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## TECHNICAL INFORMATION

### ENZYME SYSTEMS PRODUCTS

a division of



### Technical Data Sheet

#### Method for Assay of Tryptase (EC 3.4.21.59) with Ac-Lys-Pro-Arg

##### Materials:

50 mM Hepes, pH 7.6, 120 mM NaCl

##### – Buffer

10 mM solution of Ac-Lys-Pro-Arg-AFC, AMC (Catalog # AFC- or AMC130) in DMSO

##### – Substrate

Tissue homogenate or approximately 0.2 units/ml purified enzyme in cold deionized water

##### – Enzyme

80  $\mu$ M free AFC or AMC (Catalog #, T07 or T02) in DMSO

##### – Fluorescence Standard

##### Method:

- Add 10  $\mu$ l of enzyme sample or d.H<sub>2</sub>O to 480  $\mu$ l of buffer. Mix by inversion and equilibrate to 25° C.
- Set the fluorometer to appropriate wavelengths (refer to ESP catalog), and add 10  $\mu$ l of substrate.
- Mix by inversion and record increase in fluorescence for approximately five minutes (record increase in fluorescence from T<sub>0</sub> to T<sub>end</sub> where fluorescence units generated at T<sub>end</sub> are significantly different from those at T<sub>0</sub>).

##### Preparing Calibration Curve:

80  $\mu$ M free AFC, AMC, or MNA (DMSO or DMF) stock solution is diluted in enzyme assay buffer to give 0.5ml final volumes as follows: 1/50 dilution (8 x 10<sup>-4</sup>  $\mu$ Moles AFC), 2/50 (16 x 10<sup>-4</sup>  $\mu$ Moles AFC) and 3/50 (24 x 10<sup>-4</sup>  $\mu$ Moles AFC). The three dilutions are measured on the fluorometer. A calibration curve is prepared with x =  $\mu$ Mole free substrate and y = fluorescence units (FU). The slope of the calibration curve is calibrated.

##### Calculation of FU to Units of Enzyme Activity:

$FU \times 1 = \text{Units enzyme}$

Time (min) x slope x ml enzyme sample assayed min · ml

##### Units Definition:

One unit is the amount of enzyme that cleaves one micromole of AFC, AMC or MNA per minute, per milliliter at above described conditions.

##### Specificity:

Other trypsin-like enzymes are cleaved by tripeptide synthetic substrates. The absence of inhibition of Tryptase by classical inhibitors or serine esterase, including soybean trypsin inhibitor, clearly distinguishes tryptase from pancreatic trypsin and from most other serine esterases. Tryptase is inhibited by leupeptin, PMSF, and diisopropyl fluorophosphate.

##### Storage:

Desiccate AFC- or AMC130 in solid form at room temperature. Store DMSO/DMF solutions at -20° C. Material stable for at least one year, if stored as recommended.

##### Reference:

- Cushman, D.W. (1971). *Biochem. Pharm.* **20**: 1637