Technical Data Sheet

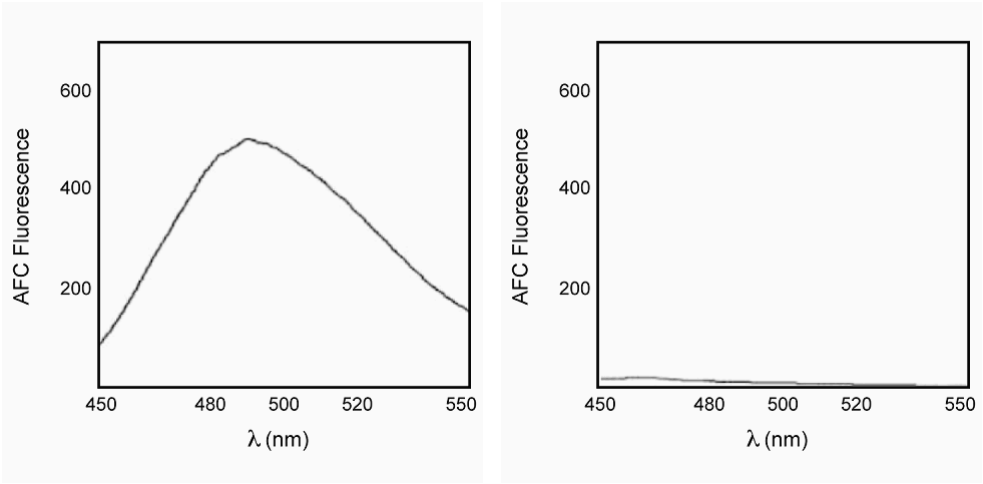
Active Caspase-3 Set

Product Information

Material Number:	**556473**
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
Component:	**51-67201U**
Description:	Ac-DEVD-AFC, Caspase-3 Fluorogenic Substrate
Size:	1.0 mg (1 ea)
Storage Buffer:	Lyophilized in dimethyl sulfoxide (DMSO).
Component:	**51-66221U**
Description:	Ac-DEVD-CHO, Caspase-3 Inhibitor
Size:	1.0 mg (1 ea)
Storage Buffer:	Lyophilized in dimethyl sulfoxide (DMSO).

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. Active caspase-3, consisting of a 12 kDa and 17 kDa subunit derived from a 32 kDa pro-enzyme (pro- caspase-3), has been shown to cleave PARP [poly (ADP ribose) polymerase], an enzyme that is involved in DNA repair and genomic maintenance. Proteolysis of the 116 kDa intact form of PARP into 85 and 25 kDa subunits 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 the highly specific caspase-3 substrate Ac(N-acetyl) -DEVD-AFC (7-amino-4-trifluoromethylcoumarin). Caspase-3 cleaves the tetrapeptide between D and AFC, thus releasing the fluorogenic AFC which can be quantified by UV spectrofluorometry. When coupled to an aldehyde group (CHO), the DEVD peptide functions as a potent inhibitor of caspase-3 activity and can be used to block caspase-3 mediated cleavage of Ac-DEVD-AFC. These tetrapeptide substrates can be used to identify and quantitate caspase-3 activity in apoptotic cell lysates.



Activity of recombinant human active caspase-3. *Ac-DEVD-AFC is a synthetic tetrapeptide substrate that is cleaved by active human caspase-3. This substrate is cleaved between D and AFC, releasing the fluorogenic AFC, which is detected by spectrofluorometry. When coupled to an aldehyde group (CHO), the DEVD tetrapeptide functions as a potent inhibitor of caspase activity and can be used to block caspase cleavage of Ac-DEVD-AFC.* ***Left panel:*** *In the presence of active caspase-3, fluorogenic AFC is released from Ac-DEVD-AFC, demonstrating the activity of caspase-3 enzyme.* ***Right panel:*** *In the presence of active caspase-3, Ac-DEVD-AFC and Ac-DEVD-CHO, fluorogenic AFC is not released, indicating that Ac-DEVD-AFC was not cleaved and that caspase-3 activity was blocked by Ac-DEVD-CHO.*

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Preparation and Storage

Avoid multiple freeze-thaws of product.
Store recombinant human active caspase-3 at -80°C prior to use or for long term storage of stock solutions.
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.
Store the lyophilized Ac-DEVD-AFC substrate and the Ac-DEVD-CHO inhibitor at -20°C. Once reconstituted in DMSO, store at -20°C for up to 2 months. Avoid multiple freeze-thaw cycles by aliquoting samples for storage.

Application Notes

Application

Enzyme assay	Routinely Tested

Recommended Assay Procedure:
Enzyme Evaluation: The rate of caspase-3 enzymatic hydrolysis can be measured by the 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.

1. Reconstitute the caspase-3 fluorogenic substrate and inhibitor:

(a) Ac-DEVD-AFC [N-acetyl-Asp-Glu-Val-Asp-AFC (7-amino-4-trifluoromethylcoumarin)]: Reconstitute 1 mg of the lyophilized powder in 1 ml DMSO so that the concentration is at 1 mg/ml in DMSO. Ac-DEVD-AFC is a synthetic tetrapeptide fluorogenic substrate for active caspase-3. It contains the amino acid sequence of the PARP cleavage site at Asp-216. The Ac-DEVD-AFC substrate was made on a peptide synthesizer and purified according to standard protocols: purity ≥ 98%, MW=729 Daltons. Ac-DEVD-AFC has been reported to have linear Michaelis-Menton kinetics with a Km of 10 µM for Caspase-3.

(b) Ac-DEVD-CHO [N-acetyl-Asp-Glu-Val-Asp-CHO (aldehyde)]: Reconstitute 1 mg of the lyophilized powder in 1 ml DMSO so that the concentration is at 1 mg/ml in DMSO. Ac-DEVD-CHO is a synthetic tetrapeptide inhibitor for active caspase-3. It contains the amino acid sequence of the PARP cleavage site at Asp-216.3 The Ac-DEVD-CHO inhibitor was made on a peptide synthesizer and purified according to standard protocols: purity ≥98%, MW=502.5 Daltons. Ac-DEVD-CHO has been previously reported to have linear Michaelis-Menton kinetics with a Ki of <1 µM for Caspase-3.

2. To one tube, add 10 µl of Ac-DEVD-AFC into 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.
3. In a separate tube, add 10 µl of Ac-DEVD-AFC and 10 µl Ac-DEVD-CHO into 1 ml assay buffer.
4. Add 50 ng of recombinant human active caspase-3 to each tube.
5. Incubate for 1 hour at 37°C.
6. Measure the AFC liberated from the Ac-DEVD-AFC using a spectrofluorometer with an excitation wavelength of 400 nm and an emission wavelength of 480-520 (peak at 505) 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

Mashima T, Naito M, Kataoka S, Kawai H, Tsuruo T. Aspartate-based inhibitor of interleukin-1 beta-converting enzyme prevents antitumor agent-induced apoptosis in human myeloid leukemia U937 cells. *Biochem Biophys Res Commun.* 1995; 209(3):907-915.(Biology)
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Stennicke HR, Salvesen GS. Biochemical characteristics of caspases-3, -6, -7, and -8. *J Biol Chem.* 1997; 272(41):25719-25723.(Biology)