Caspases - Executioner of Apoptosis & Judges of Inflammation

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Caspases - Introduction

Caspases, a family of proteases, are best known as executioners of apoptotic cell death and their activation are considered as the "point of no return" commitment to cell death. Based on structure and function, caspases are grouped into two main classes: initiator/apical caspases that are activated through oligomerization and effector caspases, which require cleavage by an initiator caspase to be activated (reviewed by [1]). So far 13 mammalian caspases have been identified (see Table 1 & 2) and in addition caspases have been also cloned e.g. in *Caenorhabditis elegans* (nematode), *Drosophila melangoster* (fly), *Spodoptera frugiperda* (butterfly), zebrafish and even in sponges (*Geodia cydonium*), the phylogenetically oldest metazoan phylum.

Caspase	Other Name(s)
Caspase-1	ICE, EC 3.4.22.36
Caspase-2	Nedd2, ICH-1
Caspase-3	CPP32, Yama, Apopain, SCA-1
Caspase-4	ICE_{rel}-II, TX, ICH-2
Caspase-5	ICE_{rel}-III, TY, ICH-3
Caspase-6	Mch2
Caspase-7	Mch3, ICE-LAP3, CMH-1, LICE2, SCA-2
Caspase-8	MACH, FLICE, Mch5
Caspase-9	ICE-LAP6, Mch6, Apaf-3
Caspase-10	Mch4, FLICE2
Caspase-11	ICH-3 (mouse)
Caspase-12*	(mouse)
Caspase-13**	ERICE (bovine)
Caspase-14	MICE

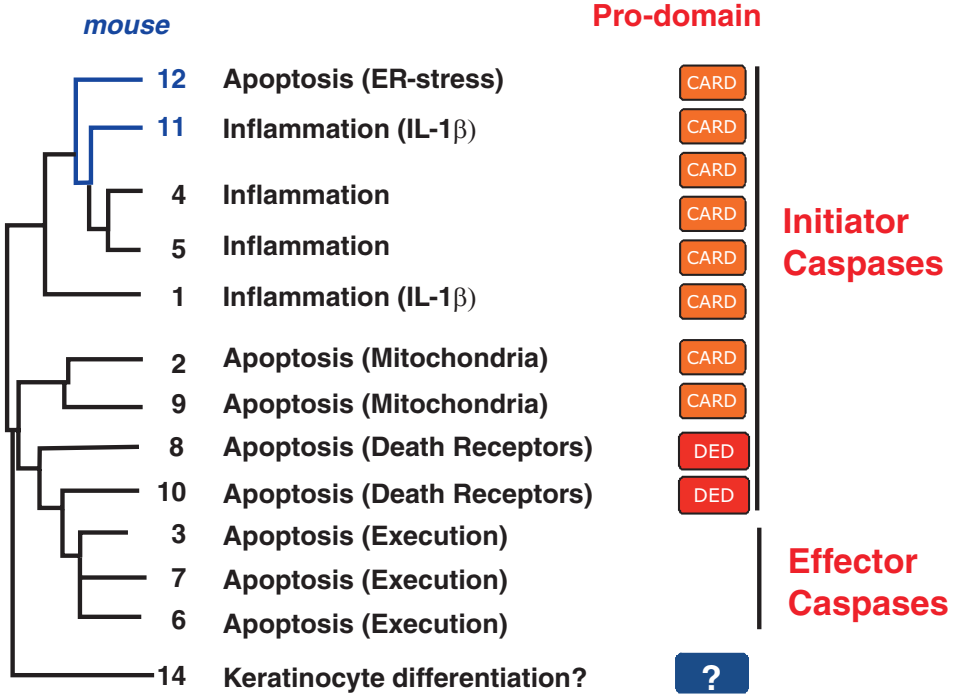
* The human CASP-12 gene does not appear to be expressed except in some African population and is probably functionally replaced by caspase-4 and -5.
** Human caspase-13 as reported by E. Humke, et al. (J. Biol. Chem. **273**, 15702 (1998)) has been shown to be bovine caspase-4 (*Evidence that caspase-13 is not a human but a bovine gene*: U. Koenig, et al.; BBRC **285**, 1150 (2001)).

Caspases can also be grouped according to their preferred cleavage sites after an aspartic acid residue and a recognition of normally four consecutive amino acids: group I (caspase-1, -4 and -5) with preference to Trp-Glu-His-Asp (WEHD), group II (caspase-2, -3 and -7) Asp-Glu-X-Asp (DE-X-D) and group III (caspases-6, -8, -9 and -10) (Leu/Val)-Glu-X-Asp (L/V-E-X-D). As of to date more than 280 different proteins have been identified that are cleaved by caspases (reviewed in [2]).

Apoptosis involves a cascade of events and two common pathways exist: an intrinsic pathway which is caused by cellular stress and mediated through the release of cytochrome c from the mitochondrion and an extrinsic pathway that relies on a cell surface stimulus and is initiated by binding of death ligands to death receptors or granzyme B. This extrinsic pathway employs also the mitochondrion pathway as an amplification loop.

Caspases also play important roles in immune reactions that culminate in cytokine production rather than apoptosis. For example it is well established that caspase-1 (first described as interleukin-1β converting enzyme; ICE) is essential for the production of mature IL-1β, IL-1α, and IL-18 in the context of lipopolysaccharide-initiated inflammatory reactions. Nitric oxide (NO), a pleiotropic signalling molecule produced at sites of inflammation, can inhibit inflammation by S-nitrosylation of the active site cysteine of caspases (for a review see [3]). NO also inhibits caspase-dependent T cell proliferation, underlining the role of caspases as critical mediators of T cell activation [4]. For a review of non-apoptotic functions of caspases see [5].

Lit. [1] *Caspase activation*: K.M. Boatright & G.S. Salvesen; Biochem. Soc. Symp. **70**, 233 (2003) [2] *Many cuts to ruin: a comprehensive update of caspase substrates*: U. Fischer, et al.; Cell Death Differ. **10**, 76 (2003) [3] *Regulation of caspases by nitric oxide*: P.K. Kim, et al.; Ann. NY Acad. Sci. **962**, 42 (2002) [4] *Nitric oxide-mediated inhibition of caspase-dependent T lymphocyte proliferation*: R.S. Mahidhara, et al.; J. Leukoc. Biol. **74**, 403 (2003) [5] *Non-apoptotic functions of caspases in cellular proliferation and differentiation*: C. Schwerk & K. Schulze-Osthoff; Biochem. Pharmacol. **66**, 1453 (2003)

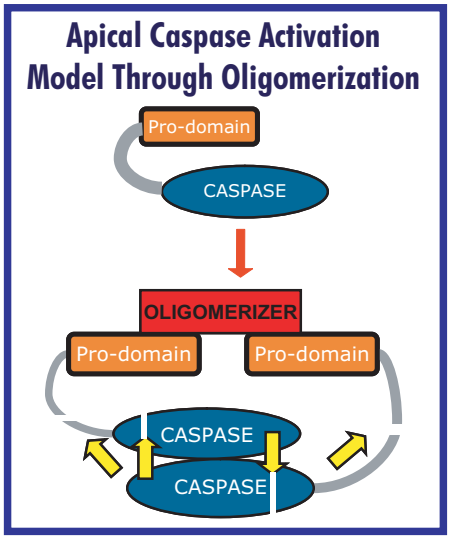


Initiator/Apical Caspases

Caspases exist as inactice procaspases (zymogens), which comprise an N-terminal prodomain of variable length and a C-terminal protease domain that can be further divided into the large and small subunits, the constituents of mature caspases. Conversion of procaspases to mature caspases involves at least one cleavage event that separates the large and small subunits but often also another cleavage event that separates the prodomain and large subunit. During apoptosis, caspase activation occurs sequentially with long prodomain-containing caspases (initiator caspases, including caspase-2, -8, -9, and -10) being activated first, which then cleave and activate short prodomain-containing caspases (effector caspases, including caspase-3, -6, and -7). Mature effector caspases cleave a wide range of intracellular structural and regulatory proteins, leading to a set of stereotypic changes in cell morphology and eventual cell death.

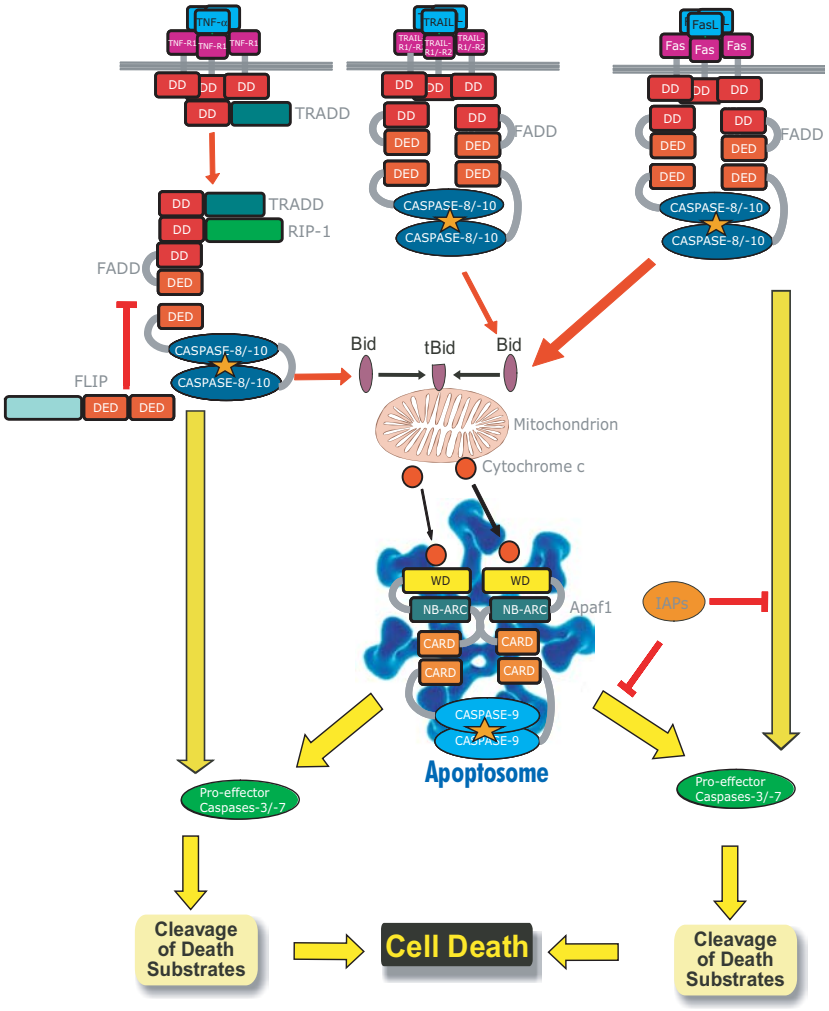
As there are no upstream proteases that cleave the initiator caspases, an unresolved question is how initiator caspases achieve catalytic competence in their recruitment/activation complexes. Based on the induced proximity model, it is suggested that the initiator procaspases exist as monomers, demonstrating only a weak equilibrium with the dimeric form. Oligomerization by an adapter allows the monomers to overcome the weak interaction equilibrium and adopt the dimeric formation, leading to cross-cleavage among the caspase dimer to fully activate the enzyme [1-5].

Lit. [1] *Caspase activation: the induced-proximity model*: G.S. Salvesen & V.M. Dixit; PNAS **96**, 10964 (1999) (Review) [2] *A unified model for apical caspase activation*: K.M. Boatright, et al.; Mol. Cell **11**, 529 (2003) [3] *Oligomerization is a general mechanism for the activation of apoptosis initiator and inflammatory procaspases*: D.W. Chang, et al.; J. Biol. Chem. **278**, 16466 (2003) [4] *Caspase activation*: K.M. Boatright & G.S. Salvesen; Biochem. Soc. Symp. **70**, 233 (2003) (Review) [5] *Initiator caspases in apoptosis signalling pathways*: M. Chen & J. Wang; Apoptosis **7**, 313 (2002) (Review)



Caspase-8 & -10

Caspase-8 & -10 Pathways

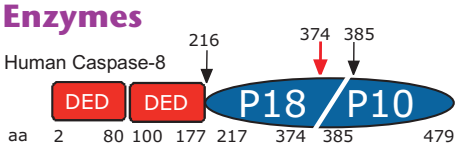


Caspase-8 & -10 *continued*

The initiator caspases-8 and -10 are activated in response to ligation of death receptors. Death receptors are a subfamily of the tumor necrosis factor (TNF) receptor superfamily compromising Fas (CD95), TNF-R1 and TNF-related apoptosis-inducing ligand receptor 1(TRAIL-R1) and TRAIL-R2. They are characterized by a death domain (DD) motif within their intracellular domain, which is required for the induction of apoptosis. Fas-associated death domain protein (FADD) is reported to be the adapter used by death receptors Fas, TRAIL-R1 and -R2, to recruit and activate the initiator caspase-8 and -10 by interaction with their death effector domains (DED), to form the death-inducing signalling complex (DISC). Fas, TRAIL-R1 and -R2 bind FADD directly through their DD domains, whereas recruitment to TNF-R1 is indirect through another adapter TNF receptor-associated death domain protein (TRADD) [1]. TRADD then recruits RIP-1 through DD interactions. This complex then dissociates from the receptor to bind FADD and caspase-8. Binding of FADD to caspase-8 and -10 can be inhibited by FLIP. For reviews see [2-8].

Lit. *Fas-associated death domain protein and caspase-8 are not recruited to the tumor necrosis factor receptor 1 signalling complex during tumor necrosis factor-induced apoptosis*: N. Harper, et al.; J. Biol. Chem. **278**, 25534 (2003) [2] *Caspase 8: igniting the death machine*: G.S. Salvesen; Structure Fold. Des. **7**, R225 (1999) [3] *Apoptosis induced by death receptors*: P. Schneider & J. Tschopp; Pharm. Acta Helv. **74**, 281 (2000) [4] *Caspase-8 in apoptosis: the beginning of "the end"?*: M. Kruidering & G.I. Evan; IUBMB Life **50**, 85 (2000) [5] *Molecular mechanisms of death-receptor-mediated apoptosis*: U. Sartorius, et al.; Chembiochem. **2**, 20 (2001) [6] *Regulation of lymphocyte proliferation and death by FLIP*: M. Thome & J. Tschopp; Nat. Rev. Immunol. **1**, 50 (2001) [7] *The death effector domain protein family: regulators of cellular homeostasis*: M.D. Tibbetts, et al.; Nat. Immunol. **4**, 404 (2003) [8] *Live and let die: regulatory mechanisms in Fas-mediated apoptosis*: J.F. Curtin & T.G. Cotter; Cell Signal. **15**, 983 (2003)

Enzymes



Caspase-8 (active) (human) (rec.)

201-041-C005 5µg

Expressed in *E. coli*. The rate of caspase-8 enzymatic hydrolysis can be measured by the release of AMC from the caspase substrate Ac-DEVD-AMC (Prod. No. 260-031) with emission at 440nm and excitation at 380nm.

Lit. *Biochemical characteristics of caspases-3, -6, -7 and -8*: H.R. Stennicke & G.S. Salvesen; J. Biol. Chem. **272**, 25719 (1997)

Caspase-8 (active) (human) (rec.)

201-062-U025 25 Units

201-062-U100 100 Units

Expressed in *E. coli*. **Specific Activity**: ~5000U/mg.One unit of rec. caspase-8 is the enzyme activity that cleaves 1 nmole of the caspase substrate IETD-pNA per hour at 37ºC in a buffered solution.

Caspase-8 (active) (human) (rec.) (high purity)

522-054-C010 10µg

Purity: >95% (SDS-PAGE). Expressed in *E. coli*. **Specific Activity**: ~2000U/mg. One unit of rec. caspase-8 is the enzyme activity that cleaves 1 nmole of the caspase substrate Ac-DEVD-AMC (Prod. No. 260-031) per minute at 37ºC in a buffered solution.

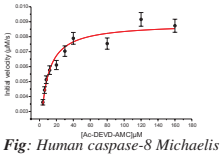


Fig: Human caspase-8 Michaelis Menten Kinetics.

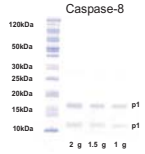


Fig: SDS-PAGE of 522-054.

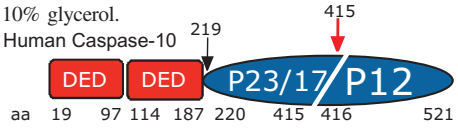
Initiator/Apical Caspases *continued*

Caspase-8 & -10 *continued*

Caspase-8 (active) (mouse) (rec.)

201-163-C020 20µg

Purity: ~90% (gel electrophoresis). Expressed in *E. coli*. **Specific Activity**: >100U/µg. One unit is defined as the enzyme activity that cleaves 1nmole of the caspase substrate Z-IETD-AMC per hour at +30°C under the following reaction conditions: 20µM Z-IETD-AMC (Prod. No. 260-042), 50mM HEPES, pH 7.4, 100mM NaCl, 0.5% CHAPS, 10mM DTT, 1mM EDTA and 10% glycerol.



Caspase-10 (active) (human) (rec.)

201-137-C005 5µg

Produced in *E. coli*. When expressed in *E. coli*, caspase-10 spontaneously undergoes autoprocessing to yield the subunits characteristic of the active enzyme. The rate of caspase-10 enzymatic hydrolysis can be measured by the release of AMC from the caspase substrate Ac-IETD-AMC (Prod. No. 260-042) as emission at 440nm and excitation at 380nm using a spectrofluorometer.

NEW Caspase-10/a [Mch4] (active) (human) (rec.) (high stability)

201-193-U050 50 Units

Purity: ≥95% (SDS-PAGE). Expressed in *E. coli*. **Specific Activity**: ~2500U/mg.One unit of rec. caspase-10/a is the enzyme activity that cleaves 1 nmole of the caspase substrate IETD-pNA per minute at 37°C in a buffered solution. The undiluted product has been shown to be stable over several freeze/thaw cycles.

NEW Caspase-10/d (active) (human) (rec.) (high stability)

201-194-U050 50 Units

Purity: ≥95% (SDS-PAGE). Expressed in *E. coli*. **Specific Activity**: ~2500U/mg.One unit of rec. caspase-10/d is the enzyme activity that cleaves 1 nmole of the caspase substrate IETD-pNA per minute at 37°C in a buffered solution.

Antibodies

MAb to Caspase-8 (human) (12F5)

804-242-C100 100µg

Clone: 12F5. **Isotype**: Mouse IgG2b. **Immunogen**: Recombinant human caspase-8 fusion protein. **Specificity**: Recognizes human procaspase-8 (p55/54), the intermediate cleavage products of 43kDa and 41kDa and the p18 active subunit of caspase-8. **Application**: IP, WB.

Lit. *Differential regulation and ATP requirement for caspase-8 and caspase-3 activation during CD95- and anticancer drug-induced apoptosis*: D. Ferrari, et al.; J. Exp. Med. **188**, 979 (1998)/*Sendai virus infection induces apoptosis through activation of caspase-8 (FLICE) and caspase-3 (CPP32)*: M. Bitzer, et al.; J. Virol. **73**, 702 (1999)/*Anticancer drugs induce caspase-8/FLICE activation and apoptosis in the absence of CD95 receptor/ligand interaction*: S. Wesselborg, et al.; Blood **93**, 3053 (1999)/*P2Z purinoreceptor ligation induces activation of caspases with distinct roles in apoptotic and necrotic alterations of cell death*: D. Ferrari, et al.; FEBS Lett. **447**, 71 (1999)/*IL-10 induces apoptosis in human monocytes involving the CD95 receptor/ligand pathway*: M. Schmidt, et al.; Eur. J. Immunol. **30**, 1769 (2000)/*Inhibition of death receptor-mediated gene induction by a cycloheximide-sensitive factor occurs at the level of or upstream of Fas-associated death domain protein (FADD)*: H. Wajant, et al.; J. Biol. Chem. **275**, 24357 (2000)

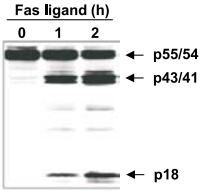


Fig.: Detection of Fas ligand-induced human caspase-8 processing and activation in human Jurkat cells.

