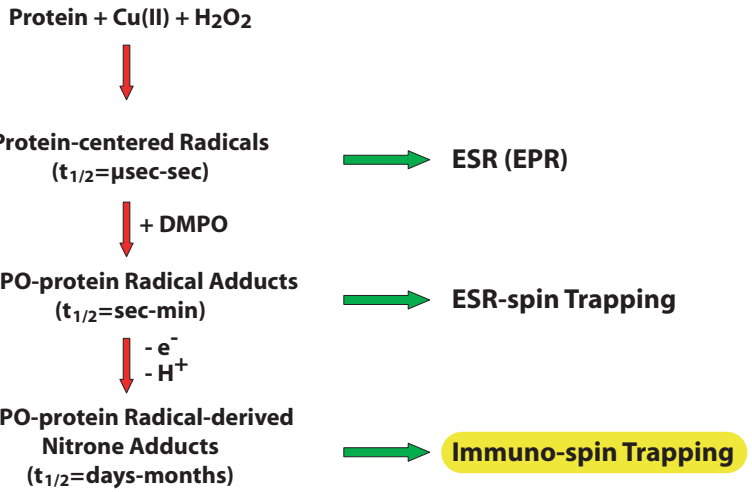


FIGURE: Schematic overview of approaches to detect protein radicals.

Product Highlight

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ALEXIS® BIOCHEMICALS *offers the widely published*

PAb to DMPO

ALX-210-530-R100 100 µl

From rabbit. **IMMUNOGEN:** DMPO-octanoic acid conjugated to ovalbumin. **SPECIFICITY:** Recognizes DMPO (Prod. No. ALX-430-090), DMPO-octanoic acid and DMPO-protein adducts. Does not cross react with non-adducted proteins. **APPLICATION:** ELISA, WB.

LIT: Immunological identification of the heart myoglobin radical formed by hydrogen peroxide: C.D. Detweiler, et al.; Free Radic. Biol. Med. **33,** 364 (2002) ▪ Immunochemical detection of hemoglobin-derived radicals formed by reaction with hydrogen peroxide: involvement of a protein-tyrosyl radical: D.C. Ramirez, et al.; Free Radic. Biol. Med. **34,** 830 (2003) ▪ Identification of free radicals on hemoglobin from its self-peroxidation using mass spectrometry and immuno-spin trapping: observation of a histidinyl radical: L. Deterding; J. Biol. Chem. **279,** 11600 (2004) ▪ Immuno-spin trapping: detection of protein-centered radicals: D.C. Ramirez, et al.; Curr. Prot. Toxicol. **2,** 17.7.1 (2005) ▪ **For a comprehensive bibliography please visit our website.**

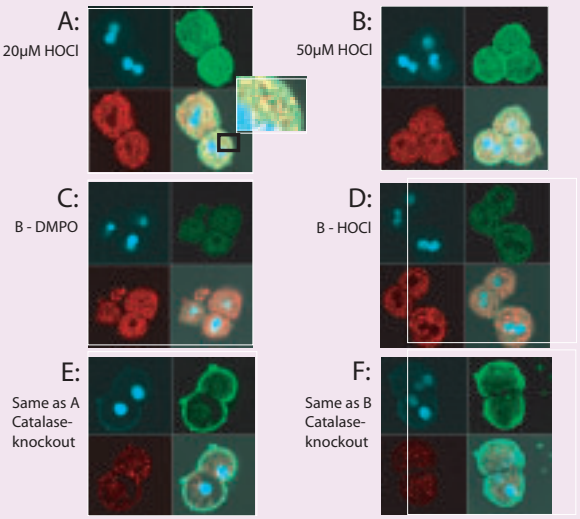


FIGURE: Confocal microscopy images of the colocalization of catalase (red stain) and protein-DMPO adducts (green stain) obtained by treating mouse hepatocytes ($2.5x10^6$ cells/ml) with HOCl.

