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ProductInformation

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

Product Number **A 6345**
Storage Temperature –20 °C

(Ac-IEPD-AFC)

Product Description

Appearance: White Powder
Molecular Formula: $C_{32}H_{38}N5O_{11}F_3$
Formula Weight: 725.7
Purity: =97 % by HPLC.

Fluorescent substrate for granzyme B.

- Fluorometric detection when AFC is cleaved from the peptide (excitation wavelength = 400 nm emission wavelength = 505 nm)
- Molar Extinction coefficient = 12,600 at 380 nm (pH 7.2)
- Spectrophotometric detection of AFC at 380 nm
- 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 by the Ames Test to be a non-mutagenic chemical

Preparation Instructions

Soluble in DMSO or 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-IEPD-AFC in DMSO
- Enzyme: Cell lysate or purified enzyme solution (~15 nanograms enzyme)
- Fluorescence Standard: 80 µM of free AFC (Product Number A 8401) in DMSO

1. Add 10 µl of enzyme to 470 µl buffer. Mix and 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 of free AFC in 490 µl (1.6 µM), 480 µl (3.2 µM), and 470 µl (4.8 µM) of buffer solution, respectively. These solutions contain 0.8, 1.6 and 2.4 nanomoles of free AFC product per 0.5 ml of solution, 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 if you further dilute the enzyme sample, and want to compare to the original solution. Very low and very high levels of enzyme may give abnormal results. It is recommended to test the enzyme at several concentrations.

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. Harris, J.L., et al., Definition and redesign of the extended substrate specificity of granzyme B. J. Biol. Chem., **273**, 27364-27373 (1998).

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