MAb to Caspase-8 (human) (C15)

804-429-C050 50µg
804-429-C100 100µg

Clone: C15. **Isotype**: Mouse IgG2b. **Immunogen**: Recombinant human caspase-8 (aa 181-478). **Specificity**: Recognizes the p18 subunit of human caspase-8. **Application**: ICC, IP, WB.

Lit. *FLICE Is Predominantly Expressed as Two Functionally Active Isoforms, Caspase-8/a and Caspase-8/b*: C. Scaffidi, et al.; J. Biol. Chem. **272**, 26953 (1997)/For a comprehensive bibliography of this well-characterized MAb please contact us.

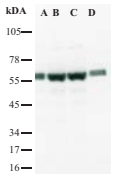


Fig: Western blot analysis with MAb to caspase-8 (C15)
*Used at a dilution of 1:500 on the following cell lysates (4 ug loaded / well) **A**: Boe cells **B**: RF3 cells transfected with cDNA for FLIPshort; **C**: Raji cells; **D**: Jurkat mutant cell line. Secondary antibody was a mouse IgG2b isotype-specific antibody conjugated to HRP and revealed by ECL.*

MAb to Caspase-8 (mouse) (1G12)

804-447-C100 100µg

Clone: 1G12. **Isotype**: Rat IgG1. **Immunogen**: Recombinant p18 subunit of mouse caspase-8. **Specificity**: Recognizes the p18 subunit of mouse caspase-8. Does not cross-react with human caspase-8. Detects bands of ~55kDa (full-length caspase-8) and ~18kDa (apoptosis-induced cleavage fragment) by WB. **Application**: ELISA, FC, ICC, WB (excellent).

MAb to Caspase-8 (mouse) (3B10)

804-448-C100 100µg

Clone: 3B10. **Isotype**: Rat IgG1. **Immunogen**: Recombinant p18 subunit of mouse caspase-8. **Specificity**: Recognizes mouse caspase-8. Does not cross-react with human caspase-8. Detects bands of ~55kDa (full-length caspase-8) and ~18kDa (apoptosis-induced cleavage fragment) by WB. **Application**: ELISA, FC, ICC (excellent), WB.

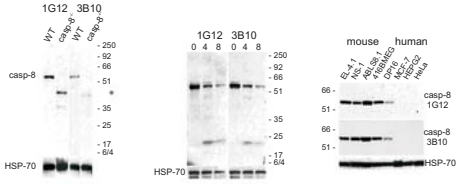


Fig.: MAbs 1G12 and 3B10.
*Both MAbs detect pro-caspase-8 in MEFs from WT mice, but not in MEFs from caspase-8$^{-/-}$ mice. Several smaller bands detected in the caspase-8$^{-/-}$ MEFs, correspond to truncated forms of caspase-8 made in the caspase-8$^{-/-}$ mice since only exons 1 and 2 of mouse caspase-8 were deleted in these knock-out mice and not the region encoding the p18 subunit. **Note:** extra bands marked by * are only seen in lysates from caspase-8$^{-/-}$ MEFs and not in lysates from any WT cell lines or mouse WT tissue. Both MAbs to caspase-8 (mouse) do not recognize human caspase-8, whereas endogenous caspase-8 can be efficiently detected in various mouse cell lines. Upon an apoptotic stimulus e.g. by cross-linked rhsFasL both MAbs to caspase-8 (mouse) do also recognize the cleaved active p18 subunit of mouse caspase-8 in addition to the caspase-8 precursor.*

Latest Insight: Caspase-8 and -10 activate NF-κB through RIP, NIK and IKKα, but not RICK or IKKβ, suggesting that caspase-8 and -10 have roles in a non- or anti-apoptotic pathway. For details see: *Caspase-8 and caspase-10 activate NF-kappaB through RIP, NIK and IKKalpha kinases*: Y. Shikama, et al.; Eur. J. Immunol. **33**, 1998 (2003)

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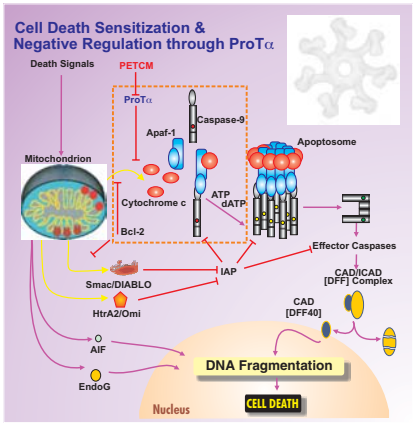
Caspase-9

The initiator caspase-9 is responsible for initiating caspase activation in response to cytotoxic stress, genome damage and some developmental cues. These signals lead to the release of mitochondrial cytochrome c to the cytoplasm, where it binds to Apaf-1 (apoptotic protease-activating factor 1) to initiate the intrinsic apoptosis pathway. Apaf-1 is a mammalian homolog of the *C. elegans* protein CED-4. In addition to a CED-4 domain Apaf-1 also has an N-terminal caspase recruitment domain (CARD) and a C-terminal WD-40 repeat domain (WDR). Cytochrome c interacts with the WDR, whereas dATP or ATP binds the nucleotide binding domain located within the CED-4 domain. Upon binding of cytochrome c and dATP or ATP Apaf-1 undergoes self-oligomerization to form the caspase-activating complex termed apoptosome. The three-dimensional structure has been determined and revealed a wheel-like particle with 7-fold symmetry (for reviews see [1-6]).



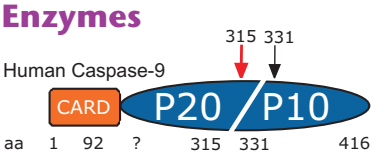
The apoptosome then recruits procaspase-9 and activates effector caspases-3 and -7. The caspase inhibitor XIAP also associates with the apoptosome inhibiting caspase-9, -3 and -7 activity [7]. Another protein called Aven binds to Apaf-1 and Bcl-X_L and thus also inhibits the formation of the apoptosome [8]. On the other hand the PHAP proteins (also called HLA-DR-associated proteins or protein phosphatase 2A inhibitors) promote caspase-9 activation after apoptosome formation, whereas prothymosin α (ProTα) negatively regulates caspase-9 activation by inhibiting apoptosome formation [9-10]. In apoptotic cells, ProTα is subject to C-terminal truncation by caspase-3 in the nucleus, which results in relocalization of the truncated protein to the cytoplasm and to cell exterior [11]. tProTα then binds cytochrome c and thus probably negatively regulates caspase activation by inhibiting apoptosome formation [12].

Lit. [1] *The mitochondrial apoptosome: a killer unleashed by the cytochrome seas*: C. Adrain and S.J. Martin; TIBS **26**, 390 (2001) [2] *Apoptotic death sensor: an organelle's alter ego?*: S.B. Bratton and G.M. Cohen; TIPS **22**, 306 (2001) [3] *Apoptosome: the seven-spoked death machine*: G.S. Salvesen and M. Renatus; Dev. Cell **2**, 256 (2002) [4] *Apoptosome: the cellular engine for the activation of caspase-9*: Y. Shi; Structure **10**, 285 (2002) [5] *The Apaf-1 apoptosome: a large caspase-activating complex*: K. Cain, et al.; Biochimie **84**, 203 (2002) [6] *Apoptosomes: engines for caspase activation*: J.M. Adams and S. Cory; Curr. Opin. Cell. Biol. **14**, 715 (2002) [7] *Recruitment, activation and retention of caspases-9 and -3 by Apaf-1 apoptosome and associated XIAP complexes*: S.B. Bratton, et al.; EMBO J. **20**, 998 (2001) [8] *Aven, a novel inhibitor of caspase activation, binds Bcl-xL and Apaf-1*: B.N. Chau, et al.; Mol. Cell **6**, 31 (2000) [9] *Distinctive roles of PHAP proteins and prothymosin-alpha in a death regulatory pathway*: X. Jiang, et al.; Science **299**, 223 (2003) [10] *Apoptosis. Life and death decisions*: D.W. Nicholson & N.A. Thornberry; Science **299**, 214 (2003) [11] *Apoptosis-related fragmentation, translocation, and properties of human prothymosin alpha*: A.G. Evstafieva, et al.; Exp. Cell Res. **284**, 211 (2003) [12] *Cytochrome c is transformed from anti- to pro-oxidant when interacting with truncated oncoprotein prothymosin alpha*: O.V. Markova, et al.; Biochim. Biophys. Acta **1557**, 109 (2003)

Initiator/Apical Caspases *continued*

Caspase-9 *continued*

Enzymes



Procaspase-9 (human) (rec.)

201-164-C050 50µg

Produced in *E. coli*. Requires activation.

Procaspase-9 (mouse) (rec.)

201-165-C050 50µg

Produced in *E. coli*. Requires activation.

Caspase-9 (active) (human) (rec.)

201-136-C005 5µg

Produced in *E. coli*. When expressed in *E. coli*, caspase-9 spontaneously undergoes autoprocessing to yield the subunits characteristic of the active enzyme. The rate of caspase-9 enzymatic hydrolysis can be measured by the release of AMC from the caspase substrate Ac-LEHD-AMC (Prod. No. 260-080) as emission at 440nm and excitation at 380nm using a spectrofluorometer.

Caspase-9 (active) (human) (rec.)

201-047-U025 25 Units

201-047-U100 100 Units

Expressed in *E. coli*. **Specific Activity**: ~400U/mg. One unit of rec. caspase-9 is the enzyme activity that cleaves 1 nmole of the caspase substrate LEHD-pNA per hour at 37°C in a buffered solution.

Antibodies

PAb to Caspase-9 (Bur 49)

210-014-R050 50µl

From rabbit. **Immunogen**: Recombinant human caspase-9 (catalytic subunit). **Specificity**: Recognizes human, mouse, rat and dog caspase-9. Detects both procaspase-9 and the 15kDa small subunit of activated caspase-9. **Application**: ICC, IHC (PS), WB.

Lit. *Release of caspase-9 from mitochondria during neuronal apoptosis and cerebral ischemia*: S. Krajewski, et al.; PNAS **96**, 5752 (1999)

PAb to Caspase-9

210-815-C100 100µg

From rabbit. **Immunogen**: Synthetic peptide corresponding to aa 287-306 of human caspase-9. **Specificity**: Recognizes human, mouse, rat, rabbit, monkey, dog, pig, bovine and hamster procaspase-9 and a cleaved fragment (35kDa). **Application**: WB.

PAb to Caspase-9 (active)

210-816-C100 100µg

From rabbit. **Immunogen**: Synthetic peptide around the cleavage site of human caspase-9. **Specificity**: Recognizes human and rat cleavage fragment (37kDa) of caspase-9. Does not cross-react with procaspase-9. **Application**: ICC, IP, WB.

PAb to Caspase-9 (human)

210-838-R100 100µl

From rabbit. **Immunogen**: His-tagged full-length recombinant human caspase-9. **Specificity**: Recognizes human procaspase-9 and the p37/p35 cleavage products of activated caspase-9. **Appli-cation**: IP, WB.

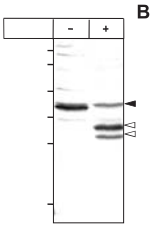
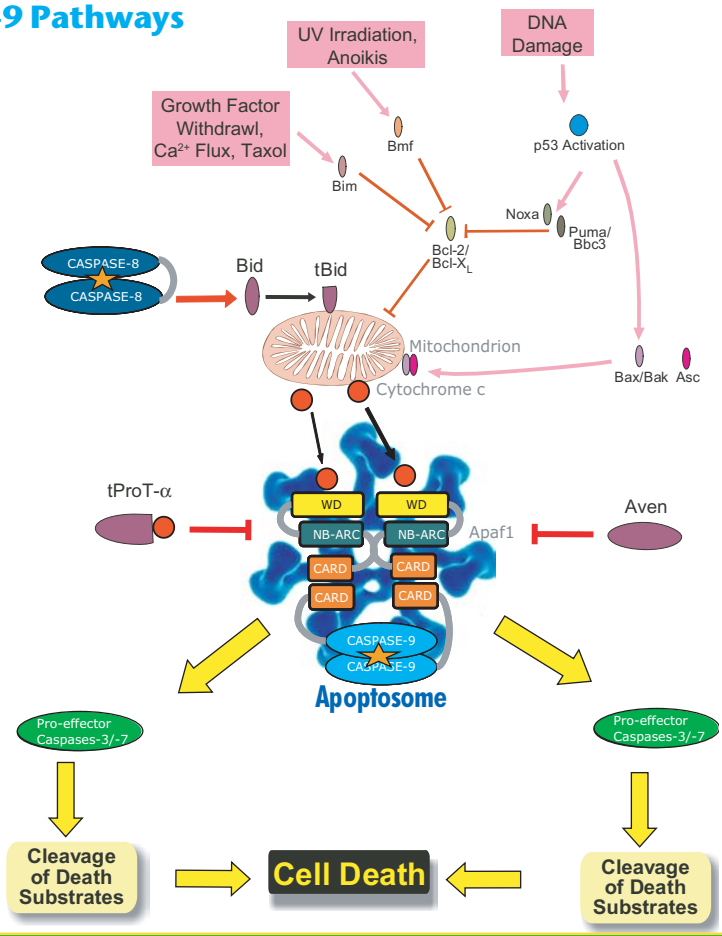


Fig: (B) Detection of caspase-9 processing during apoptosis. The antiserum detects procaspase-9 (46 kDa) and the intermediate cleavage products of 37 kDa and 35 kDa. No cross-reactivity with other caspases is observed.

Caspase-9 Pathways



NEW Caspase-9 Related Products

Enzymes

Apaf-1 (rat) (rec.) (His-tagged)

201-161-C025 25µg

Purity: ~95% (gel electrophoresis). Produced in *E. coli*.

Prothymosin α (truncated) [tProTα] (1-99) (human) (rec.)

201-125-C050 50µg

Purity: >90%. Produced in *E. coli*. Corresponding to the caspase-3 cleavage product of prothymosin α (ProTα) isolated from apoptotic human cells (no affinity-tags, no added additional (linker) sequences). Purified from overproducing bacterial strains by a phenol extraction procedure, DEAE chromatography, and ethanol precipitation.

Lit. *Overproduction in Escherichia coli, purification and properties of human prothymosin alpha:* A.G. Evstafieva, et al.; Eur. J. Biochem. **231**, 639 (1995)/*Sensing prothymosin alpha origin, mutations and conformation with monoclonal antibodies:* E.A. Sukhacheva, et al.; J. Immunol. Methods **266**, 185 (2002)/*Apoptosis-related fragmentation, translocation, and properties of human prothymosin alpha:* A.G. Evstafieva, et al.; Exp. Cell Res. **284**, 209 (2003)/*Cytochrome c is transformed from anti- to pro-oxidant when interacting with truncated oncoprotein prothymosin alpha:* O.V. Markova, et al.; Biochim. Biophys. Acta **1557**, 109 (2003)

Prothymosin α [ProTα] (1-109) (human) (rec.)

201-126-C050 50µg

Purity: >90%. Produced in *E. coli*. Native sequence representing mature prothymosin α (ProTα) isolated from human cells (no affinity-tags, no additional added (linker) sequences). Purified from overproducing bacterial strains by a phenol extraction procedure, DEAE chromatography, and ethanol precipitation.

Lit. *See above (Prod. No. 210-125)*

XIAP, Soluble (human) (rec.)

522-065-C050 50µg

Purity: >90%. Produced in bacteria. Human XIAP is fused to a N-terminal tag. **Application**: Positive control for PAb to XIAP (human) (Prod. No. 210-327).

Antibodies

NEW MAb to Prothymosin α [ProTα] (NT) (2F11)

804-486-C100 100µg

Clone: 2F11. **Isotype:** Mouse IgG1. **Immunogen:** Recombinant human prothymosin α (ProTα) (aa 1-99) fusion protein. **Specificity:** Recognizes human, mouse, rat and bovine ProTα. Recognized epitope includes aa 1-31 of human ProTα. Does not cross-react with human parathymosin. **Application:** ICC (excellent), IP, WB.

Lit. *Overproduction in Escherichia coli, purification and properties of human prothymosin alpha:* A.G. Evstafieva, et al.; Eur. J. Biochem. **231**, 639 (1995)/*Sensing prothymosin alpha origin, mutations and conformation with monoclonal antibodies:* E.A. Sukhacheva, et al.; J. Immunol. Meth. **266**, 185 (2002)/*Apoptosis-related fragmentation, translocation, and properties of human prothymosin alpha:* A.G. Evstafieva, et al.; Exp. Cell Res. **284**, 209 (2003)

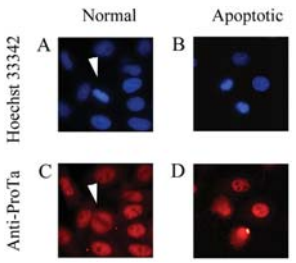


***Figure:** Localization of endogenous ProTα in healthy and apoptotic cells using anti-ProTα MAb 2F11.*

Initiator/Apical Caspases *continued*

NEW Caspase-9 Related Products

MAb to Prothymosin α [ProTα] (human) (4F4)

804-487-C100 100µg

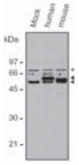
Clone: 4F4. **Isotype:** Mouse IgG1. **Immunogen:** Full-length recombinant human prothymosin α (ProTα). **Specificity:** Recognizes human ProTα. Recognized epitope includes aa 52-89 of human ProTα. Does not cross-react with human parathymosin. **Application:** ICC, IP, WB (excellent).

Lit. *See above (Prod. No. 804-486).*

PAb to XIAP

210-909-R050 50µl
210-909-R100 100µl

From rabbit. **Immunogen**: N-terminal recombinant human XIAP (BIR1 domain; aa 1-168). **Specificity**: Recognizes human and mouse XIAP. **Application**: WB.



Lit. *A serine protease, HtrA2, is released from the mitochondria and interacts with XIAP, inducing cell death:* Y. Suzuki, et al.; Mol. Cell **8**, 613 (2001)

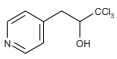
***Fig.**: HeLA cells were transfected with pcDNA3-FLAG-human XIAP or -mouse XIAP plasmid. Whole cell lysates (20µg protein in each lane) were analyzed by Western blot using PAb to XIAP (Prod. No. 210-909). Closed triangle: Exogenously expressed XIAP. Open triangle: Endogenous XIAP. Asterisk: Non-specific band.*

Inhibitor

NEW PETCM

[α-(Trichloromethyl)-4-pyridineethanol]

420-031-M010 10mg
420-031-M050 50mg



Activator of caspase-3 in cell extracts. Relieves prothymosin α inhibition and promotes apoptosome formation at a physiological concentration of deoxyadenosine triphosphate.

Lit. *Distinctive roles of PHAP proteins and prothymosin-alpha in a death regulatory pathway:* X. Jiang, et al.; Science **299**, 223 (2003)

Latest Insight I: TNF-α induced apoptosis can activate caspase-9 independent of cytochrome c by direct cleavage through caspase-8. For details see: *Caspase-9 is activated in a cytochrome c-independent manner early during TNFalpha-induced apoptosis in murine cells*: M.A. McDonnell, et al.; Cell Death Differ. **10**, 1005 (2003)

PAb to HtrA2/Omi

210-906-R050 50µl
210-906-R100 100µl

From rabbit. **Immunogen**: Recombinant human HtrA2/Omi. **Specificity**: Recognizes human and mouse HtrA2/Omi. **Application**: ICC, WB.