Picture courtesy of Dr. M. G. Bonini, National Institute of Environmental Health Sciences, Research Triangle Park (RTP), NC, USA.

Latest Insight

Immuno-spin Trapping of DNA radicals

The detection of DNA radicals by immuno-spin Trapping (IST) is based on the trapping of radicals with 5,5-dimethyl-1-pyrroline N-oxide (DMPO), forming stable nitrone adducts that are then detected using an anti-DMPO serum. IST combines the simplicity, reliability, specificity and sensitivity of spin trapping with heterogeneous immunoassays for the detection of DNA radicals, and complements existing methods for the measurement of oxidatively generated DNA damage.

LIT: Immuno-spin trapping of DNA radicals: D.C. Ramirez, S.E. Mejiba, R.P. Mason; Nat. Method. **3,** 123 (2006)

Nitric Oxide Spin-trapping Reagents

Diethyldithiocarbamic acid . Na . $3H_2O$ (high purity)

[DETC; Diethyldithiocarbamate]

ALX-400-003-G005 5 g
ALX-400-003-G025 25 g

Nitric oxide (NO) spin-trapping reagent. Thiol and iron chelator. Inhibits induction of macrophage nitric oxide synthase (NOS). Has been shown to be an inhibitor of the nuclear transcription factor κB (NF-κB).

LIT: On-line detection of nitric oxide formation in liquid aqueous phase by electron paramagnetic resonance spectroscopy: P. Mordvintcev, et al.; Anal. Biochem. **199,** 142 (1991) ▪ NO accounts completely for the oxygenated nitrogen species generated by enzymic L-arginine oxygenation: A. Mülsch, et al.; Biochem. J. **288,** 597 (1992) ▪ Dithiocarbamates as potent inhibitors of nuclear factor kappa B activation in intact cells: R. Schreck, et al.; J. Exp. Med. **175,** 1181 (1992) ▪ Diethyldithiocarbamate inhibits induction of macrophage NO synthase: A. Mülsch, et al.; FEBS Lett. **321,** 215 (1993) ▪ The relationship between L-arginine-dependent nitric oxide synthesis, nitrite release and dinitrosyl-iron complex formation by activated macrophages: A. Vanin, et al.; Biochim. Biophys. Acta **1177,** 37 (1993) ▪ Iron diethyldithiocarbamate as spin trap for nitric oxide detection: A.F. Vanin; Methods Enzymol. **301,** 269 (1999)

MGD . Na . H_2O

[N-(Dithiocarbamoyl)-N-Methyl-D-glucamine]

ALX-400-014-M050 50 mg
ALX-400-014-M250 250 mg

Together with $FeSO_4$ MGD is a useful component for the formation of the MGD_2-Fe^{2+}complex, which is an excellent nitric oxide (NO) spin-trapping reagent. The MGD_2-Fe^{2+}complex is quite unstable, especially in the presence of dissolved oxygen. Thus, the complex should be used immediately after being made. An excess (usually 5-fold excess), of MGD to Fe^{2+} is used for making the complex with $FeSO_4$ to give a more stable complex solution. Acidic conditions should be avoided because dithiocarbamate tends to decompose forming toxic carbon disulfide. It was reported that MGD and $Fe(MGD)_2$ do not exhibit toxicity up to 8mmol/kg and 0.3mmol/kg, respectively.

