Technical Data Sheet

Ac-DEVD-AMC Caspase-3 Fluorogenic Substrate

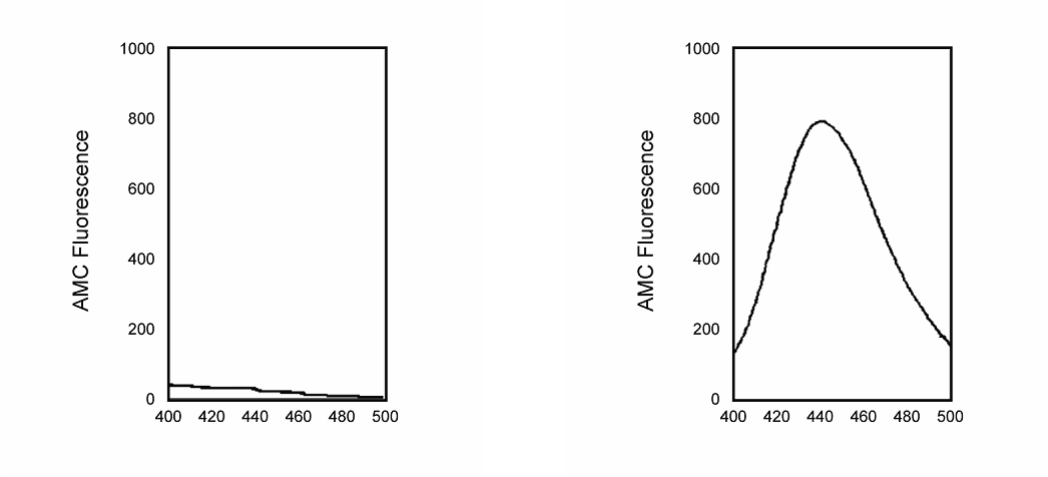
Product Information

Material Number: **556449**
Size: 1.0 mg
Storage Buffer: Lyophilized powder

Description

Members of the ICE/CED-3 cysteine protease family have key roles in inflammation and mammalian apoptosis. The ICE family member Caspase-3 (also known as CPP32, Yama, apopain) is activated early in apoptosis and appears to be involved in the proteolysis of several important molecules, including poly (ADP ribose) polymerase (PARP). Activated Caspase-3 cleaves PARP from its 116 kDa to an 85 kDa residual fragment. The cleavage site in PARP is C-terminal to Asp-216. The upstream sequence of the cleavage site, DEVD (Asp-Glu-Val-Asp), is utilized as a basis for the highly specific Caspase-3 substrate, Ac (N-acetyl)-DEVD-AMC (7-amino-4-methylcoumarin).

Ac-DEVD-AMC is a synthetic tetrapeptide fluorogenic substrate for Caspase-3 (CPP32) and contains the amino acid sequence of the PARP cleavage site at Asp-216. The tetrapeptide substrate can be used to identify and quantify the Caspase-3 activity in apoptotic cells. Caspase-3 cleaves the tetrapeptide between D and AMC, thus releasing the fluorogenic AMC, which can be quantified in a spectrofluorometer. The substrate can also be used to study the inhibition of Caspase-3 by the tetrapeptide aldehyde, Ac-DEVD-CHO or any other inhibitor of Caspase-3.



Protease Assay Using Substrate for Caspase-3 (CPP32), Ac-DEVD-AMC (Cat. No. 556449). *Non-apoptotic (left panel) and apoptotic HPB-ALL leukemia cell lysates (right panel) were incubated in Ac-DEVD-AMC protease assay buffer at 37°C for 1 hour. In each assay, the AMC liberated from the fluorogenic substrate was measured in a spectrofluorometer with an excitation wavelength of 380 nm and emission wavelength range of 430-460 nm. The results show that the Ac-DEVD-AMC substrate was cleaved by apoptotic cell lysate but not by the non-apoptotic lysate. In the right panel, cells were induced to undergo apoptosis using an Anti-Human Fas monoclonal antibody, clone DX2 (Cat. No. 555670). Apoptosis was measured after 5 hour of incubation with 2 µg/ml Fas mAb and 1 µg/ml Protein G. We have found that the addition of Protein G can significantly enhance the effieciency of Fas mAb to induce apoptosis.*

Preparation and Storage

Store undiluted at -20°C.
Reconstitute the substrate before use. Reconstitute in 1 ml DMSO to yield 1 mg/ml peptide in DMSO.
Store the reconstituted substrate at –20°C for up to 1-2 months and avoid repeated freeze-thaw cycles, which greatly alter product stability.

