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ProductInformation

N-ACETYL-LEU-GLU-HIS-ASP 7-AMIDO-4-TRIFLUOROMETHYLCOUMARIN
Product Number **A 5845**
Storage Temperature –20 °C

(Ac-LEHD-AFC)

Product Description
Appearance: White Powder
Molecular Formula: $C_{33}H_{38}N_7O_{11}F_3$
Formula Weight: 765.70
Purity: ≥95% by HPLC.

Fluorescent substrate for caspase 9.

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

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.

Preparation instructions
Soluble in DMSO/DMF at 20 mM

Procedure

- 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-LEHD-AFC in DMSO
- Enzyme -Cell lysate or purified enzyme solution (~15 nanograms enzyme)
- Fluorescence Standard - 80 µM free AFC (Product No. A8401) in DMOS

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 ΔFLU/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, respectively, of free AFC product 0.5 ml of standard 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 AFC from the standard curve.
7. Calculate activity as follows:

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

D**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 determine4d from standard curve in step 6

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

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