Lit. *A serine protease, HtrA2, is released from the mitochondria and interacts with XIAP, inducing cell death:* Y. Suzuki, et al.; Mol. Cell **8**, 613 (2001)

Latest Insight II: HtrA2/Omi is a serine protease residing in the mitochondria of healthy cells, but during apoptosis is released in the cytosol, where it can bind to inhibitors of apoptosis (IAPs) HtrA2/Omi thus promotes apoptosis by relieving the inhibition of caspases imposed by XIAP, at least *in vitro*. But new studies suggest that the primary role of HtrA2/Omi is to maintain mitochondrial function by refolding and degarding misfolded proteins in the mitochondria. For details see: *HtrA2/Omi, a Sheep in Wolf's Clothing*: D.L. Vaux & J. Silke; Cell **115**, 251 (2003)

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www.alexis-e.biz

Caspase-2

Caspase-2 was the second mammalian caspase described and discovered as the first apoptotic caspase [1,2]. Caspase-2 is most closely related to the *Caenorhabditis elegans* CED-3 of all mammalian caspases and is the most evolutionarily caspase within the family [3]. Even though caspase-2 bears sequence homology to the initiator caspase-9, its cleavage specificity is closer to the effector caspase-3 and -7 [4]. In mouse and man two forms of caspase-2 are found, a long proapoptotic form and a shorter antiapoptotic form, although it is not clear whether this form is expressed as a protein [2]. Caspase-2 is unique among all caspases, as crystal structures revealed that caspases exists as a $(p19/p12)_2$ dimer in solution, stabilized through a disulfide bridge between the central cysteine pair Cys^{390} and $Cys^{390\#}$ ($^{\#}$ referring to the second monomer) [5].

A further unique feature of caspase-2 is that caspase-2 is completely inactive toward other caspase zymogens, unlike all other caspases [6]. But the lack of a dramatic phenotype in the caspase-2 null mouse somewhat damped the interest in caspase-2 and its implication in apoptosis. Recent advances refueled interest in caspase-2, as it seems that caspase-2 is an initiator caspase for the intrinsic pathway and an executioner caspase in neuronal cells.

Caspase-2 can also be activated by forming an Apaf-1 and dATP independent complex, eventhough the components of this complex have not yet been indentified [7].

However, events subsequent to caspase-2 activation remain largely unknown. Like caspase-8, physiological levels of purified caspase-2 can cleave cytosolic Bid protein, which in turn can trigger the release of cytochrome c from isolated mitochondria. Caspase-2, however, can also induce directly the release of cytochrome c, AIF (apoptosis inducing factor) and Smac/DIABLO from isolated mitochondria independent of Bid or other cytosolic factors. The caspase-2-released cytochrome c is sufficient to activate the Apaf-1/caspase-9 apoptosome *in vitro*. Caspase-2 is required to translocate Bax to the mitochondria.

Caspase-2 appears to be an initiator caspase responding to BH3-only proteins that sensor cellular damage [8-11] at least in some cell types. Thus while much remains to be deciphered about caspase-2, most critically the mode of activation, it is clear that caspase-2 plays critical and singular roles in the control of programmed cell death (for a latest review see [14]).

Continued on next page.

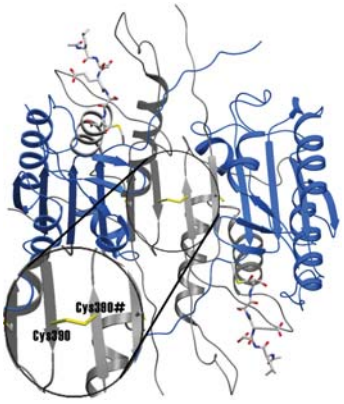
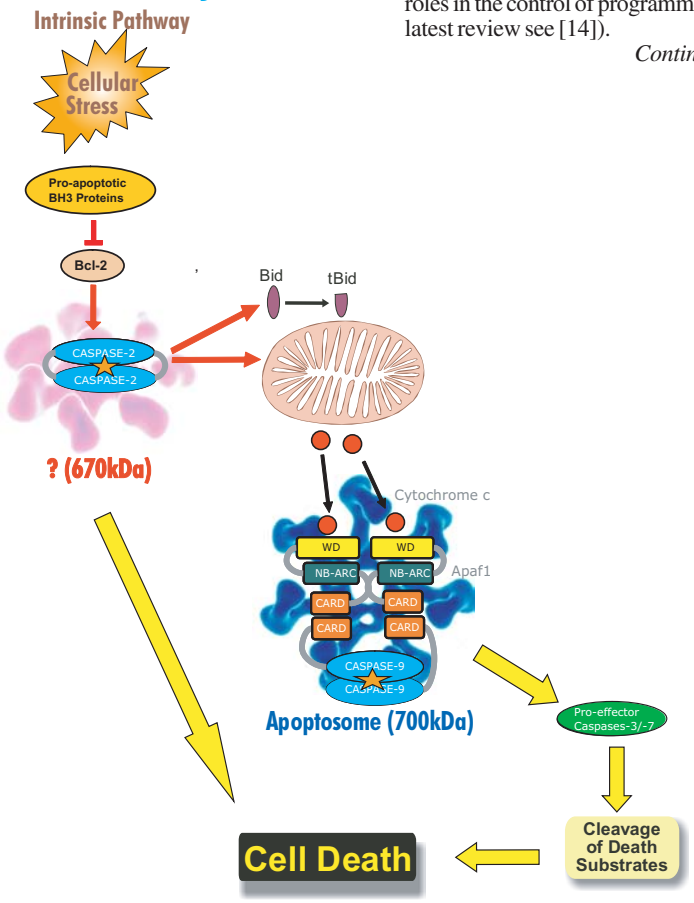


***Figure**: Crystal structure of caspase-2 in complex with the inhibitor Ac-LDESD-CHO showing the central disulfide bridge. Picture kindly provided by and courtesy of Prof. M.G. Grütter, University of Zürich, Switzerland.*

Caspase-2 Pathways



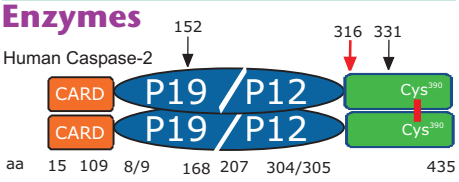
Initiator/Apical Caspases *continued*

Caspase-2 *continued*

An earlier suggested involvement of caspase-2 in the extrinsic pathway, in which caspase-2 interacts with the CARD domain of CRADD (RAIDD, an adapter protein for Fas and TNFR) [12,13] could not be confirmed under physiological conditions [14].

Lit. [1] *Induction of apoptosis by the mouse Nedd2 gene, which encodes a protein similar to the product of the Caenorhabditis elegans cell death gene ced-3 and the mammalian IL-1 beta-converting enzyme*: S. Kumar, et al.; Genes Dev. **8**, 1613 (1994) [2] *Ich-1, an Ice/ced-3-related gene, encodes both positive and negative regulators of programmed cell death*: L. Wang, et al.; Cell **78**, 739 (1994) [3] *Alice in caspase land. A phylogenetic analysis of caspases from worm to man*: M. Lamkanfi, et al.; Cell Death Differ. **9**, 358 (2002) [4] *A combinatorial approach defines specificities of members of the caspase family and granzyme B. Functional relationships established for key mediators of apoptosis*: N.A. Thornberry, et al.; J. Biol. Chem. **272**, 17907 (1997) [5] *Crystal structure of caspase-2, apical initiator of the intrinsic apoptotic pathway*: A. Schweizer, et al.; J. Biol. Chem. **278**, 42441 (2003) [6] *Caspase-2 induces apoptosis by releasing proapoptotic proteins from mitochondria*: Y. Guo, et al.; J. Biol. Chem. **277**, 13430 (2002) [7] *A novel Apaf-1-independent putative caspase-2 activation complex*: S.H. Read, et al.; J. Cell Biol. **159**, 739 (2002) [8] *Caspase-2 acts upstream of mitochondria to promote cytochrome c release during etoposide-induced apoptosis*: J.D. Robertson, et al.; J. Biol. Chem. **277**, 29803 (2002) [9] *Requirement for caspase-2 in stress-induced apoptosis before mitochondrial permeabilization*: P. Lassus, et al.; Science **297**, 1352 (2002) [10] *Apoptosis. A cinderella caspase takes center stage*: S. Kumar & D.L. Vaux; Science **297**, 1290 (2002) [11] *Caspase-2 redux*: C.M. Troy & M.L. Shelanski; Cell Death Differ. **10**, 101 (2003) [12] *RAIDD is a new 'death' adaptor molecule*: H. Duan & V.M. Dixit; Nature **385**, 86 (1997) [13] *CRADD, a novel human apoptotic adaptor molecule for caspase-2, and FasL/tumor necrosis factor receptor-interacting protein RIP*: M. Ahmad, et al.; Cancer Res **57**, 615 (1997) [14] *Induction of TNF receptor I-mediated apoptosis via two sequential signaling complexes*: O. Micheau & J. Tschopp; Cell **114**, 181 (2003)

Enzymes



Caspase-2 (active) (human) (rec.)

201-135-C005 5µg

Produced in *E. coli*. When expressed in *E. coli*, caspase-2 spontaneously undergoes autoprocessing to yield the subunits characteristic of the active enzyme. The rate of caspase-2 enzymatic hydrolysis can be measured by the release of AFC from the caspase substrate Ac-VDVAD-AFC (Prod. No. 260-112) as emission at 505nm and excitation at 400nm using a spectrofluorometer.

Caspase-2 (active) (human) (rec.)

201-057-U025 25 Units
201-057-U100 100 Units

Expressed in *E. coli*. **Specific Activity**: ≥10'000U/mg. One unit of rec. caspase-2 is the enzyme activity that cleaves 1 nmole of the caspase substrate VDVAD-pNA per hour at 37°C in a buffered solution.

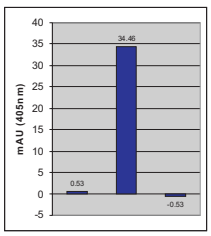
Caspase-2 (active) (human) (rec.) (high purity)

522-083-C005 5µg

Purity: >95% (SDS-PAGE). Expressed in *E. coli*. **Specific Activity**: ~80'000U/mg. One unit of rec. caspase-2 is the enzyme activity that cleaves 1 nmole of the caspase substrate VDVAD-pNA per hour at 37°C in a buffered solution.

Lit. *Crystal structure of caspase-2, apical initiator of the intrinsic apoptotic pathway*: A. Schweizer, et al.; J. Biol. Chem. **278**, 42441 (2003)

***Fig**: The activity of recombinant caspase-2 was determined by cleaving VDVAD-pNA. The cleavage activity was effectively inhibited by the corresponding peptide inhibitor (Ac-VDVAD-CHO) as indicated. **1)** 0nM caspase-2, **2)** 20nM caspase-2 **3)** 20nM caspase-2 + Ac-VDVAD-CHO. Measurements were done in triplicates and the bars represent the averages.*



Antibodies

MAb to Caspase-2 (10C6)

804-355-C100 100µg

Clone: 10C6. **Isotype**: Rat IgG2a. **Immunogen**: His-tagged p19 fragment of recombinant human caspase-2. **Specificity**: Recognizes an epitope in the p19 subunit of human, mouse, rat, monkey and dog caspase-2. **Application**: FC, ICC, IHC (FS).

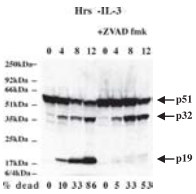
MAb to Caspase-2 (11B4)

804-356-C100 100µg

Clone: 11B4. **Isotype**: Rat IgG2a. **Immunogen**: His-tagged p19 fragment of recombinant human caspase-2. **Specificity**: Recognizes an epitope in the p19 subunit of human, mouse, rat, monkey and dog caspase-2. **Application**: IP, WB.

Lit. *Requirement for caspase-2 in stress-induced apoptosis before mitochondrial permeabilization*: P. Lassus, et al.; Science **297**, 1352 (2002)

***Fig**: Detection of procaspase-2 (p51) with anti-Caspase-2 MAb (11B4) (Prod. No. 804-356). Procaspase-2, an intermediate cleavage product of caspase-2 (p33), and activated caspase-2 (p19) was detected in FDC-P1 cells (mouse IL-3 dependent promyelocytic line) during growth factor withdrawl induced apoptosis. Activation of caspase-2 is inhibited in the presence of the caspase inhibitor Z-VAD-FMK (Prod. No. 260-020 or 260-138 (Ready-to-Use)).*



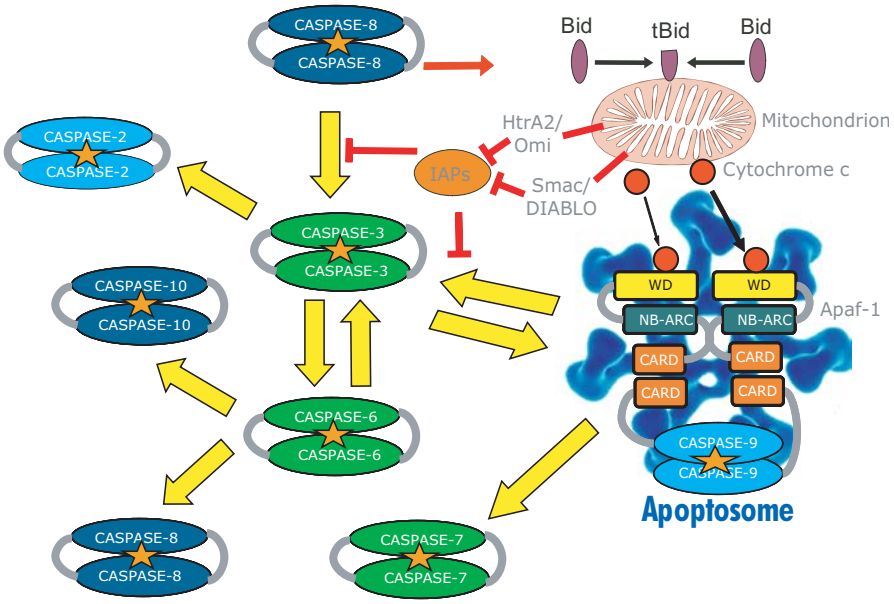
MAb to Caspase-2 (human) (G310-1248)

804-304-C100 100µg

Clone: G310-1248. **Isotype**: Mouse IgG. **Immunogen**: Recombinant human caspase-2. **Specificity**: Recognizes both human caspase-2 long (48kDa) and short forms. **Application**: WB.

Effector Caspases

Caspase-3, -6 & -7



While the initiator caspases can be activated by oligomerization, effector caspases are activated by other proteases, most commonly by initiator caspases, but also by other proteases (*trans* activation, e.g. activation caspase-3 by granzyme B). In contrast to the initiator caspases, which have extended prodomains (>90 aa, CARD or DED domains), effector caspases contain only 20-30 aa in their prodomains. The effector caspases orchestrate the direct dismantling of cellular structures, disruption of cellular metabolism, inactivation of cell-death inhibitory proteins and activation of additional destructive enzymes, like cleavage of initiator caspases as a positive feedback loop.

While many substrates cleaved by initiator caspases are relevant for the effective execution of apoptosis, many substrates seem to be just cleaved as bystanders because they happen to contain a caspase cleavage domain in their sequence. Some proteins might be cleaved very late and less completely during apoptosis and some substrates are cleaved in certain cells and not in others, while some yet other substrates are cleaved at different sites in different cell types. An intriguing question is how in certain cells active caspases are restricted to cleave certain substrates (e.g. cell cycle regulators), while leaving other vital proteins intact. In order to answer this question more needs to be known about the subcellular compartmentalization of caspases, the existence of scaffold proteins, different accessibility of cleavable substrates and the various positive and negative feedback loops of caspase activation. As the caspases emerge to have roles in other biological processes than apoptosis, such as cell cycle regulation and cellular differentiation, but also in various diseases upon increased activation, much remains to be learned about the potential dual role of caspases.

Most caspase substrates identified so far are cleaved by caspase-3, but several substrates that are efficiently cleaved by caspase-3 can also be targeted by caspase-7, suggesting at least partial redundance of both caspases.

Effector Caspases *continued*

Caspase-3, -6 & -7 *continued*

Selected caspase signalling pathways (for an excellent and extensive review see: *Many cuts to ruin: a comprehensive update of caspase substrates*: U. Fischer, et al.; Cell Death Differ. **10**, 76 (2003)):

Execution of Apoptosis:

- **DNA fragmentation and chromatin condensation**

  In the nucleus caspase-3 cleaves the DNase inhibitor ICAD to liberate active CAD nuclease that mediates DNA fragmentation. In addition, cleavage of acinus and the DNA helicase helicard, contributes to chromatin condensation and nuclear remodeling.

- **Deconstruction of the Cytoskeleton**

  Caspases destroy several proteins involved in maintenance of the cytoskeletal architecture such as vimentin, plectin and in epithelial cells cytokeratins.

- **Positive Feedback Loops**

  Caspases can turn off cell-protective mechanisms by cleaving apoptosis inhibitors such as FLIP and the anti-apoptotic members of the Bcl-2 proteins like Bcl-2 and Bcl-X_L. Caspase-8, the initiator caspase of the extrinisc pathway, also cleaves Bid to an active C-terminal fragment that induces the release of cytochrome c from the mitochondrion to use the intrinsic pathway as an amplifier.

- **Signal Transduction**

  Akt (protein kinase B) and Raf-1 are anti-apoptotic kinases which can be cleaved by caspase-3. On the other hand members of the PKC and MAP kinase pathway are activated through caspase cleavage leading to activation of JNK, which then phosphorylates and thus inactivates Bcl-2. Proteinphosphatase 2A (PP2A), which counteracts the survival function of kinases, is also activated by caspases.
  Interestingly low levels of caspase activity, such as those observed in differentiating cells, are associated with protective mechanisms. For instance, it was reported that the partial cleavage of RasGAP, a GTPase in the Ras signalling pathway, owing to low caspase activity first generates an N-terminal fragment that is antiapoptotic by activating the PI_3K pathway. Increased caspase levels, in contrast, result in the further cleavage of RasGAP into two proapoptotic fragments.

- **Caspases & Transcription**

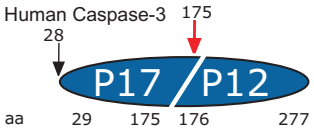
  Antiapoptotic transcription factors inhibited by caspases include the cAMP-responsive factor CREB, heat-shock factor HSF-1 and NF-κB. The NF-κB pathway is a paradigm of how caspase cleavage may result in a complete loss of the transcription factor's antiapoptotic function: (i) Cleavage of NF-κB subunit p65 (RelA) generates a dominant-negative fragment that is still able to bind to DNA but looses its transactivating activity, and therefore functions as a dominant-negative inhibitor. (ii) The NF-κB inhibitor IκBα is normally inducibly degraded by the proteasome. The N-terminal cleavage of IκBα by caspases generates a constitutive super-repressor that can no longer be removed by the proteasome. (iii) The cleavage of the adapter proteins TRAF-1 and RIP-1 that are involved in receptor-mediated pathways also contributes to impaired NF-κB activation and antiapoptotic capacity.

- **Prevention of Necrosis**

  PARP inactivation by caspase-3 is important for turning off an energetically expensive DNA repair pathway and for maintaining ATP levels that are required for the execution of apoptosis. PARP is rapidly activated during oxidative stress and DNA damage. Activated PARP then transfers more than 100 ADP-ribose moieties to each acceptor site in target proteins, and each cycle of ADP-ribosylation is coupled with consumption of one NAD molecule, which is metabolically equivalent to four ATP molecules. Hence, it can be imagined that excessive activation of PARP will quickly deplete cellular energy stores. In the absence of an energy pool sufficient to execute apoptosis or to maintain ionic homeostasis, cells can die quickly by necrosis. Indeed, when cells engineered to express caspase-resistant PARP are treated with apoptotic stimuli, they undergo extensive necrosis instead of apoptosis.

Latest Insight: As caspases emerge to play important roles in T cell proliferation (for a review see [1]), caspase-3 now has been shown to regulate the cell cycle in B cells [2]. For details see: [1] *Caspases and T lymphocytes: a flip of the coin?*: S. Lakhani & R.A. Flavell; Immunol. Rev. **193**, 22 (2003) [2] *Caspase-3 regulates cell cycle in B cells: a consequence of substrate specificity*: M. Woo, et al.; Nat. Immunol. **4**, 1016 (2003)

Enzymes



Procaspase-3 (human) (rec.)

