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-CBZ-GLY-PRO-ARG 7-AMIDO-4-METHYLCOUMARIN
Hydrobromide salt

Product Number **C 0855**
Storage Temperature –20 °C

(Z-GPR-AMC) HBr

Product Description
Appearance: White Powder
Molecular Formula: $C_{31}H_{37}N_7O_7 \bullet HBr$
Formula Weight: 700 (includes salt)
Purity: =85 % by TLC.

Fluorescent substrate for granzyme A.

- Fluorometric detection when AMC is cleaved from peptide (excitation wavelength = 380 nm emission wavelength = 460 nm)
- Molar Extinction coefficient = 17,800 (EtOH).
- Absorption maximum: 354 nm (EtOH)
- AMC is soluble in DMF or absolute ethanol
- Sensitivity of the enzyme fluorescence assay is greatly increased over 4-Methoxy-2-Naphthylamide.
- AMC has been shown by the Ames Test 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 DMF/ethanol solutions at –20 °C for up to 6 months.

Procedure
Fluorometric Enzyme Assay

- Buffer: Of choice, refer to literature.
- Substrate: 20 mM stock solution of Z-GPR-AMC in DMF
- Enzyme: Cell lysate or purified enzyme solution (~15 ng enzyme)
- Fluorescence Standard: 80 µM of free AMC (Product Number A 9891) in DMF

1. Add 10 µl of enzyme to 470 µl buffer. Mix and 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 of the free AMC standard and 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 AMC product in 0.5 ml, 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 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. Smyth, M.J., et al., Purification and cloning of a novel serine protease, RNK-Met-1, from the granules of a rat natural killer cell leukemia. J. Biol. Chem., **267**, 24418-24425 (1992).
2. Velotti, F., et al., Differential expression of granzyme A and granzyme B proteases and their secretion by fresh rat natural killer cells (NK) and lymphokine-activated killer cells with NK phenotype (LAK-NK). Eur. J. Immunol., **22**,1049-1053 (1992).

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