LIT: Sodium N-methyl-D-glucamine dithiocarbamate and cadmium intoxication: L.A. Shinobu, et al.; Acta Pharmacol. Toxicol. **54,** 189 (1984) ▪ In vivo spin trapping of nitric oxide in mice: A. Komarov, et al.; BBRC **195,** 1191 (1993) ▪ Spin trapping of nitric oxide produced in vivo in septic-shock mice: C.-S. Lei & A.M. Komarov; FEBS Lett. **345,** 120 (1994) ▪ Spin trapping isotopically-labelled nitric oxide produced from [15N]L- arginine and [17O]dioxygen by activated macrophages using a water soluble Fe(++)-dithiocarbamate spin trap: Y. Kotake, et al.; Free Rad. Res. **23,** 287 (1995) ▪ Continuous monitoring of cellular nitric oxide generation by spin trapping with an iron-dithiocarbamate complex: Y. Kotake, et al.; Biochim. Biophys. Acta **1289,** 362 (1996) ▪ Continuous and quantitative monitoring of rate of cellular nitric oxide generation: Y. Kotake; Methods Enzymol. **268,** 222 (1996) ▪ Redox properties of iron-dithiocarbamates and their nitrosyl derivatives: implications for their use as traps of nitric oxide in biological systems: A.F. Vanin, et al.; Biochim. Biophys. Acta **1474,** 365 (2000) ▪ EPR spectroscopy of common nitric oxide - spin trap complexes: S. Nedeianu & T. Pali; Cell. Mol. Biol. Lett. **7,** 142 (2002)

Trimethylammonio-PTIO

ALX-430-085-M010 10 mg
ALX-430-085-M050 50 mg

Water soluble nitric oxide (NO) spin trap that is non-cell permeable, allowing study of extracellular NO release.

LIT: Spin trapping of nitric oxide by nitronylnitroxides: measurement of the activity of no synthase from rat cerebellum: Y.Y. Woldman, et al.; BBRC **202,** 195 (1994) ▪ ESR study of free decomposition of N,N-bis(arylsulfonyl)hydroxylamines in organic solution: M.Y. Balakirev & V.V. Khramtsov; J. Org. Chem. **61,** 7263 (1996)

Selected Latest Review Articles

Electron spin resonance spin-trapping detection of superoxide generated by neuronal nitric oxide synthase: J. Vasquez-Vivar, et al.; Methods Enzymol. **301,** 169 (1999)

Detection and imaging of endogenously produced nitric oxide with electron paramagnetic resonance spectroscopy: S. Fujii & T. Yoshimura; Antioxid. Redox Signal. **2,** 879 (2000)

In vivo spin trapping of nitric oxide: L.J. Berliner & H. Fujii; Antioxid. Redox Signal. **6,** 649 (2004)

Simultaneous detection of NO and ROS by ESR in biological systems: Y. Cao, et al.; Methods Enzymol. **396,** 77 (2005)

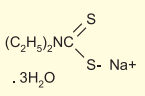
The ESR method to determine nitric oxide in plants: Y. Xu, et al.; Methods Enzymol. **396,** 84 (2005)

Application of electron spin resonance spin-trapping technique for evaluation of substrates and inhibitors of nitric oxide synthase: K. Saito & M. Kohno; Anal. Biochem. **349,** 16 (2006)

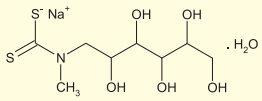
Inefficient spin trapping of superoxide in the presence of nitric-oxide: implications for studies on nitric-oxide synthase uncoupling: M. Pignitter, et al.; Free Radic. Biol. Med. **41,** 455 (2006)

Electron paramagnetic resonance (EPR) spin trapping of biological nitric oxide: A.L. Kleschyov, et al.; J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. (in press) (2006)

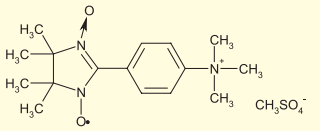
Chemical Structures



Diethyldithiocarbamic acid . Na . $3H_2O$ (high purity)



MGD . Na . H_2O



Trimethylammonio-PTIO

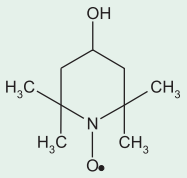
Antioxidant Spin Probe – A Key Standard Compound

TEMPOL

[4-Hydroxy-TEMPO; 4-Hydroxy-2,2,6,6-tetramethylpiperidinyloxy, free radical]

ALX-430-081-M250 250 mg
ALX-430-081-M500 500 mg
ALX-430-081-G001 1 g

Free radical scavenger useful for both *in vivo* and *in vitro* experiments.