201-082-C005 5µg

Purity: >90% (SDS-PAGE). Expressed in *E. coli*. **Application**: Very useful in studying mechanisms of caspase activation and regulation, as well as investigating caspase-3-mediated signal transduction pathways.

Caspase-3 (active) (human) (rec.)

201-038-C005 5µg

Expressed in *E. coli*. The rate of caspase-3 enzymatic hydrolysis can be measured by the release of AMC from the caspase substrate Ac-DEVD-AMC (Prod. No. 260-031) with emission at 440nm and excitation at 380nm using a spectrofluorometer.

Lit. *Biochemical characteristics of caspases-3, -6, -7 and -8*: H.R. Stennicke & G.S. Salvesen; J. Biol. Chem. **272**, 25719 (1997)

Caspase-3 (active) (human) (rec.)

201-059-U025 25 Units
201-059-U100 100 Units

Expressed in *E. coli*. **Specific Activity**: ≥300'000U/mg. One unit of rec. caspase-3 is the enzyme activity that cleaves 1 nmole of the caspase substrate DEVD-pNA per hour at 37°C in a buffered solution.

Caspase-3 (active) (human) (rec.) (high purity)

522-069-C005 5µg

Purity: >95% (SDS-PAGE). Expressed in *E. coli*. **Specific Activity**: ≥50'000U/mg. One unit of recombinant caspase-3 is the enzyme activity that cleaves 1nmole of the caspase substrate Ac-DEVD-AMC (Prod. No. 260-031) per minute at 37°C in a buffered solution.

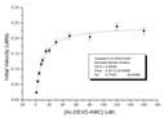


Fig: Caspase-3 Michaelis Menten Kinetics.

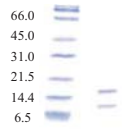


Fig: SDS-PAGE of 522-069.

Caspase-3 (active) (mouse) (rec.)

201-162-C050 50µg

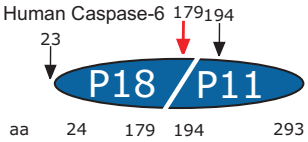
Purity: ~95% (gel electrophoresis). **Specific Activity**: 1500U/µmg. One unit is defined as the enzyme activity that cleaves 1 nmole of the caspase substrate Z-DEVD-AMC per hour at 30°C under the following reaction conditions: 20µM Z-DEVD-AMC, 50mM HEPES, pH 7.4, 100mM NaCl, 0.5% CHAPS, 10mM DTT, 1mM EDTA and 10% glycerol.

Caspase-3 (active) (rat) (rec.)

201-078-C005 5µg

Purity: >95% (SDS-PAGE). **Specific Activity**: ≥350U/µg. One unit is defined as the amount of enzyme that cleaves 1 nmole of the caspase substrate Z-DEVD-AMC per hour at 20°C. During the initiation of apoptosis the procaspase-3 is processed at aspartate residues to form the active enzyme. Includes both the proenzyme and the processed form.

Lit. *Presence of DNA fragmentation and lack of neuroprotective effect in DFF45 knockout mice subjected to traumatic brain injury*: A.G. Yakovlev, et al.; Mol. Med. **7**, 205 (2001)



Caspase-6 (active) (human) (rec.)

201-039-C005 5µg

Expressed in *E. coli*. The rate of caspase-6 enzymatic hydrolysis can be measured by the release of AMC from the caspase substrate Ac-DEVD-AMC (Prod. No. 260-031) with emission at 440nm and excitation at 380nm using a spectrofluorometer.

Lit. *Biochemical characteristics of caspases-3, -6, -7 and -8*: H.R. Stennicke & G.S. Salvesen; J. Biol. Chem. **272**, 25719 (1997)

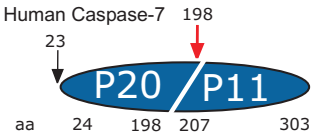
Caspase-6 (active) (human) (rec.)

201-060-U025 25 Units
201-060-U100 100 Units

Expressed in *E. coli*. **Specific Activity**: ≥13'000U/mg. One unit of rec. caspase-6 is the enzyme activity that cleaves 1 nmole of the caspase substrate VEID-pNA per hour at 37°C in a buffered solution.

Effector Caspases *continued*

Caspase-3, -6 & -7 *continued*



Caspase-7 (active) (human) (rec.)

201-040-C005 5µg

Expressed in *E. coli*. The rate of caspase-7 enzymatic hydrolysis can be measured by the release of AMC from the caspase substrate Ac-DEVD-AMC (Prod. No. 260-031) with emission at 440nm and excitation at 380nm using a spectrofluorometer.

Lit. *Biochemical characteristics of caspases-3, -6, -7 and -8*: H.R. Stennicke & G.S. Salvesen; J. Biol. Chem. **272**, 25719 (1997)

Caspase-7 (active) (human) (rec.)

201-061-U025 25 Units
201-061-U100 100 Units

Expressed in *E. coli*. **Specific Activity**: ~20'000U/mg. One unit of rec. caspase-7 is the enzyme activity that cleaves 1 nmole of the caspase substrate DEVD-pNA per hour at 37°C in a buffered solution.

Antibodies

MAb to Caspase-3 (human) (31A1067)

804-305-C100 100µg

Clone: 31A1067. **Isotype**: Mouse IgG1. **Immunogen**: Recombinant human caspase-3. **Specificity**: Recognizes human procaspase-3 and cleaved 12 and 17kDa catalytic subunits. **Application**: WB.

PAb to Caspase-3

210-806-C100 100µg

From rabbit. **Immunogen**: Synthetic peptide corresponding to aa 163-175 of human caspase-3. **Specificity**: Recognizes human, mouse and rat caspase-3. Detects bands of ~32kDa (procaspase-3) and ~20kDa (cleavage product) by WB. **Application**: WB.

PAb to Caspase-3 (active)

210-807-C100 100µg

From rabbit. **Immunogen**: Synthetic peptide around the cleavage site of human caspase-3. **Specificity**: Recognizes human, mouse and rat active caspase-3 (p20 subunit). **Application**: IHC (PS), WB.

MAb to Caspase-6 (human) (B93-4)

804-307-C100 100µg

Clone: B93-4. **Isotype**: Mouse IgG1. **Immunogen**: Synthetic peptide corresponding to aa 271-285 of human caspase-6. **Specificity**: Recognizes human procaspase-6 (34kDa) and active caspase-6 (11kDa). **Application**: WB.

PAb to Caspase-6 (human)

210-012-R050 50µl

From rabbit. **Immunogen**: Recombinant human caspase-6 (catalytic subunit). **Specificity**: Recognizes human caspase-6. Detects both procaspase-6 and active caspase-6. **Application**: WB.

Lit. *Investigation of glucocorticoid-induced apoptotic pathway: processing of caspase-6 but not caspase-3:* T. Miyashita, et al.; Cell Death Differ. **5**, 1034 (1998)

PAb to Caspase-6

210-810-C100 100µg

From rabbit. **Immunogen**: Synthetic peptide corresponding to aa 250-264 of human caspase-6. **Specificity**: Recognizes human, mouse, rat, hamster, monkey, rabbit, dog, pig, sheep and bovine caspase-6. Detects bands of ~34kDa (caspase-6) and ~22kDa (alternately spliced β-isoform) by WB. **Application**: WB.

PAb to Caspase-7 (human)

210-013-R050 50µl

From rabbit. **Immunogen**: Recombinant human caspase-7 (large catalytic subunit). **Specificity**: Recognizes human caspase-7. Detects both procaspase-7 and active caspase-7. **Application**: WB.

Lit. *Characterization of caspase processing and activation in HL-60 cell cytosol under cell-free conditions. Nucleotide requirement and inhibitor profile:* P.W. Mesner Jr., et al.; J. Biol. Chem. **274**, 22635 (1999)

PAb to Caspase-7

210-811-C100 100µg

From rabbit. **Immunogen**: Synthetic peptide corresponding to aa 264-279 of human caspase-7. **Specificity**: Recognizes human, mouse, rat, rabbit, monkey, dog, pig, sheep and hamster procaspase-7 (34kDa) and the p10 subunit of active caspase-7 (10kDa). **Application**: WB.

PAb to Caspase-7 (active)

210-813-C100 100µg

From rabbit. **Immunogen**: Synthetic peptide around the cleavage site of human caspase-7. **Specificity**: Recognizes human, mouse and rat caspase-7 p20 subunit. Does not cross-react with procaspase-7. **Application**: ICC, IHC, IP, WB.

Related Products

M30-Apoptosense® ELISA Kit

850-270-KI01 1 Kit
850-270-5001 5 Kits

Quantity: 96 wells. **Sensitivity**: 12U/l. **Measuring Range**: 50-1,000U/l. No high dose hook effect at 42,000U/l, which is high above the measuring range and obtainable values. Suitable for serum and plasma samples. Contains NEW improved Manual.

ELISA Kit for quantitative measurement of apoptosis in epithelial cells, especially when monitoring apoptotic carcinoma cell death in human serum or plasma samples. Recognizes the neo-epitope at the cleavage site DALD in the C-terminal domain of cytokeratin 18 (CK18) (aa 387-396), which is exposed after cleavage by caspases during apoptosis.

PAb to RasGAP (CT)

210-781-R100 100µl

From rabbit. **Immunogen**: Synthetic peptide corresponding to aa 1034-1047 of human RasGAP. **Specificity**: Recognizes human, mouse, rat and monkey full-length RasGAP as well as the C-terminal (CT) fragment generated by caspase cleavage (RasGAP aa 456-1047). Detects a band of ~150 kDa by WB. Does not cross-react with the N-terminal portion of RasGAP (including the SH2 and SH3 domains). **Application**: IP, WB.

Lit. *Caspase-dependent cleavage of signalling proteins during apoptosis. A turn-off mechanism for anti-apoptotic signals:* C. Widmann, et al.; J. Biol. Chem. **273**, 7141 (1998)/*Antiapoptotic signalling generated by caspase-induced cleavage of RasGAP:* J.-Y.Yang, et al.; Mol. Cell. Biol. **21**, 5346 (2002)/*The RasGAP N-terminal fragment generated by caspase cleavage protects cells in a Ras/PI3K/Akt-depepndent manner that does not rely on NF-kB activation:* J.-Y.Yang, et al.; J. Biol. Chem. **277**, 14641 (2002)/*Antiapoptotic signalling generated by caspase-induced cleavage of RasGAP:* J.Y.Yang & C.Widmann; Mol. Cell. Biol. **21**, 5346 (2001)/*Ras GTPase-activating Protein Binds to Akt and Is Required for Its Activation:* Y.Yue, et al.; J. Biol. Chem. **279**, 12883 (2004)

PAb to RasGAP (NT)

210-860-R100 100µl

From rabbit. **Immunogen**: Recombinant fusion protein corresponding to aa 158-455 (fragment N2) of human RasGAP. **Specificity**: Recognizes full-length human, mouse and rat RasGAP as well as the N-terminal fragment (that comprises SH2 and SH3 domains) generated by caspase cleavage (fragments N and N2). Does not cross-react with the C-terminal portion of RasGAP. **Application**: WB.

Lit. *Antiapoptotic signalling generated by caspase-induced cleavage of RasGAP:* J.Y.Yang & C.Widmann; Mol. Cell. Biol. **21**, 5346 (2001)

Caspases in Inflammation

Caspase-1, -4, -5 & -11

Caspase-1, also known as interleukin-1β-converting enzyme (ICE), is a specific intracellular cysteine protease required for the processing of some cytokines lacking a signal peptide to allow for release of the mature proteins from the intracellular compartment. The precursors of IL-1β and IL-18 have been identified as substrates for caspase-1. Both pro-IL-1β and pro-IL-18 are inactive until cleavage by caspase-1 occurs. Therefore, mice deficient in caspase-1 have a defective production and release of mature, bioactive IL-1β and IL-18, whereas the precursor forms are normally synthesized. Caspase-1 itself exists as inactive precursor and requires two internal cleavages before becoming enzymatically active; this activation can be induced by a variety of proinflammatory stimuli. IL-18 is constitutively expressed in the inactive precursor form mainly in monocytes macrophages and epithelial cells. IL-18 acts as an important costimulus for production of IFN-γ and other T helper type TH1 cytokines. In addition, IL-18, together with IL-12, facilitates T lymphocyte activation and the production of IFN-γ.

Although it is well established that the generation of IL-1β via cleavage of its pro-form requires the activity of caspase-1 (and caspase-11 in mice), the mechanism involved in the activation of the pro-inflammatory caspases remains elusive. Recent studies report the identification and initial characterization of a caspase-activating complex the so called inflammasome comprised of caspase-1, caspase-5, Asc (Pycard; CARD5) and NALP1 (NACHT-, LRR- and PYD-containing protein) or DEFCAP (NAC; CARD7), a pyrin domain-containing protein sharing structural homology with NODs and Apaf-1 [1-3]. Upon binding of the adapter protein Asc, NALP1 simultaneously recruits and activates caspase-1 and -5. Under cell-free conditions, the inflammasome assembly reproduces specific aspects of the activation and maturation of IL-1 *in vitro*. Inflammatory caspase activation and proIL-1β processing is lost upon prior immunodepletion of Asc, or in the presence of Asc antagonist antibodies (Prod. No. 210-905). A dominant-negative form of Asc blocks proIL-1β maturation and activation of inflammatory caspases induced by LPS.

The leucine-rich regions (LRRs) of NALP1 (like those found in the TLRs and NODs) may recognize pathogen associated molecular patterns (PAMPS) and/or endogenous non-foreign 'alarm signals' (e.g. mammalian DNA and heat shock proteins) and thereby trigger inflammasome assembly. LRRs hereby may act as a NALP regulatory unit.

ICEBERG and pseudo-ICE (COP) are proteins containing a single CARD domain that compete for binding to the caspase-1 prodomain, thus preventing inflammasome formation [4,5]. Pyrin inhibits the formation of the inflammasome by binding to the pyrin domain of Asc [6], while the proteinase inhibitor-9 (PI9) binds to the active site of caspase-1 [7,8].

Even though caspase-4 was discovered in 1995 [9-11], much less is known about it other than caspase-4 can process the same substrates as caspase-1 (including IL-1β), but less efficiently.

Caspases in Inflammation *continued*

Caspase-1, -4, -5, & -11 *continued*

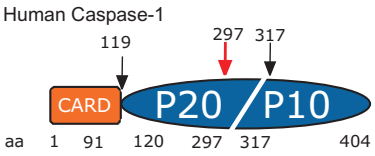
Human caspase-4 and -5 share the most sequence similarity to mouse caspase-11. Caspase-11 not only activates caspase-1, that is required for the maturation of proinflammatory cytokines such as interleukin-1 (IL-1) and IL-18, but also activates caspase-3, leading to cellular apoptosis under pathological conditions. In most cells, caspase-11 is only expressed upon induction with pro-inflammatory stimuli.

Caspase-11 is an essential mediator of endotoxic shock and caspase-11-deficient mice are resistant to endotoxic shock. LPS-induced caspase-11 regulates lymphocyte apoptosis by activating both caspase-3 and caspase-7. Human caspase-4, thought to be the homolog of mouse caspase-11, may be an effective therapeutic target for treatment of septic shock.

Lit. [1] *The Inflammasome. A Molecular Platform Triggering Activation of Inflammatory Caspases and Processing of proIL-beta*: F. Martinon,et al.;*Mol. Cell.* **10**,417 (2002) [2] *PYPAF7, a novel PYRIN-containing Apaf1-like protein that regulates activation of NF-kappa B and caspase-1-dependent cytokine processing*: L. Wang, et al.; J.Biol. Chem. **277**, 29874 (2002) [3] *The PYRIN-CARD protein ASC is an activating adapter for caspase-1*: S.M. Srinivasula, et al. J. Biol. Chem. **277**, 21119 (2002) [4] *Regulation of IL-1beta generation by Pseudo-ICE and ICEBERG, two dominant negative caspase recruitment domain proteins*: A. Druilhe, et al.; Cell Death Differ. **8**, 649 (2001) [5] *Cop, a caspase recruitment domain-containing protein and inhibitor of caspase-1 activation processing*: S.H. Lee, et al.; J. Biol. Chem. **276**, 34495 (2001) [6] *Interaction between pyrin and the apoptotic speck protein (ASC) modulates ASC-induced apoptosis*: N. Richards, et al.; J. Biol. Chem. **276**, 39320 (2001) [7] *Caspase-1 (interleukin-1beta-converting enzyme) is inhibited by the human serpin analogue proteinase inhibitor 9*: R.R. Annand, et al.; Biochem. J. **342**, 655 (1999) [8] *Modulators of inflammation use nuclear factor-kappa B and activator protein-1 sites to induce the caspase-1 and granzyme B inhibitor, proteinase inhibitor 9*: P. Kannan-Thulasiraman & D.J. Shapiro; J. Biol. Chem. **277**, 41230 (2002) [9] *A novel human protease similar to the interleukin-1 beta converting enzyme induces apoptosis in transfected cells*: C. Faucheu, et al.; EMBO J. **14**, 1914 (1995) [10] *Molecular cloning and pro-apoptotic activity of ICErelII and ICErelIII, members of the ICE/CED-3 family of cysteine proteases*: N.A. Munday, et al.; J. Biol. Chem. **270**, 15870 (1995) [11] *Identification and characterization of ICH-2, a novel member of the interleukin-1 beta-converting enzyme family of cysteine proteases*: J. Kamens, et al.; J. Biol. Chem. **270**, 15250 (1995) [12] *Expression analysis of the human caspase-1 subfamily reveals specific regulation of the CASP5 gene by lipopolysaccharide and interferon-gamma*: X.Y. Lin, et al.; J. Biol. Chem. **275**, 39920 (2000)

Selected Review Articles: *An innate sense of danger*: P. Matzinger; Ann. NY Acad. Sci. **961**, 341 (2002)/*The danger model: a renewed sense of self*: P. Matzinger; Science **296**, 301 (2002)/*Innate immune recognition*: C.A. Janeway, Jr. & R. Medzhitov; Annu. Rev. Immunol. **20**, 197 (2002)/*New insights into the mechanism of IL-1beta maturation*: K. Burns, et al.; Curr. Opin. Immunol. **15**, 26 (2003)/*NALPS: a novel protein family involved in inflammation*: J. Tschopp, et al.; Nat. Rev. Mol. Cell Biol. **4**, 95 (2003)/*Caspase-1 activation by Salmonella*: H.A. Jarvelainen, et al.; Trends Cell Biol. **13**, 204 (2003)

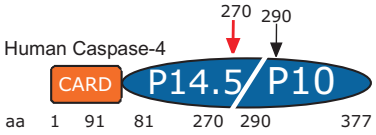
Enzymes



Caspase-1 (active) (human) (rec.)

