COSMO BIO CO., LTD.

PRODUCT DATA SHEET

Product: Ac-VDVAD-AFC (Fluorogenic caspase-2, 3, 7 substrate)

Cat. No: **AC-008 (5 mg)**

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

Molecular Weight: 772

Description:
Peptide substrate labeled at the carboxy end with AFC (7-amino-4-trifluoromethyl coumarin). Designed to measure Caspase-2 activity *in vitro*.

Introduction:
Caspase-2 (also known as ICH-1) is a member of the caspase family of cysteine proteases involved in apoptosis. It is a member of the Group II caspases, along with caspases-3 and -7. Group II caspases prefer peptides of the DEXD-type as substrates. However, unlike caspases-3 and -7, Caspase-2 requires a P5 amino acid in the peptide for efficient cleavage. The similar substrate specificities of the Group II caspases suggests that their roles in cells are at least overlapping, if not completely redundant. The requirement for a fifth amino acid in substrates for Caspase-2 means that the DEVD-type, while serving as substrates for caspases-3 and -7, do not work with Caspase-2. For this reason, the Ac-VDVAD-AFC substrate is excellent for studying Caspase-2.

Principal:
A synthetic peptide substrate, Ac-Val-Asp-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-2 activity. Caspase-2 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-2 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:
Highly specific substrate for Caspase-2. Will also serve as a substrate for Caspases-3 and -7.

Applications:
For *in vitro* assays of Caspase-2 activity, or Caspases-3 and -7 although the Caspase-3 Fluorogenic Substrate (Ac-DEVD-AFC, Cat. No. AC-003) is preferable for these two enzymes. The Caspase-2,3 Fluorogenic Substrate can be used with purified or partially purified enzymes or possibly with crude cell lysates (if the Caspase-2,3 Inhibitor 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 enzyme 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 Caspase-2,3 Inhibitor (Cat. No. AB-008).

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:
- 20 mM Ac-Val-Asp-Val-Ala-Asp-AFC stock solution in DMSO. Dilute 1:10 in DMSO.
- 20 mM Caspase-2,3 Inhibitor (Z-VDVAD-FMK) stock solution in DMSO. Dilute 1:10 in DMSO.
- Caspase-2,3 buffer: 100 mM HEPES, 10% sucrose, 10 mM DTT, 0.5 mM EDTA, adjust to pH 7.5 w/ conc. NaOH.

2. Prepare several dilutions of sample using Caspase-2,3 buffer (1/10, 1/100, 1/1000).

3. Ideally, each sample dilution should be assayed using 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 using known amounts of AFC. (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 460 µl Caspase-2,3 buffer, 10 µl 2.0 mM inhibitor solution, vortex, then add 20 µl sample. Mix gently, incubate at 4°C for 30 min and then at 37°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 490 µl Caspase-2,3 buffer, 10 µl 2.0 mM substrate solution.

6b. Group 2 reactions: Add 10 µl 2.0 mM substrate solution.

6c. Group 3 reactions: Add 470 µl Caspase-2,3 buffer, 10 µl 2.0 mM substrate solution, vortex, then add 20 µl sample.

7. Groups 1, 2, and 3 : Mix gently, incubate at 4°C for 30 minutes, then measure fluorescence for time 0 (T_0).

8. Continue incubation at 37°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 samples for T_1. If activity is low, assay should be run for a longer time (up to 24 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-2 activity = 1 µmol of free AFC/min.
Units Caspase-2 = [(ΔFU/min) / (std. curve slope)] x [1 Unit/ (1×10^{-6} µmoles AFC/min)]

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

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

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

For a $\Delta FU = 7.8$ ($T_1 - T_0$); $T_1 = 60$ min
Units Caspase-2 = $(7.8/60) \times (8 \times 10^{-6}) \times (1 \times 10^{6}) = 1.04$

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-2,3 Fluorogenic Substrate in a desiccator at room temperature or 4°C. The Caspase-2,3 Fluorogenic Substrate 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:

1. Talanian, R.V. *et al*. (1997). J. Biol. Chem. **272**: 9677-82.
2. Thornberry, N.A. *et al*. (1997). J. Biol. Chem. **272**: 17907-911.
3. Wang, L. *et al.* (1994). Cell **78**: 739-50.

Limitations:

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