

PRODUCT DATA SHEET

Product: Ac-YVAD-AFC (CASPASE-1 / ICE FLUOROGENIC SUBSTRATE 2)

Cat. No: AC-001 (10 mg)

Chemical Name:

Ac-Tyr-Val-Ala-Asp-AFC

Molecular Weight: 720

Description:

Peptide substrate labeled at the carboxy end with AFC (7-amino-4-trifluoromethyl coumarin). Designed to measure Caspase-1 or Caspase-4 activity *in vitro*.

Introduction:

Interleukin-1 β Converting Enzyme (ICE), now termed Caspase-1, is a cytoplasmic cysteine protease that cleaves inactive 31 kDa pro-IL-1 β to generate the active 17.5 kDa proinflammatory cytokine IL-1 β , the predominant form of IL-1 produced by human monocytes. This cytokine has been implicated in the pathogenesis of several diseases such as rheumatoid arthritis, inflammatory bowel disease, and septic shock.

Caspase-1/ICE mRNA is found in a variety of cells such as peripheral blood monocytes, peripheral blood lymphocytes, peripheral blood neutrophils, and resting and activated peripheral blood T lymphocytes. The tissue distribution of Caspase-1/ICE suggests that the enzyme may have other substrates in addition to IL-1 β .

Current hypotheses suggest that Caspase-1/ICE is able to cause apoptosis as well as activate inflammation in animal cells. Experiments have shown that Caspase-1/ICE has sequence homology with other mammalian apoptosis genes and that activation of Caspase-1/ICE or other ICE-related proteases (caspases) is required for anti-Fas mAb-induced apoptosis.

Specificity:

Serves as a substrate for Caspase-1 and as a weak substrate for Caspase-4. Not cleaved by Caspases-2, -3, -6, or -7.

Principal:

A synthetic peptide substrate, Ac-Tyr-Val-Ala-Asp, has been labeled with AFC (7-amino-4-trifluoromethyl coumarin) at the carboxy end. AFC is a fluorescent molecule whose release from the substrate can be used to measure

Caspase-1/ICE activity. Caspase-1/ICE enzyme activity in the sample is proportional to the amount of free AFC produced.

When AFC is attached to the peptide substrate, it produces a blue fluorescence upon exposure to UV light (400 nm). Caspase-1/ICE enzymatically cleaves the AFC-substrate and releases free AFC, which produces a yellow-green fluorescence at 505 nm when exposed to UV light.

AFC has two advantages over other fluorogenic labels. The wide Stokes' shift between bound and free AFC enables the substrate to be both chromogenic (yellow-green color visible to the naked eye) and fluorogenic (emission at 505 nm). The wide Stoke's shift also makes the assay more sensitive.

Applications:

For *in vitro* assays of Caspase-1 and Caspase-4 activities. Can be used with purified or partially purified enzymes, or possibly with crude cell lysates (if the Caspase-1/ICE Inhibitor 2 is included to determine background protease activity).

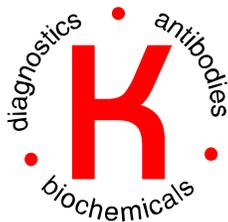
Protocol:

Fluorometer calibration: The fluorometer is calibrated using known concentrations of free AFC (Excitation = 400 nm, Emission = 505 nm) to generate a standard curve of fluorescence versus μ moles AFC.

Samples: Can be either purified or partially purified caspase preparations. Application to crude cell lysates has not been confirmed. If crude cell lysates are to be assayed, the non-specific protease background must be determined using our specific Caspase-1/ICE Inhibitor 2 (Cat. No. AB-011).

General Fluorometric Assay Procedure:

CAUTION: The following procedure is provided only as an example for reference purposes. The user should determine the optimal conditions for their system. For example, see: Nancy A. Thornberry, Interleukin-1 β Converting Enzyme, *Methods in Enzymology*, 244:615-631 (1994).



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1. Prepare:

- 25 mM Caspase-1/ICE Fluorogenic Substrate 2 (Ac-Tyr-Val-Ala-Asp-AFC) stock solution in DMSO. Dilute 1:10 in DMSO.
- 25 mM Caspase-1/ICE Inhibitor 2 (Z-YVAD-FMK) stock solution in DMSO. Dilute 1:10 in DMSO.
- 100 mM DTT, prepare immediately before use in Caspase-1/ICE buffer.
- Caspase-1/ICE buffer: 50 mM HEPES, 10% sucrose, 0.1% CHAPS, adjust pH to 7.5 with conc. NaOH.

Storage and Stability:

Store Caspase-1/ICE Fluorogenic Substrate 2 in a desiccator at room temperature or 4°C. For long term, 4°C is recommended. The Caspase-1/ICE Fluorogenic Substrate 2 has a shelf life of up to 6 months if stored at 4°C. DMSO stock solutions have a shelf life of 1 year if stored at 4°C.

References:

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2. Buttler, D.J. *et al.* (1992). Biochem. J. **281**:175-177.
3. Cerretti, D.P. *et al.* (1992). Science. **256**:97-100.
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5. Hughes, F. M., *et al.* (1997). J. Biol. Chem. **272**(48): 30567-30576.
6. Kronheim, S.R., *et al.* (1993). Arch. Biochim. Biophys. **296**(2):698-703.
7. Kuida, K. *et al.* (1995). Science. **267**:2000-2003.
8. Talanian, R.V. *et al.* (1997). J. Biol. Chem. **272**: 9677-82.
9. Thornberry, N.A. *et al.* (1992). Nature. **356**:768-774.

Limitations:

For *in vitro* research use only. Not for use in diagnostics or in humans.

Warranty:

No warranties, expressed or implied, are made regarding the use of this product. KAMIYA BIOMEDICAL COMPANY is not liable for any damage, personal injury, or economic loss caused by this product.