201-056-U025 25 Units
201-056-U100 100 Units

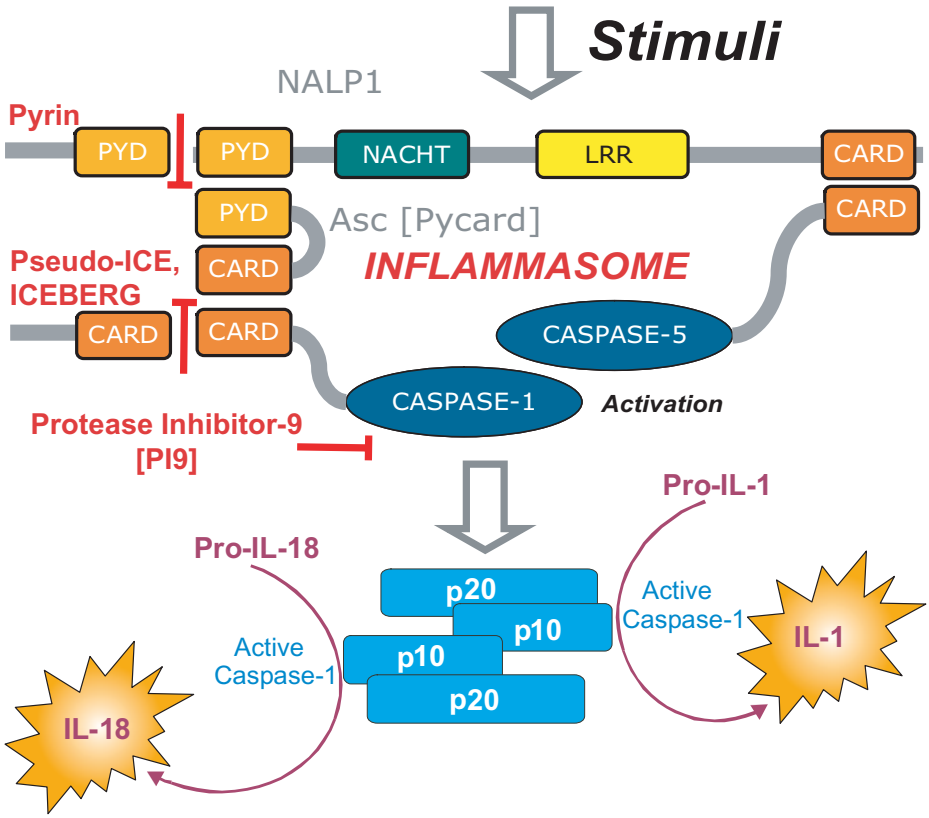
Expressed in *E. coli*. **Specific Activity**: ≥5'000U/mg. One unit of rec. caspase-1 is the enzyme activity that cleaves 1 nmole of the caspase substrate YVAD-pNA per hour at 37°C in a buffered solution.

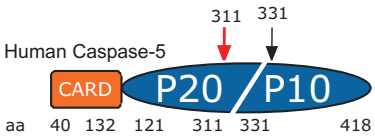


Caspase-4 (active) (human) (rec.)

201-093-U025 25 Units
201-093-U100 100 Units

Expressed in *E. coli*. **Specific Activity**: ≥5'000U/mg. One unit of rec. caspase-4 is the enzyme activity that cleaves 1 nmole of the caspase substrate WEHD-pNA per hour at 37°C in a buffered solution.

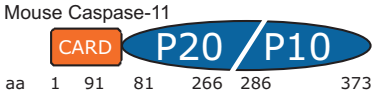




Caspase-5 (active) (human) (rec.)

201-094-U025 25 Units
201-094-U100 100 Units

Expressed in *E. coli*. **Specific Activity**: ≥5'000U/mg. One unit of rec. caspase-5 is the enzyme activity that cleaves 1 nmole of the caspase substrate WEHD-pNA per hour at 37°C in a buffered solution.



Antibodies

NEW MAb to Caspase-1 (mouse) (4G8)

804-531-C100 100µg

Clone: 4G8. **Isotype**: Rat IgG1. **Immunogen**: Mouse caspase-1 p20 fragment and a synthetic peptide corresponding to aa 206-220 of mouse caspase-1. **Specificity**: Recognizes mouse caspase-1. **Application**: ELSIA, FC, WB.

NEW MAb to Caspase-1 (mouse) (1H11)

804-530-C100 100µg

Clone: 1H11. **Isotype**: Rat IgG1. **Immunogen**: Mouse caspase-1 p20 fragment and a synthetic peptide corresponding to aa 206-220 of mouse caspase-1. **Specificity**: Recognizes mouse caspase-1. **Application**: ELSIA, FC, WB.

PAb to Caspase-1

210-804-C100 100µg

From rabbit. **Immunogen**: Synthetic peptide corresponding to aa 129-152 of human caspase-1. **Specificity**: Recognizes human, mouse and rat procaspase-1 (45kDa) and the 20kDa cleaved product. **Application**: ICC, IP, WB.

PAb to Caspase-4

210-808-C100 100µg

From rabbit. **Immunogen**: Synthetic peptide corresponding to aa 85-101 of human caspase-4. **Specificity**: Recognizes human and monkey procaspase-4. Detects a band of ~43kDa by WB. **Application**: WB.

PAb to Caspase-5

210-809-C100 100µg

From rabbit. **Immunogen**: Synthetic peptide corresponding to aa 159-176 of human caspase-5. **Specificity**: Recognizes human, mouse, rat, rabbit, bovine, monkey and hamster procaspase-5 (47kDa) and two cleavage intermediates (30kDa & 38kDa). **Application**: WB.

MAb to Caspase-11 (mouse) (8A5)

804-494-C100 100µg

Clone: 8A5. **Isotype**: Rat IgG1. **Immunogen**: p20 fragment of mouse caspase-11. **Specificity**: Recognizes mouse caspase-11 (epitope in the p20 fragment). Cross-reacts with rat caspase-11. Detects two bands at ~38 and ~43kDa by WB. **Application**: FC, WB.

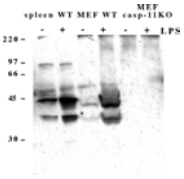


***Figure**: MAb 8A5 detects pro-caspase-11 in mouse embryonic fibroblasts (MEFs) from WT mice, but not in MEFs from caspase-11$^{-/-}$ mice, even after co-culture with LPS.*

Caspases in Inflammation cont.

Caspase-1, -4, -5, & -11 cont.

MAb to Caspase-11 (mouse) (4E11)

804-507-C100 100µg

Clone: 4E11. **Isotype**: Rat IgG1. **Immunogen**: p20 fragment of mouse caspase-11. **Specificity**: Recognizes mouse caspase-11 (epitope in the p20 fragment). Cross-reacts with rat caspase-11. Detects two bands at ~38 and ~43kDa by WB. **Application**: FC, WB.

PAb to Caspase-11

210-818-C100 100µg

From rabbit. **Immunogen**: Synthetic peptide corresponding to aa 202-217 of mouse caspase-11. **Specificity**: Recognizes mouse and rat caspase-11. Detects full length caspase-11 and cleavage fragments by WB. **Application**: WB.

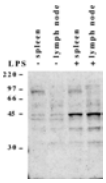


Figure: MAb 4E11 detects endogenous caspase-11 in mouse spleen and lymph node as two bands of 43 and 38 kDa. After 16 hrs co-culture with LPS 20 µg/mL elevated levels of caspase-11 are detected in mouse spleen and lymph node.

Related Products

Enzymes

Interleukin-1β, Soluble (human) (rec.)

522-056-C010 10µg

Produced in bacteria. Human Interleukin-1β (IL-1β) (aa 117-270) is fused to a linker peptide (10aa) and an N-terminal FLAG-tag.

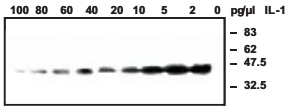


Figure: IL-1β activity was assessed by its ability to induce activation of the NF-κB pathway. IκBα degradation was detected.

Interleukin-1β (human) (rec.) (cell culture grade)

520-001-C010 10µg

Produced in bacteria. **Specific Activity**: >3x10^7 units/mg. Exerts *in vitro* biological activity in the range of 0.1 to 10ng/ml.

ELISA Kits

Interleukin-1β Precursor (human) ELISA Kit

850-058-KI01 1 Kit

Quantity: 96 wells (~80 tests). **Sensitivity**: 15pg/ml. **Application**: The kit is suitable for use in cell culture supernatants, human serum, plasma and other biological fluids.

Interleukin-1β (human) ELISA Kit

850-209-KI01 1 Kit

Quantity: 96 wells (~80 tests). **Sensitivity**: 15pg/ml. **Application**: To investigate the complex regulation of IL-1β measurement of both precursor and mature isoforms is necessary. Measurement of IL-1β precursor with IL-1β Precursor ELISA Kit (Prod. No. 850-058) in conjunction with 850-209 enables detection of total IL-1β levels in samples. The kit is suitable for use in cell culture supernatants, human serum, plasma and other biological fluids.

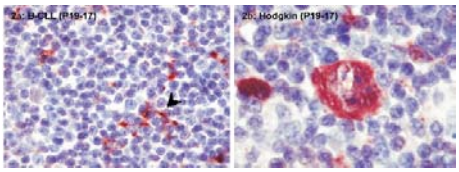


Figure: The following tumors were stained for PI9: a) B-CLL and b) classical Hodgkin disease, nodular sclerosing subtype.

Related Products continued

PAb to Asc (human) (AL177)

[anti-Pycard PAb (AL177)]

210-905-R100 100µl

From rabbit. **Immunogen**: N-terminal human Asc (G^{2}R-ARDAILDALENLTAEELKKFKLKL^{27}). **Specificity**: Recognizes human Asc. **Application**: ICC, IP, WB.

Lit. *The Inflammasome. A Molecular Platform Triggering Activation of Inflammatory Caspases and Processing of proIL-beta*: F. Martinon, et al.; Mol. Cell. **10**, 417 (2002)

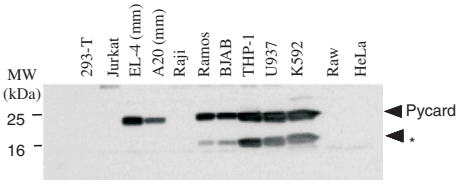


*Figure: Western blot analysis of human and mouse cell lines with the anti-human Asc antibody (AL177). **Method**: Total protein extracts from various human (293-T, Jurkat, Raj, Ramos, BJAB, THP-1, U937, K562, Raw, HeLa) and mouse (EL-4, A20) cell lines were run on SDS-PAGE and Pycard detected by Prod. No. 210-905 at 1:1000 dilution. Anti-rabbit IgG coupled horse radish peroxidase was used at 1:5000 dilution for ECL detection.*

MAb to NALP1 (human) (Nalpy1-4)

[anti-CARD7 MAb (Nalpy1-4)]

804-803-C100 100µg

Clone: Nalpy1-4. **Isotype**: Mouse IgG1. **Immunogen**: Recombinant human NALP1 (pyrin domain). **Specificity**: Recognizes the pyrin domain (PYD) of human NALP1. **Application**: ICC, IHC, (FS, PS), IP, WB.

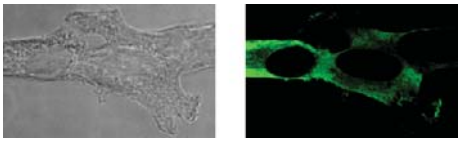


Figure: Detection of NALP1 in 293T cells transfected with a human NALP1 expression plasmid. Left: Phase contrast. Right: Staining with MAb to NALP1 (Nalpy1-4).

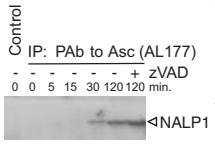


*Figure: Western blot analysis of the time course of assembly of the inflammasome with MAb to NALP1 (Nalpy1-4) (Prod. No.804-803) in THP-1 macrophages. **Method**: Assembly of the inflammasome was induced by shifting the temperature to 30°C after hypotonic lysis. THP-1 cell extracts were immunoprecipitated with PAb to Asc (AL177) (Prod. No. 210-905) and run on SDS-PAGE. NALP1 was detected by MAb to NALP1 (Nalpy1-4) (Prod. No. 804-803) at 1:1'000 dilution. Anti-mouse IgG coupled horse radish peroxidase was used at 1:5'000 dilution for ECL detection. For more information about the "time course assembly of inflammasome" method see F. Martinon; Mol. Cell. **10**, 417 (2002).*

PAb to NALP1 (human) (AL176)

[anti-CARD7 PAb (AL176)]

210-904-R100 100µl

From rabbit. **Immunogen**: N-terminal human NALP1 (A^{2}GGAWGRLACYLEFLKKEELKEFQ^{25}). **Specificity**: Recognizes human NALP1. **Application**: WB.

Lit. *The Inflammasome. A Molecular Platform Triggering Activation of Inflammatory Caspases and Processing of proIL-beta*: F. Martinon, et al.; Mol. Cell. **10**, 417 (2002)

MAb to Proteinase Inhibitor 9 [PI9] (human) (PI9-17)

804-457-C125 125µg

Clone: PI9-17. **Isotype**: Mouse IgG1. **Immunogen**: Human full-length recombinant proteinase inhibitor 9 (PI9). **Specificity**: Recognizes human PI9. Does not cross-react with other homologous serpins (PI6, PI8 and PAI-2). Detects bands of ~42kDa (native PI9) and ~63kDa (PI9 complexed to granzyme B) by WB. **Application**: IHC (PS), WB.

Caspase-12 & -14

Caspase-12 has been implicated in an endoplasmic reticulum (ER) stress-associated caspase cascade, in which caspase-12 functions as a initiator caspase in the context of ER stress, but caspase-12 might also be activated through cleavage from m-calpain, a representative of another cysteine protease family. But elucidating the roles of caspase-12 has been hampered by the fact that a) mice deficient in caspase-12 have no noticeable developmental or behavioral defects and b) by the observation that the human CASP-12 gene does not appear to be expressed, as most of the individuals examined to date possessed CASP-12 genes that contain frameshift mutations that introduce a stop codon and produce an inactive truncated protein, thus undermining the existence of a caspase-12-dependent ER stress pathway of apoptosis.

MAb to Caspase-12 (mouse) (11F10)

804-509-C100 100µg

Clone: 11F10. **Isotype**: Rat IgG1. **Immunogen**: P20 fragment of mouse caspase-12 and a synthetic peptide corresponding to aa 183-205 of mouse caspase-12. Detects a band of ~51kDa by WB. **Specificity**: Recognizes mouse caspase-12. **Application**: ELISA, FC, WB.

MAb to Caspase-12 (mouse) (12G6)

804-511-C100 100µg

Clone: 12G6. **Isotype**: Rat IgG1. **Immunogen**: P20 fragment of mouse caspase-12 and a synthetic peptide corresponding to aa 183-205 of mouse caspase-12. Detects a band of ~51kDa by WB. **Specificity**: Recognizes mouse caspase-12. **Application**: ELISA, FC, WB.

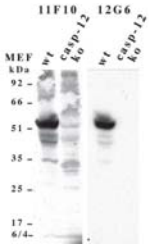


Figure: Western blot analysis of procaspase-12 in wild type and caspase-12 KO mice.

PAb to Caspase-12 (IN)

PSC-2327-C100 100µg

From rabbit. **Immunogen**: Synthetic peptide corresponding to aa 100-116 of mouse caspase-12. **Specificity**: Recognizes mouse and rat caspase-12. Detects a band of ~51kDa by WB. **Application**: IHC (PS), WB. **Blocking Peptide**: Prod. No. PSC-2327P.

PAb to Caspase-12 (NT)

PSC-2325-C100 100µg

From rabbit. **Immunogen**: Synthetic peptide corresponding to aa 2-17 of mouse caspase-12. **Specificity**: Recognizes mouse and rat caspase-12. Detects a band of ~51kDa by WB. **Application**: WB. **Blocking Peptide**: Prod. No. PSC-2325P.

Caspase-14 possesses an unusally short prodomain (therefore also called mini-ICE or MICE) and is highly expressed in embryonic tissue and epidermal keratinocytes. Caspase-14 is not activated during apoptosis induced by UV irradiation or cytotoxic substances and is thought to play an important role in the physiological cell death of keratinocytes leading to skin barrier formation.

PAb to Caspase-14 (mouse)

210-842-C200 200µg

From rabbit. **Immunogen**: Synthetic peptide corresponding to aa 165-185 (A^{165}VLKNNPQSIPTY-TDTLHIYS^{185}) of mouse caspase-14. **Specificity**: Recognizes mouse caspase-14. **Application**: ICC, IHC (PS), WB. **Blocking Peptide**: Prod. No. 153-044.

Noncaspase Proteases

Granzymes

Granzymes are granule-stored lymphocyte serine proteases that are implicated in T and natural killer cell-mediated cytotoxic defense reactions after target cell recognition. All granzymes are synthesized as pre-pro-enzymes in the endoplasmatic reticulum and converted into active enzymes in a two-step process by cleavage of the signal peptide and subsequent removal of the propeptide by a similar, presumably identical dipeptidyl aminopeptidase of cytosolic granules, called cathepsin C.

Granzyme A is necessary for target cell lysis in cell-mediated immune response and expressed in all cytolytic T and natural killer cells. Although granzyme A does not activate oligonucleosomal DNA fragmentation, it causes single-stranded DNA nicking and other characteristic features of apoptosis, including membrane perturbation, chromatin condensation, and loss of mitochondrial inner membrane potential. Granzyme A cleaves preferentially after Lys or Arg and has been found to act on lamins A, B, and C, disrupting the nuclear lamina. It also degrades histone H1 and proteolyzes the tails of the core histones, opening up chromatin to exogenous DNases.

Granzyme B is a 27kDa serine protease stored in granules of activated cytotoxic T cells and NK cells. Upon target cell contact, granzyme B is directionally exocytosed and enters the target cell assisted by perforin. With its unique substrate specificity (cleaving after Asp), granzyme B processes and activates various pro-caspases, thereby inducing apoptosis in the target cell. It is the only mammalian serine protease that prefers acidic side chains, a finding of relevance for its role as a pro-apoptotic enzyme as it allows cleavage of Bid and the pro-caspases.

Although recombinant granzyme K has been produced by several groups, little is known about its activities. In one report, purified native granzyme K induced caspase-independent cell death without apoptotic nuclear morphology, but with disruption of the mitochondrial potential and mitochondrial dysfunction, as measured by the generation of reactive oxygen species.

Selected Review Articles: *Granzymes (lymphocyte serine proteases): characterization with natural and synthetic substrates and inhibitors*: C.M. Kam, et al.; Biochim. Biophys. Acta **1477**, 307 (2000)/*Noncaspase proteases in apoptosis*: D.E. Johnson; Leukemia **14**, 1695 (2000)/*Granzymes: a family of lymphocyte granule serine proteases*: J.A. Trapani; Genome Biol. **2**, 3014 (2001)/*Lymphocyte-mediated cytotoxicity*: J.H. Russell & T.J. Ley; Annu. Rev. Immunol. **20**, 323 (2002)/*A view to a kill: signals triggering cytotoxicity*: J.Y. Djeu, et al.; Clin. Cancer Res. **8**, 636 (2002)/*Cytotoxic T lymphocytes: all roads lead to death*: M. Barry & R.C. Bleackley; Nat. Rev. Immunol. **2**, 401 (2002)/*The ABCs of granule-mediated cytotoxicity: new weapons in the arsenal*: J. Lieberman; Nat. Rev. Immunol. **3**, 361 (2003)/*Mitochondria at the heart of the cytotoxic attack*: D.L. Roberts, et al.; BBRC **304**, 513 (2003)

Enzymes

NEW Granzyme A (human) (rec.)

201-118-C010 10µg

Active human granzyme A expressed in *E. coli*.

NEW Granzyme B (human) (rec.)

201-112-C010 10µg

Active human granzyme B expressed in *E. coli*.

Lit. *Crystal structure of the caspase activator human granzyme B, a proteinase highly specific for an Asp-P1 residue*: E. Estebanez-Perpina, et al.; Biol. Chem. **381**, 1203 (2000)

NEW Granzyme K (human) (rec.)

201-117-C010 10µg

Active human granzyme K expressed in *E. coli*.

Lit. *Generation of catalytically active granzyme K from Escherichia coli inclusion bodies and identification of efficient granzyme K inhibitors in human plasma*: E. Wilharm, et al.; J. Biol. Chem. **274**, 27331 (1999)

Antibodies

NEW MAb to Granzyme A (human) (GA6)

804-142-C200 200µg

Clone: GA6. **Isotype**: Mouse IgG1. **Immunogen**: Human recombinant granzyme A. **Specificity**: Recognizes human granzyme A. **Application**: IHC (PS).

Lit. *Production and characterization of monoclonal antibodies raised against recombinant human granzymes A and B and showing cross reactions with the natural proteins*: J.A. Kummer, et al.; J. Immunol. Meth. **163**, 77 (1993)

MAb to Granzyme B (human) (B18.1)

804-121-C100 Purified 100µg

804-121F-T100 FITC 100 tests

Clone: B18.1. **Isotype**: Mouse IgG1. **Immunogen**: Human recombinant granzyme B (aa 7-227). **Specificity**: Recognizes human granzyme B. **Application**: FC, ICC, IHC (FS, PS), IP, WB.