LIT: Measurement of intracellular oxygen concentration using the spin label TEMPOL: P.D. Morse, 2nd & H.M. Swartz; Magn. Reson. Med. **2,** 114 (1985) ▪ Inhibition of oxygen-dependent radiation-induced damage by the nitroxide superoxide dismutase mimic, tempol: J.B. Mitchell, et al.; Arch. Biochem. Biophys. **289,** 62 (1991) ▪ Tempol, a stable free radical, is a novel murine radiation protector: S.M. Hahn, et al.; Cancer Res. **52,** 1750 (1992) ▪ Protective effect of 4-hydroxy-TEMPO, a low molecular weight superoxide dismutase mimic, on free radical toxicity in experimental pancreatitis: Z. Sledzinski, et al.; Int. J. Pancreatol. **18,** 153 (1995) ▪ A novel antioxidant alleviates heat hyperalgesia in rats with an experimental painful peripheral neuropathy: M. Tal; Neuroreport **7,** 1382 (1996) ▪ Stable nitroxide radicals protect lipid acyl chains from radiation damage: A.M. Samuni & Y. Barenholz; Free Radic. Biol. Med. **22,** 1165 (1997) ▪ Tempol inhibits neutrophil and hydrogen peroxide-mediated DNA damage: S.M. Hahn, et al.; Free Radic. Biol. Med. **23,** 879 (1997) ▪ Effects of the superoxide dismutase-mimic compound TEMPOL on oxidant stress-mediated endothelial dysfunction: A.I. Haj-Yehia, et al.; Antioxid. Redox. Signal. **1,** 221 (1999) ▪ The nitroxide tempol induces oxidative stress, p21(WAF1/CIP1), and cell death in HL60 cells: M.B. Gariboldi, et al.; Free Radic. Biol. Med. **29,** 633 (2000) ▪ Nitroxide TEMPOL impairs mitochondrial function and induces apoptosis in HL60 cells: E. Monti, et al.; J. Cell. Biochem. **82,** 271 (2001) ▪ Spin trapping agents (Tempol and POBN) protect HepG2 cells overexpressing CYP2E1 against arachidonic acid toxicity: M.J. Perez & A.I. Cederbaum; Free Radic. Biol. Med. **30,** 734 (2001) ▪ Systemic arterial pressure response to two weeks of Tempol therapy in SHR: involvement of NO, the RAS, and oxidative stress: L. Yanes, et al.; Am. J. Physiol. Regul. Integr. Comp. Physiol. **288,** R903 (2005) ▪ Tempol, one of nitroxides, is a novel ultraviolet-A1 radiation protector for human dermal fibroblasts: S.X. Yan, et al.; J. Dermatol. Sci. **37,** 137 (2005) ▪ Cancer chemoprevention by the antioxidant tempol acts partially via the p53 tumor suppressor: L. Erker, et al.; Hum. Mol. Genet. **14,** 1699 (2005) ▪ Antioxidant enzymes and effects of tempol on the development of hypertension induced by nitric oxide inhibition: J. Sainz, et al.; Am. J. Hypertens. **18,** 871 (2005) ▪ Neuroprotective effects of TEMPOL in central and peripheral nervous system models of Parkinson's disease: Q. Liang, et al.; Biochem. Pharmacol. **70,** 1371 (2005) ▪ The role of oxidant stress in angiotensin II-mediated contraction of human resistance arteries in the state of health and the presence of cardiovascular disease: M.B. Hussain, et al.; Vascul. Pharmacol. **45,** 395 (2006) ▪ Acute effects of the superoxide dismutase mimetic tempol on split kidney function in two-kidney one-clip hypertensive rats: G.S. Guron, et al.; J. Hypertens. **24,** 387 (2006) ▪ The effects of tempol, 3-aminobenzamide and nitric oxide synthase inhibitors on acoustic injury of the mouse cochlea: H. Murashita, et al.; Hear. Res. **214,** 1 (2006) ▪ The nitroxide Tempol modulates anthracycline resistance in breast cancer cells: M.B. Gariboldi, et al.; Free Radic. Biol. Med. **40,** 1409 (2006)

