Technical Data Sheet

Recombinant Human Active Caspase-8

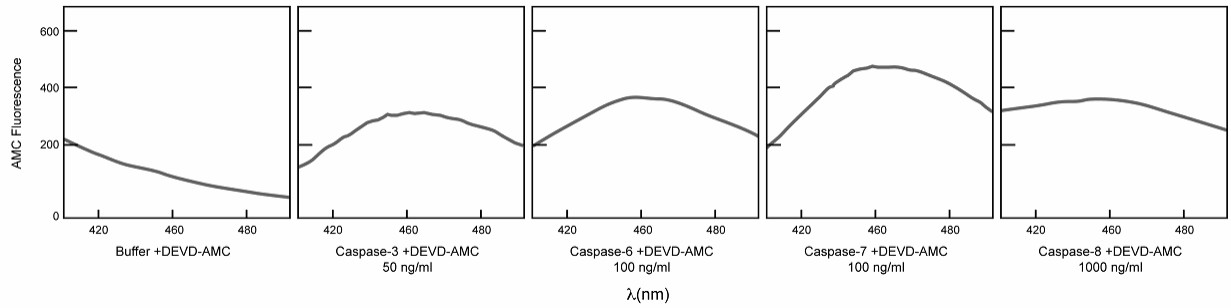
Product Information

Material Number:	**556481**
Size:	5 µg
Concentration:	0.2 mg/ml
Storage Buffer:	50 mM Tris (pH 8.0) with 100 mM NaCl and 50 mM imidazole

Description

Caspases are cysteine proteases that play a central role in apoptosis. The caspase family was discovered by searching human cDNA libraries for sequences homologous to *ced*-3, a C. *elegans* death gene that is required for normal apoptosis during development. The first mammalian homolog of *ced*-3 to be identified was ICE (interleukin-1β-converting enzyme). Subsequent numerous human *ced*-3 homologues were rapidly identified which led to multiple names for many of the molecules. To achieve consistency, "caspase" was adopted as a root name for all family members. The name was selected based on two catalytic properties of these enzymes, the "c" reflects a cysteine protease mechanism and "aspase" refers to their unique ability to cleave after aspartic acid. There are at least 10 members, caspase-1 (ICE), caspase-2 (ICH-1), caspase-3 (CPP32, Yama, apopain), caspase-4 (TX, ICH-2, ICErel-II), caspase-5 (ICErel-III), caspase-6 (Mch2), caspase-7 (Mch3, ICE-LAP3, CMH-1), caspase-8 (MACH, FLICE, Mch5), caspase-9 (ICELAP6, Mch6), and caspase-10 (Mch4). Each caspase is synthesized as an inactive proenzyme that is processed by cleavage at asparte residues by another protease or by self-proteolysis. The processed forms consist of large (17-22 kDa) and small (10-12 kDa) subunits which associate to form an active enzyme. The activation of some of these caspases has been shown to occur during apoptosis.

Caspase-3, -6, -7, and -8 have been shown to play a role in apoptosis induced by the death receptors Fas and tumor necrosis factor receptor type 1 (TNFR1). One of their substrates is poly (ADP ribose) polymerase (PARP). PARP is an enzyme that is involved in DNA repair and genomic maintenance. Activated caspases 3, 6, 7 and 8 can all cleave PARP from its 116 kDa form to an 85 kDa residual fragment. The cleavage separates the DNA-binding domain in the N-terminus of PARP from its C-terminus catalytic domain, and results in loss of normal PARP function. The cleavage site in PARP is C-terminal to Asp-216.3 The upstream sequence of the PARP cleavage site, DEVD (Asp-Glu-Val-Asp), is utilized as a basis for highly specific caspase-3 substrates such as Ac(N-acetyl)-DEVD-AFC (7-amino-4-trifluoromethylcourmarin) and Ac(N-acetyl)-DEVD-AMC (7-amino-4-methylcoumarin) as well as the caspase-3 aldehyde inhibitor Ac-DEVD-CHO.



Cleavage of the Ac-DEVD-AMC substrate by recombinant human caspases-3, -6, -7, and -8. *The activity of the caspases was analyzed by spectrofluorometry using an excitation at 380 nm and an emission wavelength of 430 - 460 nm (peak is at 440 nm). The concentration of each caspase (50, 100, or 1000 ng/ml) used in the the reactions is noted in the graphs.*

Preparation and Storage

Store product at -80°C prior to use or for long term storage of stock solutions.

The thawed active enzyme is stable at 4°C for at least a week.

Avoid multiple freeze-thaw cycles and exposure to frequent temperature changes, which can greatly alter product stability.

Caspase	Concentration	Cat. No. and Size
Caspase-3	50ng/ml	556472 (5 µg) / 556471 (10 µg)
Caspase-6	100ng/ml	556475 (5 µg) / 556474 (10 µg)
Caspase-7	100ng/ml	556478 (5 µg) / 556477 (10 µg)
Caspase-8	1000ng/ml	556481 (5 µg) / 556480 (10 µg)

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Application Notes

Application

Functional assay	Routinely Tested

Recommended Assay Procedure:

Active caspase-8 was expressed in E. coli and purified. (When expressed in E. coli, caspase-8 spontaneously undergoes autoprocessing to yield the subunits characteristic of the active enzyme). The rate of caspase enzymatic hydrolysis was measured by release of AMC from the Ac-DEVD-AMC caspase substrate as emission at 440 nm upon excitation at 380 nm using a spectrofluorometer.

The active enzyme is designed to be used in caspase assays.

This protocol is used to measure caspase enzyme activity. The synthetic fluorogenic peptide Ac-DEVD-AMC is used as the caspase enzyme substrate. The enzyme cleaves the substrate between D and AMC, releasing the fluorescent AMC. AMC release is measured by spectrofluorometry using UV excitation of 380 nm and emission wavelength of 440 nm.

1. Add 20 µM of Ac-DEVD-AMC to 1 ml of assay buffer (20 mM PIPES, 100 mM NaCl, 10mM DTT, 1mM EDTA, 0.1% (w/v) CHAPS, 10% sucrose, pH 7.2). Add the appropriate amount of the selected active caspase to the mixture (as indicated in the Enzyme Concentration table).
2. Incubate for 1 hr at 37°C.
3. Measure the AMC liberated from the Ac-DEVD-AMC using a spectrofluorometer with an excitation wavelength of 380 nm and an emission wavelength of 440 nm.

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Nicholson DW, Ali A, Thornberry NA, et al. Identification and inhibition of the ICE/CED-3 protease necessary for mammalian apoptosis. *Nature.* 1995; 376(6535):17-18.(Biology)

Patel T, Gores GJ, Kaufmann SH. The role of proteases during apoptosis. *FASEB J.* 1996; 10(5):587-597.(Biology)

Stennicke HR, Salvesen GS. Biochemical characteristics of caspases-3, -6, -7, and -8. *J Biol Chem.* 1997; 272(41):25719-25723.(Clone-specific: Functional assay)