Lit. *Granzyme B and perforin can be used as predictive markers of acute rejection in heart transplantation*: S. Legros-Maida, et al.; Eur. J. Immunol. **24**, 229 (1994)/*Perforin and granzyme B expression is associated with severe acute rejection. Evidence for in situ localization in alveolar lymphocytes of lung-transplanted patients*: M.V. Clement, et al.; Transplantation **57**, 322 (1994)/*Granzyme B and perforin lytic proteins are expressed in CD34+ peripheral blood progenitor cells mobilized by chemotherapy and granulocyte colony-stimulating factor*: C. Berthou, et al.; Blood **86**, 3500 (1995)/*Expansion of a peripheral blood perforin+ CD8+ T-cell subset in long-term surviving lung transplanted patients*: C. Berthou, et al.; Transplant. Proc. **28**, 1964 (1996)

NEW MAb to Granzyme B (human) (GB11) (R-PE)

804-143R-C100 R-PE 100µg

Clone: GB11. **Isotype**: Mouse IgG1. **Immunogen**: Human granzyme B. **Specificity**: Recognizes human, chimpanzee and rhesus monkey granzyme B. **Application**: FC. Conjugated with R-phycoerythrin (R-PE).

Lit. *Phenotypic and functional separation of memory and effector human CD8+ T cells*: D. Hamann, et al.; J. Exp. Med. **186**, 1407 (1997)/*The CD8+ granzyme B+ T-cell subset in peripheral blood from healthy individuals contains activated and apoptosis-prone cells*: P.C. Wever, et al.; Immunology **93**, 383 (1998)/*Extracellular granzymes A and B in humans: detection of native species during CTL responses in vitro and in vivo*: E.H. Spaeny-Dekking, et al.; J. Immunol. **160**, 3610 (1998)

MAb to Granzyme B (human) (GrB7)

804-198-C050 50µg

Clone: GrB7. **Isotype**: Mouse IgG2a. **Immunogen**: Human granzyme B. **Specificity**: Recognizes human granzyme B. Does not cross-react with granzyme A. **Application**: IHC (PS; not recommended for FS), WB.

Lit. *Production and characterization of monoclonal antibodies raised against recombinant human granzymes A and B and showing cross reactions with the natural proteins*: J.A. Kummer, et al.; J. Immunol. Methods **163**, 77 (1993)/*For a comprehensive bibliography please visit our website.*

MAb to Granzyme B (human) (GM-4C1)

804-483-C100 100µg

Clone: GM-4C1. **Isotype**: Mouse IgG1. **Immunogen**: Vector containing the cDNA of human granzyme B. **Specificity**: Recognizes human granzyme B. Does not cross-react with granzyme A, granzyme K or granzyme M. **Application**: FC. *Manufactured by GENOVAC GmbH.*

MAb to Granzyme K (human) (GM-24C3)

804-484-C100 100µg

Clone: GM-24C3. **Isotype**: Mouse IgG2b. **Immunogen**: Vector containing the cDNA of human granzyme K. **Specificity**: Recognizes human granzyme K. Does not cross-react with granzyme A, granzyme B or granzyme M. **Application**: FC. *Manufactured by GENOVAC GmbH.*

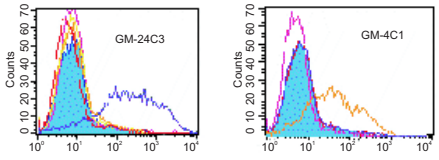


***Figure**: Specificity testing of GM-4C1 (Prod. No. 804-483) & GM-24C3 (Prod. No. 804-484).*

Related Products

The tumor metastasis suppressor and nucleoside diphosphate kinase NM23-H1 has been identified as a granzyme A-activated DNase (GAAD) [1], which possesses 3'-5' exonuclease activity [2]. While p53 upregulates NM23-H1 expression [3], an effect that can be also mimicked by dexamethasone (Prod. No. 370-002) [4], PRUNE protein interacts with NM23-H1 and promotes cancer metastasis. This effect is attributed to the nucleotide phosphodiesterase activity of prune [5]. NM23-H1 forms an endoplasmatic reticulum (ER)-associated complex with the tumor suppressor pp32 and three granzyme A substrates: the inhibitor of protein phosphatase 2A PHAP II (SET, I_2^{PP2A}), the DNA binding protein HMG2 and the base excision repair enzyme Ape1/Ref1. Granzyme A cuts PHAP II, removing its inhibitory effect on NM23-H1, which then translocates to the nucleus where it nicks chromosomal DNA and induces caspase-independent cell death.

Lit. [1] *Tumor suppressor NM23-H1 is a granzyme A-activated DNase during CTL-mediated apoptosis, and the nucleosome assembly protein SET is its inhibitor*: Z. Fan, et al.; Cell **112**, 659 (2003) [2] *The metastasis suppressor NM23-H1 possesses 3'-5' exonuclease activity*: D. Ma, et al.; J. Biol. Chem. **279**, *in press* (2004) [3] *P53 is a regulator of the metastasis suppressor gene Nm23-H1*: S.L. Chen, et al.; Mol. Carcinog. **36**, 204 (2003) [4] *Dexamethasone and medroxyprogesterone acetate elevate nm23-h1 metastasis suppressor gene expression in metastatic human breast carcinoma cells: new uses for old compounds*: T. Ouatas, et al.; Clin. Cancer Res. **9**, 3763 (2003) [5] *Prune cAMP phosphodiesterase binds nm23-H1 and promotes cancer metastasis*: A. D'Angelo, et al.; Cancer Cell **5**, 137 (2004)

NEW PAb to NM23-H1/GAAD (human) (K73)

210-911-C100 100µg

From rabbit. **Immunogen**: Recombinant human NMH23-1 (GAAD). **Specificity**: Recognizes human and mouse NM23-H1 and NMH23-H2. **Application**: ICC, IHC (PS), WB.

Lit. *Prune cAMP phosphodiesterase binds nm23-H1 and promotes cancer metastasis*: A. D'Angelo, et al.; Cancer Cell **5**, 137 (2004)

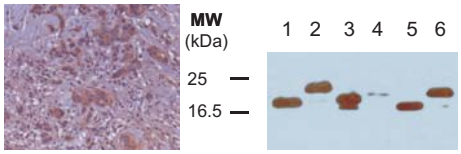


***Figure**: left) Human breast stained with PAb to NM23-H1 (human) (K73). right) Western blot analysis of NM23-H1 and NM23-H2 with PAb to NM23-H1 (human) (K73). Lane 1, rec. human NM23-H1; lane 2, His-tagged rec. human NM23-H1; lane 3, MDA H1-177 cells expressing NM23-H1; lane 4, COS-7 cells expressing NM23-H1; lane 5, rec. human NM23-H2; lane 6, His-tagged rec. human NM23-H2.*

NEW PAb to PRUNE (human) (A59)

210-912-C100 100µg

From rabbit. **Immunogen**: Recombinant human PRUNE. **Specificity**: Recognizes human PRUNE. **Application**: ICC, IHC (PS), IP, WB.

Lit. *Amplification and overexpression of PRUNE in human sarcomas and breast carcinomas-a possible mechanism for altering the nm23-H1 activity*: A. Forus, et al.; Oncogene **20**, 6881 (2001)/*Prune cAMP phosphodiesterase binds nm23-H1 and promotes cancer metastasis*: A. D'Angelo, et al.; Cancer Cell **5**, 137 (2004)

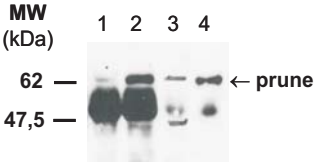


***Figure**: Immunoprecipitation and Western blot analysis of PRUNE with PAb to PRUNE (human) (A59). Lane 1, Cell extracts from MDA-MB-435 wild type; lane 2, MDA-MB-435 stable clone expressing human PRUNE; lane 3, negative control; lane 4, rec. human PRUNE.*

Noncaspase Proteases *continued*

Cathepsins, Calpain & Related Products

The cathepsin protease family consists of at least 16 known members and can be subdivided into three distinct groups: serine proteases (cathepsin A and G), aspartate proteases (cathepsin D and E) and, like the caspases, cysteine proteases (cathepsins B, C, F, H, K, L, O, S, T, V, W and X). Cathepsins are synthesized as inactive zymogens and activation involves proteolytic processing. Cathepsin B, D and L are found primarily in lysosomes and endosomes and during apoptosis they are translocated to the cytosol.

Cathepsin D triggers Bax activation resulting in the selective release of mitochondrial apoptosis inducing factor (AIF) independent from caspases [1] and thus might in some cell types act upstream of cytochrome c release and caspase activation [2]. Cathepsin B cleaves Bid resulting in a rapid release of cytochrome c triggering apoptosis [3] and, since cathepsin D can directly cleave and activate cathepsin B [4,5], the cathepsins, like the caspases, may be activated in a cascade-like fashion during apoptosis. Cystatins (stefins) are the endogenous inhibitors of cathepsins. For reviews on cathepsins and their role in apoptosis see [6-9].

Calpains are calcium-dependent thiol proteases which are widely expressed with ubiquitous and tissue specific isoforms. Calpains have been implicated in basic cellular processes including cell proliferation, differentiation and apoptosis. Examples of calpain involvement in apoptosis include cleavage of p53 [10], Bax, the proapoptotic effect of which is thereby increased [11-13] and Bid [14]. Calpastatin is the endogenous inhibitor of calpain. For reviews see [15-19].

Lit. [1] *Cathepsin D triggers Bax activation, resulting in selective apoptosis-inducing factor (AIF) relocation in T lymphocytes entering the early commitment phase to apoptosis*: N. Bidere, et al.; J. Biol. Chem. **278**, 31401 (2003) [2] *Cathepsin D mediates cytochrome c release and caspase activation in human fibroblast apoptosis induced by staurosporine*: A.C. Johansson, et al.; Cell Death Differ. **10**, 1253 (2003) [3] *Selective disruption of lysosomes in HeLa cells triggers apoptosis, mediated by cleavage of Bid by multiple papain-like lysosomal cathepsins*: T. Cirman, et al.; J. Biol. Chem. **278**, *in press* (2003) [4] *Identification of latent procathepsins B and L in microsomal lumen: characterization of enzymatic activation and proteolytic processing in vitro*: Y. Nishimura, et al.; Arch. Biochem. Biophys. **261**, 64 (1988) [5] *Rat procathepsin B. Proteolytic processing to the mature form in vitro*: A.D. Rowan, et al.; J. Biol. Chem. **267**, 15993 (1992) [6] *Noncaspase proteases in apoptosis*: D.E. Johnson; Leukemia **14**, 1695 (2000) (Review) [7] *Lysosomal cysteine proteases: more than scavengers*: B. Turk, et al.; Biochim. Biophys. Acta **1477**, 98 (2000) (Review) [8] *Lysosomal cysteine proteases: facts and opportunities*: V. Turk, et al.; EMBO J. **20**, 4629 (2001) (Review) [9] *Four deaths and a funeral: from caspases to alternative mechanisms*: M. Leist & M. Jaattela; Nat. Rev. Mol. Cell Biol. **2**, 589 (2001) (Review) [10] *Proteolytic cleavage of human p53 by calpain: a potential regulator of protein stability*: M.H. Kubbutat & K.H. Vousden; Mol. Cell Biol. **17**, 460 (1997) [11] *Bax cleavage is mediated by calpain during drug-induced apoptosis*: D.E. Wood, et al.; Oncogene **17**, 1069 (1998) [12] *N-terminal cleavage of Bax by calpain generates a potent proapoptotic 18-kDa fragment that promotes Bcl-2-independent cytochrome C release and apoptotic cell death*: G. Gao & Q.P. Dou; J. Cell. Biochem. **80**, 53 (2001) [13] *Calpain-1 regulates Bax and subsequent Smac-dependent caspase-3 activation in neutrophil apoptosis*: F. Altznauer, et al.; J. Biol. Chem. **278**, *in press* (2003) [14] *Calpain-mediated Bid cleavage and calpain-independent Bak modulation: two separate pathways in cisplatin-induced apoptosis*: A. Mandic, et al.; Mol. Cell. Biol. **22**, 3003 (2002) [15] *Calpain and caspase: can you tell the difference?*: K.K. Wang; TINS **23**, 20 (2000) (Review) [16] *Noncaspase proteases in apoptosis*: D.E. Johnson; Leukemia **14**, 1695 (2000) (Review) [17] *Four deaths and a funeral: from caspases to alternative mechanisms*: M. Leist & M. Jaattela; Nat. Rev. Mol. Cell Biol. **2**, 589 (2001) (Review) [18] *Calpain*: B.J. Perrin & A. Huttenlocher; Int. J. Biochem. Cell Biol. **34**, 722 (2002) (Review) [19] *Ischemic neuronal death in the rat hippocampus: the calpain-calpastatin-caspase hypothesis*: A. Rami; Neurobiol. Dis. **13**, 75 (2003) (Review)

Product Overview Cathepsins, Calpains & Related Products

Enzymes

Cathepsin G (human neutrophils)
200-310-C100 100µg

Cathepsin L (human)
200-314-1 1 Vial

Cystatin C (human)
200-087-C100 100µg

Antibodies

anti-Calpain MAb (156), *mouse IgG1*
804-053-R100 100µl
Reactivity: H O Application: WB

anti-µ-Calpain (domain II) MAb (2H2A7C2), *mouse IgG1*
804-051-R100 100µl
Reactivity: H O Application: IC WB

anti-µ-Calpain (domain III) MAb (9A4H8D3), *mouse IgG1*
804-050-R100 100µl
Reactivity: H O Application: IC WB

anti-Calpastatin (domain II) MAb (2G11D6), *mouse IgG2a*
804-055-R100 100µl
Reactivity: H O Application: IC WB

anti-Calpastatin (domain IV) MAb (1F7E3D10), *mouse IgG2a*
804-054-R100 100µl
Reactivity: H O Application: IC WB

anti-Cathepsin B / Procathepsin B (human) MAb (CB 59-4B11), *mouse IgG1*
LBS-AB-CB-1 200µg
Reactivity: H Application: IH WB

anti-Cathepsin L / Procathepsin L MAb (CPLH 33/2), *mouse IgG1*
LBS-AB-CL-2003 100µg
Reactivity: H O Application: EL IH WB

anti-Cathepsin L / Procathepsin L MAb (CPLH 3G10), *mouse IgG1*
LBS-AB-CL-2005 200µg
Reactivity: H M O Application: EL WB

anti-Cathepsin L / Procathepsin L (human) MAb (CPLH 33/1), *mouse IgG1*
LBS-AB-CL-2002 100µg
Reactivity: H Application: EL IH WB

anti-Cathepsin L / Procathepsin L (human) MAb (CLP 1/36), *mouse IgG1*
LBS-AB-CL-2004 200µg
Reactivity: H Application: IH WB

anti-Cathepsin V / Procathepsin V (human) MAb (CV 55-1C5), *mouse IgG1*
LBS-AB-CV-1 200µg
Reactivity: H Application: IH WB

anti-Cathepsin W / Procathepsin W (human) MAb (CW 39-1B10), *mouse IgG2b*
LBS-AB-CW-1 200µg
Reactivity: H Application: IH WB

anti-Cathepsin W / Procathepsin W (human) MAb (CW 39-2B6), *mouse IgG2b*
LBS-AB-CW-2 200µg
Reactivity: H Application: IH WB

anti-Cystatin A (human) MAb (WR 23/2/3/3), *mouse IgG1*
LBS-AB-CyB-1 200µg
Reactivity: H Application: IH WB

anti-Cystatin B (human) MAb (RJMV 2E7), *mouse IgG2a*
LBS-AB-CA-2006 200µg
Reactivity: H Application: IH WB

anti-Cystatin C (human) PAb, *from rabbit*
210-412-C100 100µg
Reactivity: H Application: EL WB

anti-Procathepsin L (human) MAb (CPLH 2D4), *mouse IgG1*
LBS-AB-CL-2001 200µg
Reactivity: H Application: EL IH WB

anti-Procathepsin W (human) MAb (CW-40 1B1), *mouse IgG1*
LBS-AB-CW-3 200µg
Reactivity: H Application: FC IH WB

ELISA Kits

Cathepsin L (human) ELISA Kit
850-240-KI01 1 Kit

Cystatin C (human) ELISA Kit
850-292-KI01 1 Kit

Inhibitors

N-Acetyl-eglin C (rec.)
201-006-MC01 0.1mg
201-006-MC05 0.5mg
201-006-M001 1mg
An effective inhibitor of chymotrypsin and subtilisin as well as of leukocyte elastase and cathepsin G.

H-Arg-Lys-Leu-Leu-Trp-NH_2
260-136-M001 1mg
260-136-M005 5mg
Highly potent peptide inhibitor of human cathepsin L.

CA-074 [N-[L-3-*trans*-Propylcarbamoyloxirane-2-carbonyl]-Ile-Pro-OH]
260-017-M001 1mg
Potent and specific inhibitor of cathepsin B *in vitro* and *in vivo*.

Chymostatin
260-005-M001 1mg
260-005-M005 5mg
Inhibitor of serine proteinases having a chymotrypsin-like specificity like α-, β-, δ- and γ-chymotrypsin, chymases, cathepsin G, and most cysteine proteinases including papain and cathepsin A, B, H, and L.

E-64
260-007-M005 5mg
260-007-M025 25mg
Irreversible inhibitor of cysteine proteinases like papain, cathepsin B and L. Acts by forming a thioether bond with the thiol of the active cysteine. Does not inhibit serine proteinases.

Calpain Inhibitor I [Ac-Leu-Leu-norleucinal]
260-037-M010 10mg
260-037-M050 50mg
Inhibits calpain I (K_i = 190 nM), calpain II (K_i = 220 nM), cathepsin B (K_i = 150 nM) and cathepsin L (K_i = 0.5 nM). Inhibits neutral cysteine proteases and proteasome.

Calpain Inhibitor II [Ac-Leu-Leu-methioninal]
260-038-M010 10mg
260-038-M050 50mg
Inhibitor of calpain I, calpain II, cathepsin B and cathepsin L.

Calpeptin
260-014-M005 5mg
260-014-M010 10mg
Membrane-permeable inhibitor of calpain I and II and cathepsin L.

Leupeptin (synthetic)
260-009-M005 5mg
260-009-M025 25mg
260-009-M100 100mg
Competitive and reversible inhibitor of serine and cysteine proteinases like calpain, porcine kallikrein, trypsin, plasmin, papain and cathepsin B. Does not inhibit chymotrypsin and thrombin.

N-(1-Naphthalenylsulfonyl)-Ile-Trp-aldehyde
260-133-M001 1mg
260-133-M005 5mg
Potent, selective and reversible inhibitor of human cathepsin L (IC_{50}=1.9nM). Inhibits release of Ca^{2+} and hydroxyproline from bone *in vitro* bone culture systems.

NCO-700 . hemisulfate
270-103-M001 1mg
270-103-M005 5mg
Specific thiol (cysteine) protease inhibitor. Active against calpains, cathepsin B and L and papain.

PD 150,606
270-234-M005 5mg
Cell permeable, selective, and non-peptide calpain inhibitor directed towards the calcium binding sites of calpain. Exhibits high specificity for calpains relative to other proteases such as cathepsin B and cathepsin L.

Pepstatin A (synthetic)
260-085-M005 5mg
260-085-M025 25mg
260-085-M100 100mg
Inhibitor of aspartate (acid) proteases, including pepsin, cathepsin D, renin, chymosin, bacterial aspartic proteinases and HIV proteases.

Z-Phe-Tyr-aldehyde
260-132-M005 5mg
Potent, reversible inhibitor of cathepsin L.

Z-Phe-Tyr(tBu)-diazomethylketone
260-134-M001 1mg
260-134-M005 5mg
Irreversible inhibitor of cathepsin L.

Substrates

Proteasome Substrate III (Fluorogenic) [Suc-LLVY-AMC]
260-070-M001 1mg
260-070-M005 5mg
Fluorogenic proteasome substrate. Also acts as a substrate for many other proteinases. AMC has an excitation maximum of 380nm and an emission maximum of 460nm.

