ALEXIS® BIOCHEMICALS

4-Hydroxynonenal [HNE]

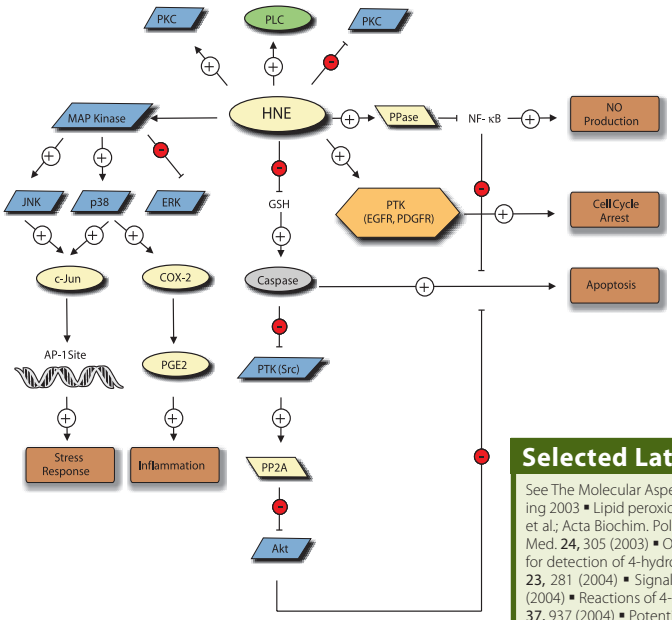
A Key Marker of Oxidative Stress-linked Pathological Events

highlight

Tomorrow's Reagents Manufactured Today™

International Version

Introduction



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 Latest 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)

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

Core Products

(E)-4-Hydroxynonenal [HNE]

ALX-270-245-M005 **5 mg** **128.00**

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**

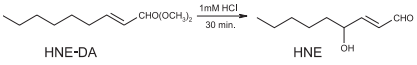
The Product Data Sheet includes detailed information for the *in situ* preparation of HNE before your experiment.

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) ▪ Formation of 8-hydroxy-2'-deoxyguanosine and 4-hydroxy-2-nonenal-modified proteins in human renal-cell carcinoma: K. Okamoto, et al.; Int. J. Cancer **58,** 825 (1994) ▪ For a comprehensive bibliography please visit our website.



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 potassium hydroxide (KOH).

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

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 1) [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 like ferrous [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 overestimation of lipid hydroperoxides. These problems are easily circumvented by performing the assay in chloroform.

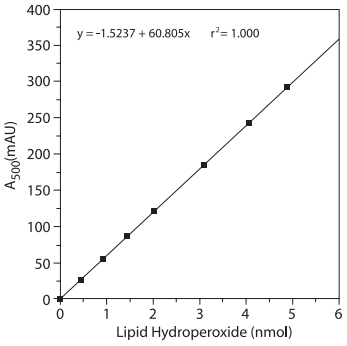
An easy to use, quantitative extraction method was developed to extract lipid hydroperoxides into chloroform and the extract is directly used in the assay. This procedure eliminates any interference caused by hydrogen peroxide or endogenous ferric ions in the sample and provides a sensitive and reliable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

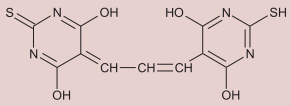
OXI-TEK TBARS Assay Kit (For Monitoring Lipid Peroxidation/ Oxidative Stress)

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.

For further information please request the Product Information Sheet/Manual.

Related Products

(E)-4-Hydroxyhexenal

ALX-270-405-M001	1 mg
ALX-270-405-M005	5 mg

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

ALX-270-407-M001	1 mg
ALX-270-407-M005	5 mg

4-Hydroperoxy-2-nonenal

CAY-10004413-M001	1 mg
CAY-10004413-M005	5 mg

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