Technical Data Sheet

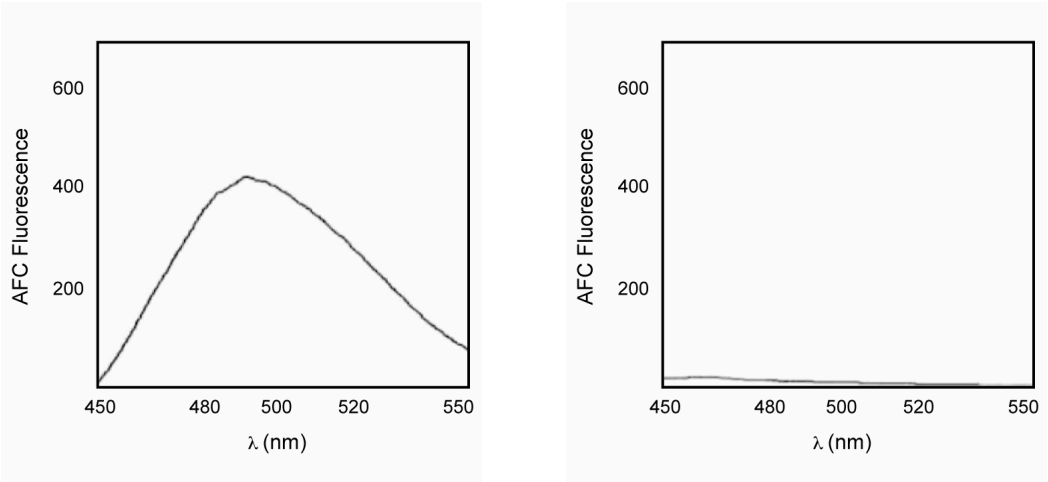
Ac-IETD-CHO, Caspase 8 Inhibitor

Product Information

Material Number:	**556554**
Size:	1.0 mg
Storage Buffer:	Lyophilized in dimethyl sulfoxide (DMSO).

Description

Members of the caspase family have key roles in inflammation and mammalian apoptosis. Caspase-8 (FLICE/MACH-1) is a 55 kDa cytosolic protein with homology to the CD95/Fas-associated signal transduction molecule, FADD, in addition to its homology with other caspases. Caspase-8 is activated early in apoptosis and is involved in the proteolysis and activation of pro-caspase-3. The upstream sequence of the site recognized by active caspase-8, IETD (Ile-Glu-Thr-Asp), is utilized as a basis for the highly specific caspase-8 substrate, Ac-IETD-AFC, and the caspase-8 inhibitor, Ac-IETD-CHO. Ac-IETD-CHO (502 Daltons) is a synthetic tetrapeptide inhibitor of caspase-8 and contains the amino acid sequence that is the target for caspase-8 mediated proteolysis. The tetrapeptide inhibitor can be used as a negative (blocking) control, in parallel with Ac-IETD-AFC, to study caspase-8 activity in apoptotic cell lysates.



Activity of recombinant human caspase-8. *Ac-IETD-AFC is a synthetic tetrapeptide substrate that is cleaved by active human caspase-8. 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 IETD tetrapeptide functions as a potent inhibitor of caspase activity and can be used to block caspase-8 mediated cleavage of Ac-IETD-AFC. In the presence of active caspase-8, fluorogenic AFC is released from Ac-IETD-AFC, demonstrating the activity of caspase-8 enzyme (left panel). In the presence of both active caspase-8 and Ac-IETD-CHO, fluorogenic AFC is not released, indicating that Ac-IETD-AFC was not cleaved and that caspase-8 activity was blocked by Ac-IETD-CHO (right panel).*

Preparation and Storage

Store the lyophilized Ac-IETD-CHO inhibitor at -20°C.
Reconstitute the Ac-IETD-CHO inhibitor with 1 ml DMSO before use.
Store the reconstituted Ac-IETD-CHO inhibitor at -20°C for up to two months and avoid repeated freeze-thaw cycles, which can greatly alter product stability.

Application Notes

Application	
Fluorescence quantitation	Routinely Tested

Recommended Assay Procedure:

The Ac-IETD-CHO inhibitor may be used in protease assays like those described in the literature. When the caspase-8 fluorogenic substrate Ac-IETD-AFC is treated with apoptotic cell lysates or with purified, active caspase-8, AFC is released. AFC release can be monitored in a spectrofluorometer at an excitation wavelength of 400 nm and an emission wavelength range of 480-520 nm. Apoptotic cell lysates yield a considerable emission as compared to non-apoptotic cell lysates, or apoptotic lysates, which also contain Ac-IETD-CHO. The amount of cell lysate required for protease assays will vary between experimental systems and should be optimized by the user. A suggested protease assay protocol follows.

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Ac-IETD-CHO PROTEASE ASSAY

Materials Required

1. Purified, Active Recombinant Caspase-8 (Cat. No. 556481). Not Included. 5 µg enzyme in 25 µl. The enzyme is buffered with 50 mM Tris, pH 8.0, with 100 mM NaCl and 50 mM imidazole.
2. Ac-IETD-AFC (Cat. No. 556552). Not Included. 1 mg peptide in DMSO; lyophilized. Reconstitute in 1 ml DMSO to yield 1 mg/ml peptide.
3. Ac-IETD-CHO (Cat. No. 556554). Included. 1 mg peptide, lyophilized. Reconstitute in 1 ml DMSO to yield 1 mg/ml peptide.
4. Protease assay buffer: Not Included. 20 mM PIPES, 100 mM NaCl, 10mM DTT, 1mM EDTA, 0.1% (w/v) CHAPS, 10% sucrose; pH 7.2.

Procedure

1. To one tube, add 10 µl of Ac-IETD-AFC into 1 ml of assay buffer. In a separate tube, add 10 µl of Ac-IETD-AFC and 1 µl Ac-IETD-CHO into 1 ml assay buffer.
2. Add 1 µg purified, active caspase-8 to each tube.
3. Incubate for 1 hr at 37°C.
4. Measure the AFC liberated from the Ac-IETD-AFC using a spectrofluorometer with an excitation wavelength of 400 nm and an emission wavelength of 480-520 nm (peak at 505 nm).

Suggested Companion Products

Catalog Number	Name	Size	Clone
556481	Purified Active Recombinant Human Caspase-8	1.0 mg	(none)
556552	Ac-IETD-AFC Caspase-8 Fluorogenic Substrate	1.0 mg	(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

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: Apoptosis, Enzyme assay)

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: Apoptosis, Enzyme assay)

Thornberry NA, Lazebnik Y. Caspases: enemies within. *Science.* 1998; 281(5381):1312-1316.(Biology: Apoptosis)