Z-Phe-Arg-AFC . TFA
260-129-M005 5mg
Fluorogenic cathepsin L substrate.

Z-Phe-Arg-pNA . HCl
260-130-M005 5mg
Chromogenic substrate for cathepsin L and papain.

Z-Phe-Arg-AMC . HCl
260-131-M005 5mg
Fluorogenic substrate for cathepsin B and L, papain, plasma kallikrein and soybean trypsin-like enzyme.

IMMUNOCHEMISTRY TECHNOLOGIES, LLC

A leading supplier of a comprehensive line of Apoptosis and Caspase Detection Kits

FAM & SR FLICA™ Caspase Assay Kits

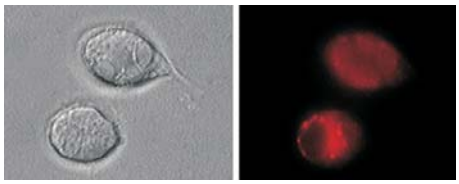
The assays are based on a unique cell-permeable and non-cytotoxic reagent called the Fluorochrome Inhibitor of Caspases (FLICA). Once inside the cell, the FLICA™ inhibitor binds covalently to the active caspase. For the FAM FLICA™ kits, which fluoresce green, a carboxyfluorescein (FAM)-labelled fluoromethyl ketone (FMK) peptide inhibitor of caspases is used. The FAM FLICA™ reagent has an optimal excitation range from 488 - 492 nm, and emission range from 515 - 535 nm; (the excitation / emission pairs which best approximate this optimal range should be used).

The SR FLICA™ Caspase Detection Assay Kits, which fluoresce red, use a sulforhodamine (SR)-labelled fluoromethyl ketone (FMK) peptide inhibitor of caspases. The SR FLICA™ reagent excites at 550 nm and has a maximum emission range of 590 – 600 nm (the excitation / emission pairs which best approximate the optimal ranges should be used).

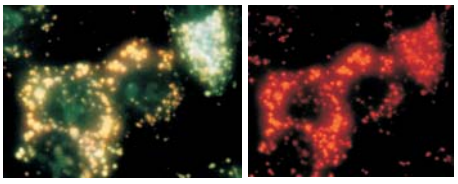
The FLICA™ Assay Kits have been shown to be specific, sensitive and convenient-to-use probes for the detection of apoptosis in time window distinctly wider than most other methods. However in light of some peculiarities in their binding feature, use of these assay kits should not be interpreted as a measure of caspase activity per se. For details see: *Interactions of fluorochrome-labeled caspase inhibitors with apoptotic cells: A caution in data interpretation*: P. Pozarowski, et al.; Cytometry **55A**, 50 (2003)

Applications

Microscope Data (Caspase Assay)



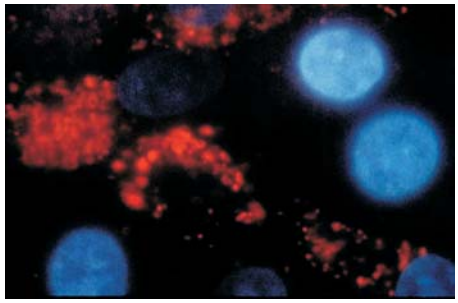
Staurosporine-induced Jurkat cells were stained with 20µM $MR(DEVD)_2$ for 60 minutes at 37°C. Intracellular structures were detected on a Nikon Eclipse E 800 photomicroscope using a 510-560nm excitation filter and a 570-620nm emission/barrier filter set at approximately 700X magnification. The photo on the left hand side shows the corresponding DIC image of the cells.



Acridine Orange (AO) staining of MCF-7 cells induced for 24 hours with 0.15µM Camptothecin at 37°C. Cells were stained with 0.5µM AO in PBS for 30 minutes, washed twice in PBS, and photographed using either blue light excitation (480nm) with 540 – 550nm emission (left photo), or green light excitation (540nm) with long pass > 640nm barrier filter setup (right photo).

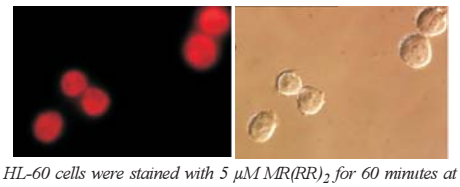
Product Overview

Prod. No.	Prod. Name	Peptide Sequence	Qty
Mitochondrial Permeability Transition [MitoPT™] Kits			
ICT-911-T100	MitoPT™-100	NA	100 Tests
FAM FLICA™ Caspase Detection Assay Kits			
ICT-91-T025	FAM FLICA™ Poly-Caspase Assay Kit	FAM-VAD-FMK	25 Tests
ICT-92-T100	FAM FLICA™ Poly-Caspase Assay Kit	FAM-VAD-FMK	100 Tests
ICT-97-T025	FAM FLICA™ Caspase-1 Assay Kit	FAM-YVAD-FMK	25 Tests
ICT-98-T100	FAM FLICA™ Caspase-1 Assay Kit	FAM-YVAD-FMK	100 Tests
ICT-918-T025	FAM FLICA™ Caspase-2 Assay Kit	FAM-VDVAD-FMK	25 Tests
ICT-919-T100	FAM FLICA™ Caspase-2 Assay Kit	FAM-VDVAD-FMK	100 Tests
ICT-93-T025	FAM FLICA™ Caspase-3 & -7 Assay Kit	FAM-DEVD-FMK	25 Tests
ICT-94-T100	FAM FLICA™ Caspase-3 & -7 Assay Kit	FAM-DEVD-FMK	100 Tests
ICT-95-T025	FAM FLICA™ Caspase-6 Assay Kit	FAM-VEID-FMK	25 Tests
ICT-96-T100	FAM FLICA™ Caspase-6 Assay Kit	FAM-VEID-FMK	100 Tests
ICT-99-T025	FAM FLICA™ Caspase-8 Assay Kit	FAM-LETD-FMK	25 Tests
ICT-910-T100	FAM FLICA™ Caspase-8 Assay Kit	FAM-LETD-FMK	100 Tests
ICT-912-T025	FAM FLICA™ Caspase-9 Assay Kit	FAM-LEHD-FMK	25 Tests
ICT-913-T100	FAM FLICA™ Caspase-9 Assay Kit	FAM-LEHD-FMK	100 Tests
ICT-922-T025	FAM FLICA™ Caspase-10 Assay Kit	FAM-AEVD-FMK	25 Tests
ICT-923-T100	FAM FLICA™ Caspase-10 Assay Kit	FAM-AEVD-FMK	100 Tests
ICT-929-T025	FAM-LEED-FMK Caspase Assay Kit	FAM-LEED-FMK	25 Tests
ICT-930-T100	FAM-LEED-FMK Caspase Assay Kit	FAM-LEED-FMK	100 Tests
SR FLICA™ Caspase Detection Assay Kits			
ICT-916-T025	Sulforhodamine (SR) Poly-Caspase Assay Kit	SR-VAD-FMK	25 Tests
ICT-917-T100	Sulforhodamine (SR) Poly-Caspase Assay Kit	SR-VAD-FMK	100 Tests
ICT-931-T025	Sulforhodamine (SR) Caspase-3 & -7 Assay Kit	SR-DEVD-FMK	25 Tests
ICT-932-T100	Sulforhodamine (SR) Caspase-3 & -7 Assay Kit	SR-DEVD-FMK	100 Tests
Magic Red™ (DEVD)2 Caspase Detection Assay Kits			
ICT-935-T025	Magic Red™-$(DEVD)_2$ Caspases-3 & -7 Assay Kit	MR-$(DEVD)_2$	25 Tests
ICT-936-T100	Magic Red™-$(DEVD)_2$ Caspases-3 & -7 Assay Kit	MR-$(DEVD)_2$	100 Tests
Magic Red™ Cathepsin Detection Assay Kits			
ICT-937-T025	Magic Red™-$(RR)_2$ Cathepsin B Assay Kit	MR-$(RR)_2$	25 Tests
ICT-938-T100	Magic Red™-$(RR)_2$ Cathepsin B Assay Kit	MR-$(RR)_2$	100 Tests
ICT-939-T025	Magic Red™-$(LR)_2$ Cathepsin K Assay Kit	MR-$(LR)_2$	25 Tests
ICT-940-T100	Magic Red™-$(LR)_2$ Cathepsin K Assay Kit	MR-$(LR)_2$	100 Tests
ICT-941-T025	Magic Red™-$(FR)_2$ Cathepsin L Assay Kit	MR-$(FR)_2$	25 Tests
ICT-942-T100	Magic Red™-$(FR)_2$ Cathepsin L Assay Kit	MR-$(FR)_2$	100 Tests



Dual staining of MCF-7 cells with Hoechst 33342 (Prod. No. ALX-620-050) stain and $MR(DEVD)_2$ following 24 hour exposure to 0.15µM Camptothecin at 37°C. Cells were stained for 30 minutes with 10µM $MR(DEVD)_2$ at 37°C, washed twice in PBS, and supravitally stained with 1µg/mL of Hoechst stain (>10 minutes). Using the Nikon Microphot FXA system containing a multi-wavelength filter pairing system (UV for the Hoechst stain and green light for the $MR(DEVD)_2$), apoptotic cells bearing orange lysosomal bodies with less intense blue nuclei can be seen intermixed with non-apoptotic cells bearing bright blue nuclei and absent or reduced lysosomal staining. As MCF-7 cells do not produce caspase-3, caspase-7 activity is detected in the apoptotic cells.

Microscope Data (Cathepsin Assay)



HL-60 cells were stained with 5 µM $MR(RR)_2$ for 60 minutes at 37°C. Intracellular localization of the hydrolyzed (fluorescent) $MR(RR)_2$ Cathepsin B substrate was detected on a Nikon Eclipse E 800 photomicroscope using a 510 – 560nm excitation filter and a 570 – 620nm emission/barrier filter set at approximately 700X magnification. The photo on the right hand side shows the corresponding differential interference contrast image of the cells.

For detailed Information & Protocols visit

www.alexis-e.biz

Application: EL = ELISA | FC = Flow Cytometry | IC = Immunocytochemistry | IH = Immunohistochemistry | IP = Immunoprecipitation | WB = Western Blot | OR = Other Application

Reactivity: H = Human | M = Mouse | O = Others **BP**: Product Number of corresponding Blocking Peptide

ALEXIS® BIOCHEMICALS

Caspase Inhibitors & Substrates

More than a bewildering 280 proteins have been described as targets for caspase cleavage [reviewed in 1]. Interestingly, more than 230 different cleavage sites have also been reported (see Box 1). Considering the fact that some noncaspase proteases can cleave the same proteins (at the same or at a different site) as caspases, it becomes evident that specific inhibitors and substrates for each protease are very rare and to date no nonpeptide, small molecule inhibitors of caspases are available. A further problem is that the known peptide inhibitors exhibit poor bioavailability and cell permeability. This then requires the treatment of living cells with inhibitors at concentrations up to 4 magnitudes higher than their inhibitory constant (K_i) evaluated *in vitro*.

The fact that unlabeled caspase inhibitors, when added after apoptosis induction, cannot prevent activation of caspases detected by binding of biotinylated inhibitors or by cleavage of fluorogenic substrates, suggests that this binding may also involve other or additional mechanisms than simply their specific attachment to the active enzyme centers of caspases [2]. Another study used substrates with the general structure Ac-XEXD-AMC (based on the optimal tetrapeptide recognition motif for each caspase enzyme) to develop continuous fluorometric assays. In some cases, enzymes with virtually identical tetrapeptide specificities had k_{cat}/K_m values for the corresponding fluorogenic substrate that differed by more than 1000-fold [3]. Taken together, it should therefore be noted that data obtained by using these reagents should be interpreted with caution.

Lit. [1] *Many cuts to ruin: a comprehensive update of caspase substrates*: U. Fischer, et al.; Cell Death Differ. **10**, 76 (2003) [2] *Interactions of fluorochrome-labeled caspase inhibitors with apoptotic cells: A caution in data interpretation*: P. Pozarowski, et al.; Cytometry **55A**, 50 (2003) [3] *Purification and catalytic properties of human caspase family members*: M. Garcia-Calvo, et al.; Cell Death Differ. **6**, 362 (1999)

Box 1: Overview on Reported Caspase Cleavage Sites: AAVD/ADID/AEPD/AETD/AEVD/AFAD/ALAD/ALDD/AMED/AQRD/ASTD/AVVD/CQND/CSTD/CYAD/DAGD/DALD/DAQD/DAVD/DCVD/DDED/DDGD/DDLD/DDRD/DDSD/DDVD/DDYD/DEAD/DEDD/DEED/DEGD/DEHD/DEID/DELD/DEMD/DEND/DEPD/DEQD/DESD/DETD/DEVD/DEVE/DFGD/DFPD/DFVD/DFVE/DGDD/DGLD/DGPD/DGTD/DGVD/DHLD/DHVD/DIND/DIPD/DITD/DLAD/DLFD/DLKD/DLLD/DLMD/DLPD/DLRD/DLVD/DLYD/DMAD/DMDD/DMED/DMVD/DNID/DNTD/DPSD/DQID/DQLD/DQMD/DQPD/DQTD/DRHD/DRLD/DSGD/DSLD/DSPD/DSQD/DSSD/DSVD/DSYD/DTAD/DTRD/DTTD/DTVD/DTYD/DVDD/DVLD/DVPD/DVTD/DYAD/DYED/DYHD/DYLD/DYPD/DYYD/DZQD/EAVD/ECVD/EDGD/EDLD/EEAD/EEED/EEID/EELD/EEMD/EERD/EESD/EETD/EEVD/EGED/EGLD/EHID/ELLD/ELPD/ENAD/EQED/ESPD/ESQD/ESVD/ETAD/ETVD/EVPD/FIQD/FPAD/GEDD/GEID/GELE/GLLD/GWAD/HLAD/IDVD/IEVE/IGGD/ILND/ILRD/IRKD/IVLD/IVPD/KESD/KLTD/KRID/LDED/LESD/LEVD/LHTD/LISD/LKTD/LQLD/LQMD/LQTD/LSPD/LSSD/LSVD/LTED/LVAD/LVRD/MDID/MDVD/MELD/METD/MMPD/NKTD/NPQD/NSPD/PAPD/PEDD/PHLD/PRED/QLED/QSVD/RAID/RKLD/RLPD/SAFD/SALD/SATD/SCTD/SDED/SEAD/SELD/SESD/SETD/SEVD/SEVT/SFPD/SGVD/SHVD/SLLD/SNHD/SQGD/SQHD/SQLD/SRVD/SSLD/SSPD/SSTD/SSYD/STPD/SVTD/SYLD/SYND/TEED/TEID/TEVD/TNLD/TQFD/TVAD/VACD/VDFD/VDVD/VEID/VEMD/VEVD/VFTD/VLGD/VSLD/VSVD/VVPD/VYRD/WEID/YLLD/YPVD/YVHD/YVPD/YWID

✓ = Reversible ✗ = Irreversible | Cell Permeable? | Other Caspases Inhibited | Literature References

Primary Caspase Target	Other Caspases Inhibited	Cell Permeable?		Literature References	Prod. No.	Size
Pan-Caspase Specific Inhibitors						
Biotinyl-VAD(OMe)-FMK	All	×	✓	[60]	260-098-M001	1mg
Boc-D(OMe)-FMK	All	×	✓	[26,42,44, 45,76]	260-071-M001 260-071-M005	1mg 5mg
NEW Q-VD-OPH [N-(2-Quinolyl)valyl-O-methylaspartyl-(2,6-difluorophenoxy)-methylketone]	1,3,8,9			[80]	260-159-M003	3mg
Z-Asp-2,6-dichlorobenzoyloxymethyl-ketone	All	×	✓	[5,16]	260-029-M010 260-029-M050	10mg 50mg
Z-VAD-FMK Ready-to-Use	All	×	✓		260-138-R020 260-138-R100	† 20µl ‡ 100µl
Z-VAD-FMK	All	×	✓	[20,56]	260-020-M001 260-020-M005	1mg 5mg
Z-VAD(OMe)-FMK	All	×	✓	[12,20,26, 28,57,62-64, 67,75,78]	260-039-M001 260-039-M005	1mg 5mg
NEW Z-VD-FMK [MX1013]	1,3,6-9	×	✓	[81]	260-162-M001	1mg
Z-VK(Biotinyl)D(OMe)-FMK	All			[31]	260-105-M001	1mg
Caspase-1						
Ac-AAVALLPAVLLALLAPYVAD-CHO		✓	✓	[18]	260-047-M001 260-047-M005	1mg 5mg
Ac-WEHD-CHO	4,5	✓	✓	[37,40]	260-055-M001 260-055-M005	1mg 5mg
Ac-YVAD-CHO	4	✓		[2-4,10,11,15, 17,22,23,46]	260-027-M001 260-027-M005	1mg 5mg
Ac-YVAD-CMK	4	×	✓	[6,10,13,14, 17,22,25,72]	260-028-M001 260-028-M005	1mg 5mg
Ac-YVAD-2,6-dimethylbenzoyloxy-methylketone	4	×	✓	[9]	260-016-M001 260-016-M005	1mg 5mg
Biotinyl-YVAD-CMK	4	×	✓	[6]	260-019-M001 260-019-M005	1mg 5mg
Z-LE(OMe)VD(OMe)-FMK					260-104-M001 260-104-M005	1mg 5mg
Z-WEHD-FMK Ready-to-Use	4,5	×	✓		260-140-R020 260-140-R100	† 20µl ‡ 100µl
Z-WE(OMe)HD(OMe)-FMK					260-101-M001 260-101-M005	1mg 5mg
Z-YVAD-FMK Ready-to-Use	4	×	✓		260-154-R020 260-154-R100	† 20µl ‡ 100µl
Z-YVAD(OMe)-FMK	4	×	✓	[32,65,78]	260-074-M001	1mg
Caspase-2						
NEW Ac-LDESD-CHO	3	✓		[82]	260-160-M001 260-160-M005	1mg 5mg
Ac-VDVAD-CHO		✓		[39]	260-058-M001 260-058-M005	1mg 5mg
Z-VDVAD-FMK Ready-to-Use		×	✓		260-139-R020 260-139-R100	† 20µl ‡ 100µl
Z-VD(OMe)VAD(OMe)-FMK		×	✓	[39,58,74]	260-099-M001 260-099-M005	1mg 5mg
Caspase-3						
Ac-AAVALLPAVLLALLAPDEVD-CHO	6,7,8,10	✓	✓	[18,19,41]	260-046-M001 260-046-M005	1mg 5mg
Ac-DEVD-CHO	7	✓		[18,22,24, 27,33,46,65]	260-030-M001 260-030-M005	1mg 5mg
Ac-DMQD-CHO		✓		[49]	260-077-M001 260-077-M005	1mg 5mg
NEW Ac-LDESD-CHO	2	✓		[82]	260-160-M001 260-160-M005	1mg 5mg
Biotinyl-DEVD-CHO	7	✓		[18]	260-034-M001 260-034-M005	1mg 5mg
Biotinyl-D(OMe)E(OMe)VD(OMe)-FMK	7			[53]	260-100-M001	1mg
NEW 5-[(S)-(-)-2-(Methoxymethyl)pyrrol-idino]sulfonylisatin	7	✓		[83,84]	270-374-M001	1mg
Z-DEVD-FMK Ready-to-Use	7	×	✓		260-141-R020 260-141-R100	† 20µl ‡ 100µl
Z-D(OMe)E(OMe)VD(OMe)-FMK	7	×	✓	[18,24,27, 35,44,61, 66,68,73,76]	260-072-M001 260-072-M005	1mg 5mg
Z-D(OMe)QMD(OMe)-FMK	6				260-103-M001 260-103-M005	1mg 5mg
Caspase-4						
Ac-LEVD-CHO	5	✓		[39]	260-065-M001 260-065-M005	1mg 5mg
Z-LEVD-FMK Ready-to-Use	5	×	✓		260-142-R020 260-142-R100	† 20µl ‡ 100µl