Fluorinated Spin Probe

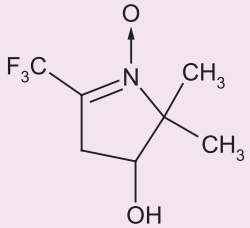
FDMPO

[4-Hydroxy-5,5-dimethyl-2-trifluoromethylpyrroline-1-oxide]

ALX-430-135-M010 10 mg
ALX-430-135-M050 50 mg

A fluorinated spin trap which allows ^{19}F-NMR detection of free radical reactions. Greatly improved signal-to-noise ratio when compared to the ^{31}P-sensitivity of the phosphorus-containing spin trap DEPMPO (Prod. No. ALX-430-093).

LIT: NMR spin trapping: detection of free radical reactions with a new fluorinated DMPO analog: V.V. Khramtsov, et al.; Free Radic. Biol. Med. **30,** 1099 (2001)



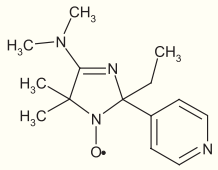
NEW pH-sensitive Spin Probes

DEDPI

[4-(Dimethylamino)-2-ethyl-5,5-dimethyl-2-pyridine-4-yl-2,5-dihydro-1*H*-imidazol-1-oxyl]

ALX-430-120-M010	10 mg
ALX-430-120-M050	50 mg

pH-sensitive nitroxide for *in vivo* studies. The presence of two ionizable groups in the side-chains extends the range of pH sensitivity. The relatively high solubility in water and broad range of pH-sensitivity makes the probe particularly suitable for pH monitoring in stomach using non-invasive low-field EPR techniques.



LIT: Grignard reagent addition to 5-alkylamino-4H-imidazole 3-oxides: synthesis of new pH-sensitive spin probes : T.G. Shevelev, et al.; Synthesis **2003,** 871 ▪ Synthesis of the tetraethyl substituted pH-sensitive nitroxides of imidazole series with enhanced stability towards reduction: I.A. Kirilyuk, et al.; Org. Biomol. Chem. **2,** 1025 (2004) ▪ In vitro and in vivo measurement of pH and thiols by EPR-based techniques: V.V. Khramtsov, et al.; Antioxid. Redox Signal. **6,** 667 (2004) ▪ Nitroxides with two pK values - useful spin probes for pH monitoring within a broad range: I.A. Kirilyuk, et al.; Org. Biomol. Chem. **3,** 1269 (2005) ▪ Real-time monitoring of drug-induced changes in the stomach acidity of living rats using improved pH-sensitive nitroxides and low-field EPR techniques: D.I. Potapenko, et al.; J. Magn. Reson. **182,** 1 (2006)

Latest Insight

Nitroxides with two pK values

The group of I. A. Grigor'ev describes useful nitroxide spin probes for sensitive pH-monitoring within a broad range. This could be useful for many biophysical and biomedical applications, including pH-monitoring in the stomach.

FOR DETAILS SEE: *Nitroxides with two pK values – useful spin probes for pH monitoring within a broad range:* I. A. Kirilyuk, et al.; Org. Biomol. Chem. **3,** 1269 (2005)

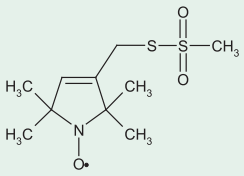
NEW Efficient Cysteine-specific Spin Labelling Compound

MTSSL

[(1-Oxyl-2,2,5,5-tetramethylpyrroline-3-methyl) methanethiosulfonate]

ALX-430-134-M010	10 mg
ALX-430-134-M050	50 mg

Highly reactive thiol-specific spin label. Has been used to label cysteine residues in proteins (site-directed labelling, SDS-labelling). Allows protein structure and protein dynamics determination as well as the study of protein-protein and protein-oligonucleotide interactions.



