Datasheet for ABIN2690897

Ac-DEVD-AFC Caspase-3 Fluorogenic Substrate

2 Publications

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Overview

Quantity:	1 mg
Application:	Functional Studies (Func)

Product Details

Brand:	BD Pharmingen™
Characteristics:	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 first mammalian homologue of ced-3 to be identified was ICE (interleukin-1β converting enzyme). Subsequently, numerous mammalian ced-3 homologues have been discovered and have each been given a variety of names. To achieve consistency, the term "caspase" was adopted as a root name for all family members. The name 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. Active caspase-3 consists of 17 and 12 kDa subunits which are derived from a 32 kDa proenzyme (pro-caspase-3). Active 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-acetyle)-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 U.V. spectrofluorometry. When coupled to an aldehyde group (CHO), the DEVD peptide functions as a potent inhibitor of

Product Details

	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 caspase-3. Ac-DEVD-AFC (MW 729 Daltons) is a synthetic tetrapeptide substrate that 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 both active caspase-3 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.

Application Details

Application Notes:	Optimal working dilution should be determined by the investigator.
Comment:	BD Pharmingen™ Ac-DEVD-AFC Caspase-3 Fluorogenic Substrate - Purified
Restrictions:	For Research Use only

Handling

Format:	Lyophilized
Reconstitution:	Reconstitute the Ac-DEVD-AFC substrate with 1 mLDMSO before use. The reconstituted substrate may be stored in small aliquots at -20 °C. Store the reconstituted Ac-DEVD-AFC substrate at -20 °C for up to two months.
Buffer:	Lyophilized powder
Storage:	-20 °C
Storage Comment:	Store undiluted at -20°C. Avoid multiple freeze-thaws of product. Reconstitute the Ac-DEVD-AFC substrate with 1 ml DMSO before use. The reconstituted substrate may be stored in small aliquots at -20°C. Store the reconstituted Ac-DEVD-AFC substrate at -20°C for up to two months.

Publications

Product cited in:	Patel, Gores, Kaufmann: "The role of proteases during apoptosis." in: **FASEB journal : official publication of the Federation of American Societies for Experimental Biology**, Vol. 10, Issue 5,

pp. 587-97, (1996) (PubMed).

Nicholson, Ali, Thornberry, Vaillancourt, Ding, Gallant, Gareau, Griffin, Labelle, Lazebnik: " Identification and inhibition of the ICE/CED-3 protease necessary for mammalian apoptosis." in: **Nature**, Vol. 376, Issue 6535, pp. 37-43, (1995) (PubMed).