

## Product Information

### N-ACETYL-LEU-GLU-GLU-ASP-7-AMIDO-4-TRIFLUOROMETHYLCOUMARIN

Product Number **A 5720**

Storage Temperature  $-20\text{ }^{\circ}\text{C}$

(Ac-LEED-AFC)

#### Product Description

Appearance: White Powder

Formula Weight: 757

Purity:  $\approx 90\%$  by HPLC.

Fluorescent substrate for caspase 13.

- 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

#### Preparation Instructions

Soluble in DMSO/DMF at 20 mM.

#### Storage/Stability

Store tightly sealed and desiccated at  $-20\text{ }^{\circ}\text{C}$ . 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  $-20\text{ }^{\circ}\text{C}$  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-LEED-AFC in DMSO
- Enzyme: Cell lysate or purified enzyme solution ( $\sim 15$  nanograms enzyme)
- Fluorescence Standard: 80  $\mu\text{M}$  of free AFC (Product Number A 8401) in DMSO

1. Add 10  $\mu\text{l}$  of enzyme to 470  $\mu\text{l}$  buffer. Mix. Incubate at  $30\text{ }^{\circ}\text{C}$  for 30 minutes.
2. With fluorometer adjusted to 400 nm excitation and 505 nm emission, add 20  $\mu\text{l}$  of substrate to enzyme solution.
3. Record increase in fluorescence (FLU) per minute from  $T_0$  to  $T_{\text{end}}$  where the fluorescence generated at  $T_{\text{end}}$  is significantly different from that of  $T_0$ .
4. Calculate the  $\Delta\text{FLU}/\text{min}$ . from the linear portion of the curve.
5. Record fluorescence units (FLU) generated by 10  $\mu\text{l}$ , 20  $\mu\text{l}$ , and 30  $\mu\text{l}$  free AFC and 490  $\mu\text{l}$  (1.6  $\mu\text{M}$ ), 480  $\mu\text{l}$  (3.2  $\mu\text{M}$ ), and 470  $\mu\text{l}$  (4.8  $\mu\text{M}$ ) 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.  $\mu\text{M}$  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:

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

**DFLU/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. Humke, E.W., et al., ERICE, a novel FLICE-activatable caspase, J. Biol. Chem., **273**, 15702-15707 (1998).

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