LIT: A novel reversible thiol-specific spin label: papain active site labeling and inhibition: L.J. Berliner, et al.; Anal. Biochem. **119,** 450 (1982) ▪ Pressure-induced thermostabilization of glutamate dehydrogenase from the hyperthermophile Pyrococcus furiosus: M.M. Sun, et al.; Protein Sci. **8,** 1056 (1999) ▪ Methods for study of protein dynamics and protein-protein interaction in protein-ubiquitination by electron paramagnetic resonance spectroscopy: H.J. Steinhoff; Front. Biosci. **7,** c97 (2002) ▪ Protein structure determination using long-distance constraints from double-quantum coherence ESR: study of T4 lysozyme: P.P. Borbat, et al.; JACS **124,** 5304 (2002) ▪ Inter- and intra-molecular distances determined by EPR spectroscopy and site-directed spin labeling reveal protein-protein and protein-oligonucleotide interaction: H.J. Steinhoff; Biol. Chem. **385,** 913 (2004) ▪ Spontaneous refolding of the pore-forming Colicin A toxin upon membrane association as studied by X-band and W-band high-field electron paramagnetic resonance spectroscopy: A. Savitski, et al.; J. Phys. Chem. B **108,** 9541 (2004) ▪ Calcium structural transition of human cardiac troponin C in reconstituted muscle fibres as studied by site-directed spin labelling: M. Nakamura, et al.; J. Mol. Biol. **348,** 127 (2005)

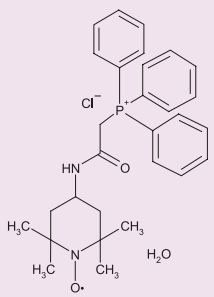
NEW Mitochondria-targeted Antioxidant

Mito-TEMPO

[(2-(2,2,6,6-Tetramethylpiperidin-1-oxyl-4-ylamino)-2-oxoethyl) triphenylphosphonium chloride . monohydrate]

ALX-430-150-M005	5 mg

Mitochondria-targeted antioxidant with superoxide and alkyl radical scavenging properties, which may be used for both *in vivo* and *in vitro* experiments.



LIT: Targeting antioxidants to mitochondria by conjugation to lipophilic cations: M.P. Murphy & R.A.J. Smith; Annu. Rev. Pharmacol. Toxicol. **47,** 629 (2007)

Product Highlight

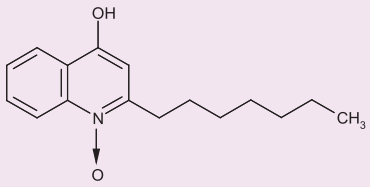
HQNO

NEW

[2-n-Heptyl-4-hydroxyquinoline N-oxide]

ALX-430-130-M010	10 mg
ALX-430-130-M050	50 mg

Naturally occurring antagonist of dihydrostreptomycin. Potent inhibitor of the respiratory chain binding to the mitochondrial cytochrome b protein. Inhibits NADH oxidase (NADH) and Na^+-dependent NADH-quinone reductase (NQR). Used in the investigation of the enzymatic pathways of elemental sulfur and thiosulfate disproportionation. Synthetic.