✓ = Reversible ✗ = Irreversible | Cell Permeable? | Other Caspases Inhibited | Literature References

Primary Caspase Target	Other Caspases Inhibited	Cell Permeable?		Literature References	Prod. No.	Size
Caspase-5						
Ac-WEHD-CHO	1,4	✓	✓	[37,40,46]	260-055-M001 260-055-M005	1mg 5mg
Z-WEHD-FMK Ready-to-Use	1,4	×	✓		260-140-R020 260-140-R100	† 20µl ‡ 100µl
Caspase-6						
Ac-VEID-CHO	8,10	✓			260-062-M001 260-062-M005	1mg 5mg
Z-VEID-FMK Ready-to-Use		×	✓		260-143-R020 260-143-R100	† 20µl ‡ 100µl
Z-VE(OMe)ID(OMe)-FMK		×	✓		260-075-M001	1mg
Z-AE(OMe)VD(OMe)-FMK	8	×	✓		260-107-M001 260-107-M005	1mg 5mg
Caspase-7						
Ac-DEVD-CHO	3	✓		[18,22,24, 27,33,65]	260-030-M001 260-030-M005	1mg 5mg
Biotinyl-DEVD-CHO	3	✓		[18]	260-034-M001 260-034-M005	1mg 5mg
NEW 5-[(S)-(-)-2-(Methoxymethyl)pyrrol-idino]sulfonylisatin	3	✓		[83,84]	270-374-M001	1mg
Z-DEVD-FMK Ready-to-Use	3	×	✓		260-141-R020 260-141-R100	† 20µl ‡ 100µl
Z-D(OMe)E(OMe)VD(OMe)-FMK	3	×	✓	[18,24,27, 35,44,61, 66,68,73,76]	260-072-M001 260-072-M005	1mg 5mg
Caspase-8 / Caspase-3 Processing Enzyme						
Ac-ESMD-CHO	6,10,GB*	✓		[33]	260-056-M001 260-056-M005	1mg 5mg
Ac-IETD-CHO	10,GB*	✓		[33]	260-043-M001 260-043-M005	1mg 5mg
Z-IETD-FMK Ready-to-Use	10,GB*	×	✓		260-144-R020 260-144-R100	† 20µl ‡ 100µl
Z-IE(OMe)TD(OMe)-FMK	10,GB*	×	✓	[50,52,54, 59,69]	260-073-M001 260-073-M005	1mg 5mg
Z-LE(OMe)TD(OMe)-FMK					260-102-M001 260-102-M005	1mg 5mg
Caspase-9						
Ac-LEHD-CHO		✓			260-079-M001 260-079-M005	1mg 5mg
Z-LEHD-FMK Ready-to-Use		×	✓		260-145-R020 260-145-R100	† 20µl ‡ 100µl
Z-LE(OMe)HD(OMe)-FMK		×	✓		260-076-M001 260-076-M005	1mg 5mg
Caspase-10						
Ac-AEVD-CHO	6,8			[46]	260-158-M001 260-158-M005	1mg 5mg
Z-AEVD-FMK Ready-to-Use	6,8	×	✓		260-146-R020 260-146-R100	† 20µl ‡ 100µl
Granzyme B						
Z-AAD-CMK Ready-to-Use					260-153-R020	† 20µl
Ac-ESMD-CHO	6,8,10	✓		[33]	260-056-M001 260-056-M005	1mg 5mg
Ac-IETD-CHO	8,10	✓		[33]	260-043-M001 260-043-M005	1mg 5mg
Z-IETD-FMK Ready-to-Use	8,10	×	✓		260-144-R020 260-144-R100	† 20µl ‡ 100µl
Z-IE(OMe)TD(OMe)-FMK	8,10	×	✓	[50,52,54, 59,69]	260-073-M001 260-073-M005	1mg 5mg
Negative Control						
Z-FA-FMK Ready-to-Use		×	✓		260-148-R020 260-148-R100	† 20µl ‡ 100µl

CMK = Chloromethylketone; FMK = Fluoromethylketone; * GB = Granzyme B
For Ready-to-Use solutions: † conc. = 10mM; ‡ conc. = 2mM

Reversible / Irreversible Inhibitors

FMK-based inhibitors are irreversible because they covalently modify the enzyme thiol group. CHO-based inhibitors form an adduct with the thiol group that is reversible, depending upon factors such as pH and metal ion concentration. They are generally slow binding.

Caspase Inhibitors & Substrates *continued*

C=chromogenic F=fluorogenic | λmax Ex/Em max | Cell Permeable? | Literature References

Substrate for Other Caspases / Primary Caspase Target					Prod. No.	Size
Pan-Caspase Specific Substrates						
Ac-VAD-AFC		F 400/505			260-109-M005 260-109-M010	5mg 10mg
Caspase-1						
Ac-WEHD-AFC	4,5	F 400/505			260-117-M005 260-117-M010	5mg 10mg
Ac-WEHD-AMC	4,5	F 380/460		[37,40]	260-057-M001 260-057-M005	1mg 5mg
Ac-WEHD-pNA	4,5	C 400		[40]	260-082-M001 260-082-M005	1mg 5mg
Ac-YVAD-AFC	4	F 400/505		[51]	260-108-M005 260-108-M010	5mg 10mg
Ac-YVAD-AMC		F 380/460		[3,8,10]	260-024-M001 260-024-M005	1mg 5mg
Ac-YVAD-pNA		C 400		[3,7,8]	260-026-M001 260-026-M005	1mg 5mg
Mca-YVADAPK(Dnp)-OH	4	F 325/392		[22]	260-023-M001	1mg
Z-YVAD-AFC		F 400/505	✓	[3,7,8,10,34]	260-035-M001 260-035-M005	1mg 5mg
Z-YVAD-pNA		C 400	✓		260-049-M001 260-049-M005	1mg 5mg
Caspase-2						
Ac-VDVAD-AFC		F 400/505			260-112-M005 260-112-M010	5mg 10mg
Ac-VDVAD-AMC		F 380/460		[39]	260-060-M001 260-060-M005	1mg 5mg
Ac-VDVAD-pNA		C 400		[39]	260-059-M001 260-059-M005	1mg 5mg
Caspase-3						
Ac-DEVD-AFC	1,4,7	F 400/505		[30,43,51]	260-032-M001 260-032-M005	1mg 5mg
Ac-DEVD-AMC	1,4,7,8	F 380/460		[18,79]	260-031-M001 260-031-M005	1mg 5mg
Ac-DEVD-pNA	1,4,7	C 400		[18,21,39]	260-033-M001 260-033-M005	1mg 5mg
Ac-DQMD-AFC	6	F 400/505			260-113-M005 260-113-M010	5mg 10mg
Ac-DMQD-AMC		F 380/460			260-078-M001 260-078-M005	1mg 5mg
Z-DEVD-pNA	1,4,7	C 400	✓		260-048-M001 260-048-M005	1mg 5mg
Caspase-4						
Ac-LEVD-AFC		F 400/505			260-084-M001 260-084-M005	1mg 5mg
Ac-LEVD-AMC		F 380/460		[40]	260-083-M001 260-083-M005	1mg 5mg
Ac-LEVD-pNA		C 400		[39]	260-061-M001 260-061-M005	1mg 5mg
Ac-YVAD-AFC	1	F 400/505		[51]	260-108-M005 260-108-M010	5mg 10mg
Caspase-5						
Ac-WEHD-AFC	1,4	F 400/505			260-117-M005 260-117-M010	5mg 10mg
Ac-WEHD-AMC	1,4	F 380/460			260-057-M001 260-057-M005	1mg 5mg
Ac-WEHD-pNA	1,4	C 400			260-082-M001 260-082-M005	1mg 5mg
Caspase-6						
Ac-AEVD-AFC	8	F 400/505			260-114-M005 260-114-M010	5mg 10mg
Ac-DQMD-AFC	3	F 400/505			260-113-M005 260-113-M010	5mg 10mg
Ac-VEID-AFC		F 400/505			260-111-M005 260-111-M010	5mg 10mg
Ac-VEID-AMC	9,10	F 380/460		[29,34,36, 39,40,79]	260-064-M001 260-064-M005	1mg 5mg
Ac-VEID-pNA		C 400		[39]	260-063-M001 260-063-M005	1mg 5mg
Caspase-8 / Caspase-3 Processing Enzyme						
Ac-AEVD-AFC	6	F 400/505			260-114-M005 260-114-M010	5mg 10mg
Ac-IEPD-AFC	GB*	F 400/505			260-115-M005 260-115-M010	5mg 10mg
Ac-IEPD-AMC	GB*	F 380/460		[48]	260-151-M001 260-151-M005	1mg 5mg
Ac-IEPD-pNA	GB*	C 400		[48]	260-152-M001 260-152-M005	1mg 5mg
Ac-IETD-AFC	10,GB*	F 400/505		[47]	260-110-M005 260-110-M010	5mg 10mg
Ac-IETD-AMC	10,GB*	F 380/460			260-042-M001 260-042-M005	1mg 5mg
Ac-IETD-pNA	10,GB*	C 400		[33]	260-045-M001 260-045-M005	1mg 5mg
Ac-LETD-AFC		F 400/505			260-118-M005 260-118-M010	5mg 10mg
Z-IETD-pNA	10,GB*	C 400	✓		260-044-M001 260-044-M005	1mg 5mg
Caspase-9						
Ac-LEHD-AFC		F 400/505			260-116-M005 260-116-M010	5mg 10mg
Ac-LEHD-AMC		F 380/460		[40]	260-080-M001 260-080-M005	1mg 5mg
Ac-LEHD-pNA		C 400			260-081-M001 260-081-M005	1mg 5mg

C=chromogenic F=fluorogenic | λmax Ex/Em max | Cell Permeable? | Literature References

Substrate for Other Caspases / Primary Caspase Target					Prod. No.	Size
Granzyme B						
Ac-IEPD-AFC		F 400/505			260-115-M005 260-115-M010	5mg 10mg
Ac-IEPD-AMC	8	F 380/460		[48]	260-151-M001 260-151-M005	1mg 5mg
Ac-IEPD-pNA	8	C 400		[48]	260-152-M001 260-152-M005	1mg 5mg
Ac-IETD-AFC	8	F 400/505		[47]	260-110-M005 260-110-M010	5mg 10mg
Ac-IETD-AMC	8,10	F 380/460			260-042-M001 260-042-M005	1mg 5mg
Ac-IETD-pNA	8,10	C 400		[33]	260-045-M001 260-045-M005	1mg 5mg
BAADT (Boc-AAD-SBzl)		C 405		[1]	260-050-M001 260-050-M005	1mg 5mg
Z-IETD-pNA	8,10	C 400	✓		260-044-M001 260-044-M005	1mg 5mg
DRONC [*Drosophila* Caspase]						
NEW Ac-TQTD-AFC		F 400/505		[85,86]	260-161-M001 260-161-M005	1mg 5mg
Reference Compounds						
AFC		F 400/505			610-027-M010	10mg
AMC		F 380/460			610-028-M010	10mg
pNA		C 400			610-043-M100 610-043-G001	100mg 1g

AFC = 7-Amido-4-trifluoromethylcoumarin; AMC = 7-Amido-4-methylcoumarin; Dnp = Dinitrophenyl; Mca = (7-Methoxycoumarin-4-yl)acetyl; pNA = p-Nitroanilide; *GB = Granzyme B

Cell Permeable Substrates / Inhibitors

Blocking the side chains of aspartic (D) or glutamic (E) acids with methyl ester (-OMe) groups makes the peptide more hydrophobic and, therefore, more cell permeable. Blocking the N-terminus with benzyloxycarbonyl (Z-), acetyl (Ac-) or biotin does likewise; in general, inhibitors or substrates with a Z-group are more cell permeable than those with Ac- or biotin. Biotin inhibitors are useful for labelling the binding site of the enzyme.

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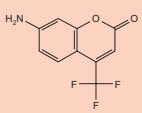
7-Amino-4-trifluoromethylcoumarin [AFC]

• Fluorometric detection (Ex=400nm; Em= 505nm) • Spectrophotometric detection at 380nm, extinction coefficient = 12,600 at pH 7.2 • Soluble in DMF or DMSO • Amino acid derivatives of AFC have blue fluorescence; free AFC has green fluorescence (more acceptable for fluorescence microscopy) • Non-mutagenic (by the Ames test) • Does not couple with 5-nitrososalicyl-aldehyde • Substrates are stable in dry form or as DMF solutions; in cold buffered solutions, stable for 2-3 days •



Caspase Assay Kits

Prod. No.	Product	Substrate	Size
850-211-KI01	Caspase-1 Colorimetric Assay Kit	YVAD-pNA	1 Kit
850-212-KI01	Caspase-1 Fluorometric Assay Kit	YVAD-AFC	1 Kit
850-213-KI01	Caspase-2 Colorimetric Assay Kit	VDVAD-pNA	1 Kit
850-214-KI01	Caspase-2 Fluorometric Assay Kit	VDVAD-AFC	1 Kit
850-215-KI01	Caspase-3 Colorimetric Assay Kit	DEVD-pNA	1 Kit
850-216-KI01	Caspase-3 Fluorometric Assay Kit	DEVD-AFC	1 Kit
850-217-KI01	Caspase-5 Colorimetric Assay Kit	WEHD-pNA	1 Kit
850-218-KI01	Caspase-5 Fluorometric Assay Kit	WEHD-AFC	1 Kit
850-219-KI01	Caspase-6 Colorimetric Assay Kit	VEID-pNA	1 Kit
850-220-KI01	Caspase-6 Fluorometric Assay Kit	VEID-AFC	1 Kit
850-221-KI01	Caspase-8 Colorimetric Assay Kit	IETD-pNA	1 Kit
850-222-KI01	Caspase-8 Fluorometric Assay Kit	IETD-AFC	1 Kit
850-223-KI01	Caspase-9 Colorimetric Assay Kit	LEHD-pNA	1 Kit
850-224-KI01	Caspase-9 Fluorometric Assay Kit	LEHD-AFC	1 Kit

Kits contain sufficient reagents for 25 assays.

Caspase Colorimetric Assay Kits

The assays are based on spectrophotometric detection of the chromophore pNA after cleavage from the pNA labelled substrate. The pNA emission can be measured using a spectrophotometer or a microtiter plate reader at 400 or 405nm. Comparison of the absorbance of pNA from an untreated control allows determination of the fold increase in caspase activity.

Caspase Fluorometric Assay Kits

The assays are based on detection of cleavage of the AFC labelled substrate. The AFC labelled substrate emits blue light (λ_{max} = 400nm). Upon cleavage of the substrate by caspases, free AFC emits a yellow-green fluorescence (λ_{max} = 505nm), which can be measured using a fluorometer or a fluorescence microtiter plate reader. Comparison of the fluorescence of AFC from a treated sample with an untreated control allows determination of the fold increase in caspase activity.

APOPTRAK™ - NEW Tool for the Characterization of Different Modes of Cell Death

APOPTRAK™ stains viable cells to a minor degree, but uptake and hence staining is greatly increased upon membrane disruption associated with cell death. ***APOPTRAK***™ is a N-oxide modification of the cell permeable DNA probe DRAQ5™ (Prod. No. BOS-889-001) benefiting from the unique fluorescence characteristics of its parental compound DRAQ5™ but with reduced cytotoxicity.

Ideal for flow cytometric multi-parameter analysis (e.g. ***APOPTRAK***™ versus light scatter versus surface binding of another probe such as FITC-tagged Annexin V). Permits assay validation and further separation of subpopulations for live/dead (necrotic or apoptotic) cell discrimination by flow cytometry.

NEW APOPTRAK™ [DRAQ5NO]

BOS-889-002-R500 500µl

Lit. *A novel deep red/low infrared fluorescent flow cytometric probe, DRAQ5NO, for the discrimination of intact nucleated cells in apoptotic cell populations*: M. Wiltshire, et al.; Cytometry **39**, 217 (2000)

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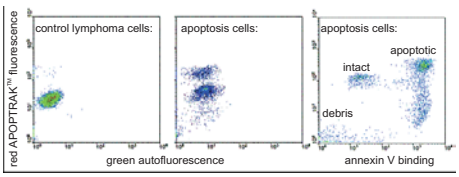

Figure: *Flow cytometry analysis with APOPTRAK™ alone or in combination with annexin V-FITC of dying lymphoma cells. APOPTRAK™ staining of normal (untreated control) (left dot blot image) versus apoptotic human lymphoma cells populations (middle dot blot image) versus APOPTRAK™ staining in combination with annexin V-FITC (Prod. No. 209-256) co-staining of cells undergoing drug-induced apoptosis (right dot blot image).*

DRAQ5™

BOS-889-001-R050 50µl
BOS-889-001-R200 200µl

DNA Damage Detection Marker

MAb to Poly(ADP-ribose) [PAR] (10H)

804-220-R100 100µl

Clone: 10H. **Isotype**: Mouse IgG3. **Immunogen**: Purified poly(ADP-ribose) (PAR). **Specificity**: Recognizes PAR synthesized by a wide range of poly(ADP-ribose) polymerases like human, mouse, rat or drosophila PARP enzyme. **Application**: ICC, IHC (PS), WB.

Lit. *Monoclonal antibodies to poly(adenosine diphosphate ribose) recognize different structures*: H. Kawamitsu, et al.; Biochemistry **23**, 3771 (1984) (Original Reference)

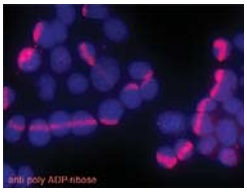

Figure: *HeLa cells irradiated with a microbeam laser. Picture courtesy of C. Spenlehauer & G. de Murcia (CNRS, Strasbourg).*

Ready-to-Use Zn-containing, Cd-free Metallothioneins

Metallothionenins have been shown to regulate apoptosis and to inhibit caspase-3 activation

Metallothioneins (MTs) form a class of ubiquitous, cysteine-rich, heavy-metal-binding proteins [Zn(II), Cu(I), Cd(II)], comprised of four major isoforms designated MT-1 through MT-4. The whole panel of metallothioneins (MT-1, MT-2, MT-3) sold by ALEXIS Corporation are non-toxic ready-to-use zinc-containing MTs essentially free of cadmium (cytotoxic), suited for life science research including cell culture studies.

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Zinc and Caspase Activation

Zinc (Zn) is co-localized with the precursor form of caspase-3, mitochondria and microtubules, suggesting that Zn is critically placed to control apoptosis. Although high concentrations of Zn, in some cells, trigger cell death by apoptosis or necrosis, the bulk of evidence indicates that Zn is a physiological suppressor of apoptosis. Zn suppresses caspase-3 activity and apoptosis *in vivo*. Zn blocks apoptosis induced by all apoptosis-inducing treatments tested, indicating that it suppresses a common event. Zn depletion on the other hand triggers caspase activation leading to apoptosis or in some cells (e.g. T cell leukemic Molt-3 cells) to necrosis. It has been suggested that Zn may interact with the sulfhydryl group of caspase-3 required for catalytic activity. ALEXIS® Biochemicals now offers two new exciting fluorescent probes for detecting low concentrations of Zn. For an extensive review on Zn and its role in apoptosis see: *The role of zinc in caspase activation and apoptotic cell death*: A.Q. Truong-Tran, et al.; Biometals **14**, 315 (2001).

ZnAF-2 . tetrahydrochloride

620-072-M001 1mg

Non cell-permeable fluorescent reagent (Ex(max): 492nm; Em(max): 514nm) for the detection of low concentration of zinc ion due to its strong affinity to zinc ion (dissociation constant: 2.7nM). The sample zinc ion can be specifically detected. Low background fluorescense supersensitizes the visualization for *in vivo* sample zinc ion.

ZnAF-2 DA

620-076-M001 1mg

Cell-permeable derivative of ZnAF-2 (Prod. No.620-072).

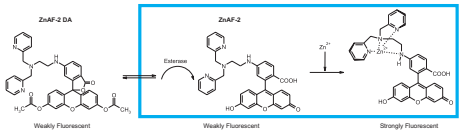

Figure: *Reaction of ZnAF-2 DA with zinc.*

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