Saint Louis, Missouri 63103 USA
Telephone (800) 325-5832 (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

N-ACETYL-VAL-GLU-ILE-ASP-7-AMIDO-4-TRIFLUOROMETHYLCOUMARIN

Product Number **A 5095**
Storage Temperature –20 °C

(Ac-VEID-AFC)

Product Description
Appearance: White Powder
Formula: $C_{32}H_{40}N_5O_{11}F_3$
Formula Weight: 727.7
Purity: =97 % by HPLC.

Fluorescent substrate for caspase 6.

- Fluorometric detection when AFC is cleaved from the peptide (excitation wavelength = 400 nm emission wavelength = 505 nm)
- Spectrophotometric detection of AFC at 380 nm. Molar Extinction Coefficient = 12,600 at 380 nm (pH 7.2)
- AFC is highly soluble in DMF or DMSO
- Sensitivity of enzyme assay is equal to AMC in purified systems which have no background blue fluorescence
- Amino acid derivatives of AFC are blue in fluorescence microscopy
- AFC has been shown to be a non-mutagenic chemical by the Ames Test

Preparation Instructions
Soluble in DMSO/DMF at 20 mM.

Storage/Stability
Store tightly sealed and desiccated at –20 °C. Allow powder to reach room temperature before opening vial. May be stored desiccated in solid form at room temperature for one year. Store DMSO/DMF solutions at –20 °C for up to 6 months.

Procedure
Fluorometric Enzyme Assay

- Buffer: 100 mM HEPES, pH 7.5, 20 % (v/v) glycerol, 5 mM DTT, 0.5 mM EDTA
- Substrate: 20 mM stock solution of Ac-VEID-AFC in DMSO
- Enzyme: Cell lysate or purified enzyme solution (~15 nanograms enzyme)
- Fluorescence Standard: 80 µM free AFC (Product Number A 8401) in DMSO

1. Add 10 µl of enzyme to 470 µl buffer. Mix. Incubate at 30 °C for 30 minutes.
2. With fluorometer adjusted to 400 nm excitation and 505 nm emission, add 20 µl of substrate to enzyme solution.
3. Record increase in fluorescence (FLU) per minute from T_0 to T_{end} where the fluorescence generated at T_{end} is significantly different from that of T_0.
4. Calculate the ? FLU/min. from the linear portion of the curve.
5. Record fluorescence units (FLU) generated by 10 µl, 20 µl, and 30 µl free AFC and 490 µl (1.6 µM), 480 µl (3.2 µM), and 470 µl (4.8 µM) buffer solution, respectively. These solution contain 0.8, 1.6 and 2.4 nanomoles, of free AFC product, respectively.
6. Graph the fluorescence units (FLU) vs. the amount of free AFC (nanomoles). The standard curve is the best line connecting the data points. Determine the value of fluorescent units per nanomole (FLU/nmole) of free AFC from the standard curve.
7. Calculate activity as follows:

$$\text{1 unit of activity (nmole/ml/min)} = \frac{(?\text{FLU/min}) \times (\text{dilution factor of sample})}{(\text{FLU/ nmole}) \times (\text{reaction volume})}$$

Note: Multiplying by the dilution factor is only necessary when the enzyme sample is diluted further, and comparison to the original solution is required. Very low and very high levels of enzyme may give abnormal results. It is recommended to test the enzyme at several concentrations.

References:

1. Talanian, R.V., et al., Substrate specificities of caspase family proteases. J. Biol. Chem., **272**, 9677-9682 (1997).
2. Takahashi, A., et al., Cleavage of lamin A by Mch2 alpha but not CPP32: multiple interleukin 1 beta-converting enzyme-related proteases with distinct substrate recognition properties are active in apoptosis. Proc. Natl. Acad. Sci. USA, **93**, 8395-8400 (1996).
3. Martins, L.M., et al., Activation of multiple interleukin-1beta converting enzyme homologues in cytosol and nuclei of HL-60 cells during etoposide-induced apoptosis. J. Biol. Chem., **272**, 7421-7430 (1997).

lpg 11/00