PRODUCT DATA SHEET

Product: Ac-VAD-AFC (Fluorogenic caspase-1, 4 substrate)

Cat. No.: **AC-021 (5 mg)**

Chemical Name:
Ac-Val-Ala-Asp-AFC

Molecular Weight: 556

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

Introduction:
Interleukin-1ß Converting Enzyme (ICE), now termed Caspase-1, is a cytoplasmic cysteine protease that cleaves inactive 31 kDa pro-IL-1ß to generate the active 17.5 kDa proinflammatory cytokine IL-1ß, 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-1ß.

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. The role of Caspase-4 in apoptosis is unclear but its substrate specificity is similar to that of Caspase-1/ICE.

Principal:
A synthetic peptide substrate, Ac-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 or -4 activity. Caspase-1/ICE or -4 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 or -4 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.

Specificity:
Serves as a substrate for Caspase-1/ICE and Caspase-4.

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 1 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 µmoles 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 1 (Cat. No. AB-001).

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.
1. Prepare:
- 25 mM fluorogenic caspase-1, 4 substrate 1 (Ac-Val-Ala-Asp-AFC) stock solution in DMSO. Dilute 1:10 in DMSO.

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• 25 mM Caspase-1/ICE Inhibitor 1 (Z-VAD-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.

2. Prepare several dilutions of sample using Caspase-1/ICE buffer (1/10, 1/100, 1/1000).

3. Ideally, each sample dilution should be tested in three different reactions:
Group 1: Substrate only (blank)
Group 2: Sample + inhibitor + substrate (background protease activity)
Group 3: Sample + substrate (enzyme assay)

4. Prepare standard curve for AFC fluorescence by measuring known amounts of AFC in a fluorometer. (Excitation = 400 nm, Emission = 505 nm)

Group 2 reactions should be started first since the inhibitor needs to react with the sample before the substrate is added. For Group 2 reactions:
Add 410 µL Caspase-1/ICE buffer, 50 µL 100 mM DTT, 10 µL 2.5 mM inhibitor solution, vortex, then add 20 µL sample. Mix gently, incubate at 30°C for 30 min to 12 hours (time should be determined by the user). After incubation is finished, proceed with step 6b.

6a. Group 1 reactions: Add 440 µL Caspase-1/ICE buffer, 50 µL 100 mM DTT, 10 µL 2.5 mM substrate solution.

6b. Group 2 reactions: Add 10 µL 2.5 mM substrate solution.

6c. Group 3 reactions: Add 420 µL Caspase-1/ICE buffer, 50 µL 100 mM DTT, and 10 µL 2.5 mM substrate solution, vortex, then add 20 µL sample.

7. Groups 1, 2, and 3: Mix gently, incubate at 30°C for 60 minutes, then measure fluorescence for time 0 (T_0).

8. Continue incubation at 30°C for another 60 min. and measure fluorescence for time 1 hr (T_1).

9. Calculate ΔFU for each sample dilution at T_1 as follows:
ΔFU= [Group 3 FU at T_1 - Group 1 FU at T_1] - [Group 3 FU at T_0 - Group 1 FU at T_0]

10. Calculate enzyme activity in sample for T_1. If activity is low, assay should be run for a longer time (up to 24 hours if necessary). For best results, use the sample dilution giving the highest Group 3 (assay) values and lowest Group 2 (background protease) values.

Unit of Caspase-1/ ICE (or Caspase-4) activity = 1 µmol of free AFC/min.
Units Caspase-1/ICE (or -4) = [(ΔFU/min) / (std. curve slope)] x [1 Unit/ (1 x 10^{-6} µmoles AFC/min)]

Example calculation:
Dilute an 80 µM AFC DMSO stock solution in Caspase-1/ICE buffer to give 0.5 ml final volumes as follows:
1 in 50 dilution = 8 x 10^{-4} µmoles AFC
2 in 50 dilution = 16 x 10^{-4} µmoles AFC
3 in 50 dilution = 24 x 10^{-4} µmoles AFC.

Plot the results with x axis = µmole AFC and y axis = Fluorescence Units (FU).

An example curve gives a slope of 8 x 10^{-6} µmoles AFC/FU.

For a ΔFU = 7.8 (T_1 - T_0); T_1 = 60 min
Units Capsase-1/ICE = (7.8/60) x (8 x 10^{-6}) x (1 x 10^6) = 1.04

The number of assays that can be run with the 10 mg of substrate provided depends upon the reaction volumes.

Storage and Stability:

Store Caspase-1/ICE Fluorogenic Substrate 1 in a desiccator at room temperature or 4°C. For long term, 4°C is recommended. The Caspase-1/ICE Fluorogenic Substrate 1 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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Limitations:

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

Warranty:

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