Technical Data Sheet

Ac-IETD-AFC Caspase-8 Fluorogenic Substrate

Product Information

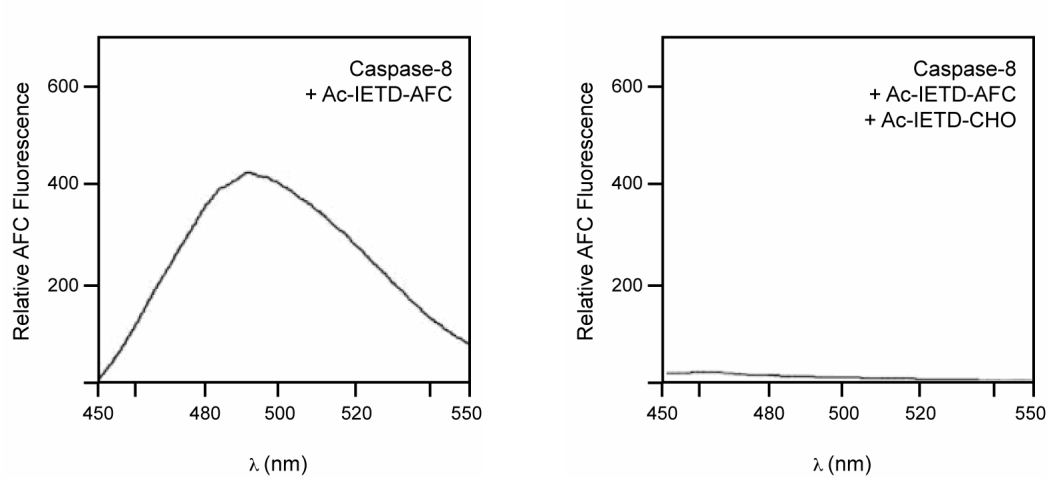
Material Number: **556552**

Size: 1.0 mg

Storage Buffer: Lyophilized in dimethyl sulfoxide (DMSO).

Description

The caspase family of cysteine proteases was discovered following a search of human cDNA libraries for sequences homologous to ced-3, a cell death gene described in the nematode worm *C. elegans*. The name caspase reflects the catalytic properties of these enzymes. The "c" denotes their cysteine protease mechanism and "aspase" refers to their ability to cleave after aspartic acid residues. These proteases are expressed as inactive proenzymes, which are proteolytically cleaved into large and small subunits, which form the active enzyme. Caspase-8 (FLICE/MACH-1) is a 55 kD 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-AFC is a synthetic tetrapeptide substrate for caspase-8 and contains the amino acid sequence which is the target for caspase-8-mediated proteolysis. The tetrapeptide substrate can be used to identify and quantify caspase-8 activity in apoptotic cell lysates and to study events downstream of caspase-8 activation.



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. Left panel: In the presence of active caspase-8, fluorogenic AFC is released from Ac-IETD-AFC, demonstrating the activity of caspase-8 enzyme. Right 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.*

Preparation and Storage

Store the lyophilized Ac-IETD-AFC substrate at -20°C. Reconstitute the Ac-IETD-AFC substrate with 1 ml DMSO before use. Store the reconstituted Ac-IETD-AFC substrate 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-AFC fluorogenic substrate may be used in protease assays like those described by Nicholson et al. and Mashima et al. When

BD Biosciences
bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	888.259.0187	32.53.720.550	0120.8555.90	65.6861.0633	55.11.5185.9995

For country-specific contact information, visit bdbiosciences.com/how_to_order/

BD
BD Biosciences

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.

Ac-IETD-AFC PROTEASE ASSAY

Ac-IETD-AFC is made on a peptide synthesizer and purified using standard protocols (purity ≥98%; MW=729 Daltons). The Ac-IETD-AFC substrate is routinely evaluated with the following protease assay protocol using purified, recombinant active caspase-8 (Cat. No. 556481).

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): ***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): ***Not Included***. 1 mg peptide in DMSO; 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	5 µg	(none)
556554	Ac-IETD-CHO, Caspase 8 Inhibitor	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: 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: Enzyme assay)

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