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ProductInformation

N-ACETYL-ILE-GLU-THR-ASP-7-AMIDO-4-METHYLCOUMARIN

Product Number **A 4188**
Storage Temperature –20 °C

(Ac-IETD-AMC)

Product Description

Appearance: White Powder
Formula Weight: 675.7
Molecular Formula $C_{31}H_{41}N_5O_{12}$
Purity: =97 % by HPLC.

Fluorescent substrate for caspase-8 and granzyme B.

- Fluorometric detection when AMC is cleaved from peptide (excitation wavelength 380 nm emission wavelength 460 nm)
- Molar Extinction coefficient: 1.78 x 10^4 liter/mole cm in EtOH.
- Absorption maximum: 354 nm in EtOH
- AMC is soluble in DMF or absolute ethanol
- Sensitivity of enzyme assay relevant to fluorescence detection is greatly increased over 4-Methoxy-2-Naphthylamide
- AMC by the Ames Test has been shown to be a non-mutagenic chemical

Preparation Instructions

Soluble in DMF or absolute ethanol 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-IETD-AMC in DMF
- Enzyme: Cell lysate or purified enzyme solution (~15 ng enzyme)
- Fluorescence Standard: 80 µM free AMC (Product Number A 9891) in DMF

1. Add 10 µl of enzyme to 470 µl buffer. Mix. Incubate at 30 °C for 30 minutes.
2. With fluorometer adjusted to 380 nm excitation and 460 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 AMC and 490 µl (1.6 µM), 480 µl (3.2 µM), and 470 µl (4.8 µM) buffer solution, respectively. These solutions contain 0.8, 1.6 and 2.4 nanomoles, respectively, of free AMC product in 0.5 ml of solution.
6. Graph the fluorescence units (FLU) vs. µM 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 AMC from the standard curve.
7. Calculate activity as follows:

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

?FLU/min = value determined for enzyme assay in step 4

Dilution factor = any dilution of original protein sample prior to addition to reaction.

FLU/ nmole = value determined from standard curve in step 6

Vol. = volume in ml of enzyme solution in the reaction

References

1. Thornberry, N.A., et al., A combinatorial approach defines specificities of members of the caspase family and granzyme B. Functional relationships established for key mediators of apoptosis.J. Biol. Chem., **272**, 17907-17911 (1997).
2. Han, Z., et al., A sequential two-step mechanism for the production of the mature p17:p12 form of caspase 3 in vitro. J. Biol. Chem., **272**, 13432-13436 (1997).

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