Application Notes

Application

Functional assay	Routinely Tested

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/**

Recommended Assay Procedure:
The Ac-DEVD-AMC substrate was made on a peptide synthesizer and purified according to standard protocols: purity ≥ 98%, MW=675 Daltons. The Ac-DEVD-AMC substrate is routinely 800 evaluated in protease assays using apoptotic and non-apoptotic cell lysates or purified, active Caspase-3 (Cat. No. 556472). Ac-DEVD-600 AMC has been previously reported to have linear Michaelis-Menton kinetics with a Km of 10 µM for Caspase-3.

The Ac-DEVD-AMC fluorogenic substrate is designed to be used in protease assays, like those described in the literature by Mashima *et al* and Nicholson *et al*. When Ac-DEVD-AMC is treated with pure and active Caspase-3 or apoptotic cell lysates, AMC is released. AMC release is monitored in a spectrofluorometer at an excitation wavelength of 380 nm and an emission wavelength range of 430-460 nm. Apoptotic cell lysates containing active Caspase-3 yield a considerable emission as compared to non-apoptotic cell lysates. Apoptosis can be induced by a number of mechanisms. For each protease assay, we typically use a 1 ml reaction buffer [20 mM HEPES (pH 7.5), 10% glycerol, 2 mM DTT], 20 µM Ac-DEVD-AMC, and cell lysate. The amount of cell lysate required for protease assays will vary between experimental systems and should be optimized by the user.

AC-DEVD-AMC PROTEASE ASSAY
The Protease Assay protocol is used at BD Biosciences Pharmingen to assay protease activity using the Ac-DEVD-AMC, Caspase-3 (CPP32) Fluorogenic Substrate (Cat. No. 556449).

Materials Required
1. Apoptotic and non-apoptotic cells.
2. PBS Wash Buffer (10X): 1.4 M NaCl, 27 mM KCl, 100 mM KH2PO4/K2HPO4, pH 7.5, dissolved in distilled, autoclaved water. Dilute to 1X with sterile distilled H20 prior to use. Adjust the pH to 7.5. Store the buffer at 4°C.
3. Cell Lysis Buffer (1X): 10 mM Tris-HCl, 10 mM NaH2PO4/NaHPO4, pH 7.5, 130 mM NaCl, 1% Triton -X-100, 10 mM NaPPi (sodium pyrophosphate); sterile filtered. Store the buffer at 4°C.
4. Caspase-3 (CPP32), Ac-DEVD-AMC Fluorogenic Substrate (Cat. No. 556449).
5. Protease Assay Buffer (1X): 20 mM HEPES (pH 7.5), 10% glycerol, 2 mM DTT. Make up fresh before use.

Procedure
Cell lysate preparation:
For adherent cells, decant the media and wash rapidly with PBS. Remove excess PBS by aspiration and lyse with Cell Lysis Buffer (~2-10 million cells/ml). For cells growing in suspension, pellet, wash with PBS and lyse for 30 min on ice with Cell Lysis Buffer (~2-10 million cells/ml).

Protease assays:
1. For each reaction, add 20 µM (final concentration) Ac-DEVD-AMC and cell lysate to 1 ml Protease Assay Buffer. The amount of apoptotic cell lysate required to cleave the Ac-DEVD-AMC will vary for each experimental system and should be titrated. We generally titrate between 10-100 µl of cell lysate per 1 ml Protease Assay Buffer. Suggested controls:
   a) Reaction mixtures with non-apoptotic cell lysates. In these lysates, there may be a basal level of apoptosis.
   b) Reaction mixtures with lysis buffer only (no cells). These lysates are used as negative controls.
2. Incubate the reaction mixtures for 1 hr 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 range of 430-460 nm. Apoptotic cell lysates containing active Caspase-3 yield a considerable emission as compared to non-apoptotic cell lysates (see figure).

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