

Technical Data Sheet

Recombinant Human Active Caspase-3

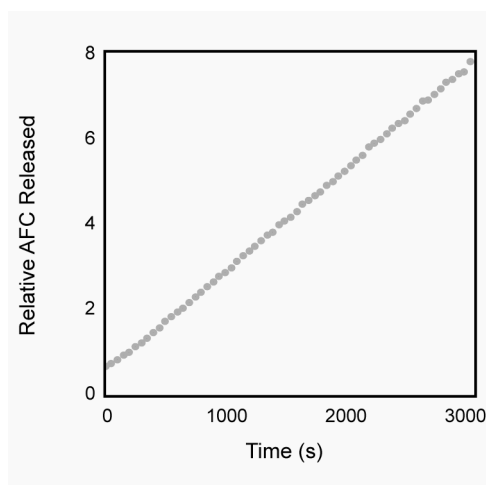
Product Information

Material Number:	556472
Size:	5 µg
Component:	51-66281V
Description:	Recombinant Human Active Caspase-3
Size:	5 µg (1 ea)
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-1 β -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 (ICE-LAP6, Mch6), and caspase-10 (Mch4). Each caspase is synthesized as an inactive proenzyme that is processed by cleavage at aspartate 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. 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-trifluoromethylcoumarin) and Ac(N-acetyl)-DEVD-AMC (7-amino-4-methylcoumarin) as well as the caspase-3 aldehyde inhibitor Ac-DEVD-CHO.



Rate of Ac-DEVD-AFC hydrolysis using 2.5 ng/ml recombinant human active caspase-3 (CPP32). The rate of enzymatic hydrolysis was measured at an emission of 505 nm upon excitation at 400 nm using a Perkin-Elmer LS50B fluorometer equipped with a thermostated plate reader.

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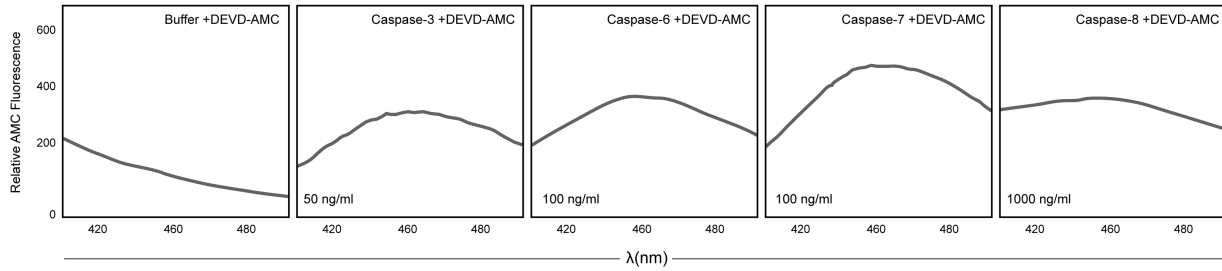
Preparation and Storage

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

Avoid multiple freeze-thaws of product.

The thawed active enzyme is generally stable at 4°C for about one week.

Active caspase-3 was expressed in E.coli and purified. When expressed in E. coli, caspase-3 will spontaneously undergo autoprocessing to yield the subunits characteristic of the active enzyme.



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 reactions is noted in the graphs.

Application Notes

Application

Enzyme assay	Routinely Tested
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Recommended Assay Procedure:

Enzyme Evaluation: The rate of caspase enzymatic hydrolysis is measured by release of AFC, from the Ac-DEVD-AFC caspase substrate, through emission at 480-520 nm (peak at 505 nm) upon excitation at 400 nm using UV spectrofluorometry. The rate of enzymatic hydrolysis can also be measured by release of AMC, from the Ac-DEVD-AMC caspase substrate, through emission at 440 nm upon excitation at 380 nm.

This protocol is used to measure caspase-3 enzymatic activity using the synthetic fluorogenic peptide, Ac-DEVD-AMC, 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 wavelength of 380 nm and emission wavelength of 440 nm.

1. Add 20 μ M of Ac-DEVD-AMC to 1 ml of assay buffer (20 mM PIPES, 100 mM NaCl, 10 mM DTT, 1 mM EDTA, 0.1% (w/v) CHAPS, 10% sucrose, pH 7.2). Add active caspase-3 to the mixture to a final concentration of 50 ng/mL.
2. Incubate for 1 hour at 37°C.
3. Measure the AMC liberated from Ac-DEVD-AMC using a spectrofluorometer with an excitation wavelength of 380 nm and an emission wavelength of 440 nm.

Suggested Companion Products

Catalog Number	Name	Size	Clone
556574	Ac-DEVD-AFC Caspase-3 Fluorogenic Substrate	1.0 mg	(none)
556449	Ac-DEVD-AMC Caspase-3 Fluorogenic Substrate	1.0 mg	(none)
556473	Active Caspase-3 Set	20 tests	(none)

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.(Biology)