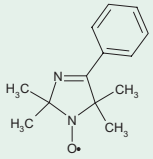
LIT: Inhibition of cytochrome system of heart muscle and of Staphylococcus aureus by 2-heptyl-4-hydroxyquinoline-N-oxide, an antagonist of dihydrostreptomycin: J.W. Lightbown & F.L. Jackson; Biochem. J. **58,** 15 (1954) ▪ Structure of a naturally occurring antagonist of dihydrostreptomycin: J.W. Cornforth & A.T. James; Biochem. J. **63,** 124 (1956) ▪ Binding of HQNO to beef-heart sub-mitochondrial particles: G. Van Ark & J.A. Berden; Biochim. Biophys. Acta **459,** 119 (1977) ▪ Inhibitor studies of a new antibiotic, korormicin, 2-n-heptyl-4-hydroxyquinoline N-oxide and Ag+ toward the Na+-translocating NADH-quinone reductase from the marine Vibrio alginolyticus: Y. Nakayama, et al.; Biol. Pharm. Bull. **22,** 1064 (1999) ▪ FTIR spectroscopic evidence for the involvement of an acidic residue in quinone binding in cytochrome bd from Escherichia coli: J. Zhang, et al.; Biochemistry **41,** 4612 (2002) ▪ A stable isotope dilution assay for the quantification of the Pseudomonas quinolone signal in Pseudomonas aeruginosa cultures: F. Lepine, et al.; Biochim. Biophys. Acta **1622,** 36 (2003) ▪ Sulfite-oxido-reductase is involved in the oxidation of sulfite in Desulfocapsa sulfoexigens during disproportionation of thiosulfate and elemental sulfur: T.M. Frederiksen & K. Finster; Biodegradation **14,** 189 (2003) ▪ Enzymatic properties of the membrane-bound NADH oxidase system in the aerobic respiratory chain of Bacillus cereus: M.S. Kim & Y.J. Kim; J. Biochem. Mol. Biol. **37,** 753 (2004) ▪ Involvement of sulfide:quinone oxidoreductase in sulfur oxidation of an acidophilic iron-oxidizing bacterium, Acidithiobacillus ferrooxidans NASF-1: S. Wakai, et al.; Biosci. Biotechnol. Biochem. **68,** 2519 (2004) ▪ Physiological roles of three Na+/H+ antiporters in the halophilic bacterium Vibrio parahaemolyticus: T. Kuroda, et al.; Microbiol. Immunol. **49,** 711 (2005) ▪ Quinone reduction by Rhodothermus marinus succinate:menaquinone oxidoreductase is not stimulated by the membrane potential: A.S. Fernandes, et al.; BBRC **330,** 565 (2005) ▪ Purification and characterization of succinate:menaquinone oxidoreductase from Corynebacterium glutamicum: T. Kurokawa and J. Sakamoto; Arch. Microbiol. **183,** 317 (2005)

New Spin Probe

NEW PTMIO

[4-Phenyl-2,2,5,5-tetramethyl-3-imidazoline-1-oxyl nitroxide]

ALX-430-145-M050	50 mg
ALX-430-145-M250	250 mg
ALX-430-145-G001	1 g



Alphabetical Product Overview

Alphabetical Product Overview *continued*

NEW

Carbonyl ELISA Kit

Protein Carbonyl ELISA Kit (BioCell)

ALX-850-312-KI01 1 Kit

QUANTITY: 96 wells (~80 tests). For quantitative detection of carbonylated protein levels in plasma, other body fluids, cell and tissue extracts. **For research use only.**

- **Assay Principle: Protein sample reacts with DNP then adsorbs to an ELISA plate. The adsorbed protein is reacted with a biotinylated anti-DNP antibody**
- **Advantages over colorimetric assays**
- **Higher sensitivity**
- **Less labour-intensive**
- **Handles more samples per day**

LIT: Protein carbonyl measurements show evidence of early oxidative stress in critically ill patients: C.C. Winterbourn, et al.; Crit. Care Med. **28,** 143 (2000) ▪ Protein carbonyl groups as biomarkers of oxidative stress: I. Dalle-Donne, et al.; Clin. Chim. Acta **329,** 23 (2003) ▪ Elevated protein carbonyls as plasma markers of oxidative stress in acute pancreatitis: C.C. Winterbourn, et al.; Pancreatology **3,** 375 (2003) ▪ Oxidative stress and high density lipoprotein function in type 1 diabetes and end stage renal disease: G. Kalogerakis, et al.; Clin. Sci. (Lond), **108,** 497 (2005)

Superoxide Dismutase [SOD] Activity Assay Kit

NEW **Superoxide Dismutase [SOD] Activity Assay Kit**

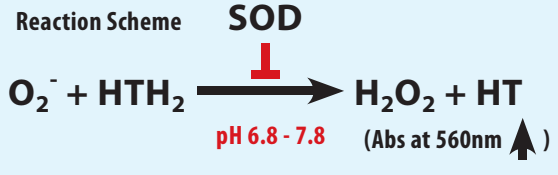
ABL-SOD-560-KI01 1 Kit

- **Colorimetric Assay Kit**
- **Based on inhibition of hematoxylin oxidation**
- **More convenient assay than the coupled cytochrome c reduction method**
- **Determines total SOD activity. Can be used to distinguish CuZnSOD or MnSOD activity separately**

Manufactured by Applied Bioanalytical Labs

SELCECTED LITERATURE REFERENCES: Superoxide dismutase assays: L. Flohe & F. Otting; Meth. Enzymol. **105,** 93 (1984) ▪ Negative and positive assays of superoxide dismutase based on hematoxylin autoxidation: J.P. Martin, Jr., et al.; Arch. Biochem. Biophys. **255,** 329 (1987)

Reaction Scheme



Kit for Monitoring Lipid Peroxidation/Oxidative Stress

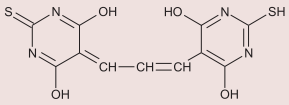
OXI-TEK TBARS Assay Kit (ZeptoMetrix)

ALX-850-287-KI01 1 Kit

QUANTITY: 160 tests.

The sensitivity of measuring thiobarbituric acid reactive substances (TBARS) has made this assay the method of choice for screening and monitoring lipid peroxidation, a major indicator of oxidative stress. This rapid, easy-to-use procedure has been modified by researchers for use with many types of samples including drugs, food products and human and animal biological tissues. The assay has provided important information regarding free radical activity in disease states and has been used for measurement of antioxidant activity of several compounds. The kit is designed to provide standardized, reproducible results.

Principles of the kit: malondialdehyde (MDA) forms a 1:2 adduct with thiobarbituric acid:



The adduct can be measured by fluorometry or spectrophotometry. Biological specimens contain a mixture of thiobarbituric acid reactive substances (TBARS), including lipid hydroperoxides and aldehydes, which increase as a result of oxidative stress. TBARS return to normal levels over time, depending upon the presence of antioxidants. In this assay, an MDA standard is used to construct a standard curve against which unknown samples can be plotted.

ADMA ELISA Kit

NEW

ADMA ELISA Kit

ALX-850-321-KI01 1 Kit

QUANTITY: 96 wells. **SENSITIVITY:** ~0.1-0.5µM.

For quantitative detection asymmetric dimethylarginine (ADMA) in EDTA-plasma and serum. Competitive enzyme linked immunoassay. **For research use only.**

- **Quantitative determination of ADMA in plasma and serum**
- **Small sample volume needed (25 µl)**
- **Competitive ELISA with microtiter plate format**
- **Good linearity between 0.1µM – 5.0 µM**
- **No crossreactivity to L-arginine**

Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of nitric oxide (NO) synthesis. NO is one of the major endothelium-derived vasoactive mediators and is involved in the modulation of blood flow and the pressure. Even small changes of the ADMA concentration alter vascular NO production, vascular tone, and systemic vascular resistence. Elevated ADMA concentrations are found in patients with diabetes mellitus, hyperchoesterolemia, hypertension, pulmonary hypertension, peripheral arterial disease, chronic renal failure, preeclampsia or erectile dysfunction. ADMA is therefor a cardiovascular risk factor.

For a fast determination of ADMA in plasma and serum please inquire for ADMA Direct ELISA Kit.

01-Apr-07

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