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TECHNICAL INFORMATION

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Technical Data Sheet

Method for Assay of Urokinase (EC 3.4.21.31) with glut-Gly-Arg

Materials:

- **Buffer** 100 mM Tris HCl, adjusted to pH 7.5-7.7; 100 mM NaCl, 0.1% PEG(6000), 20 mM D-Mannitol
- **Substrate** 20 mM solution of glut-Gly-Arg-AFC, AMC, MNA (AFC, AMC, MNA050) in DMSO
- **Enzyme** Tissue homogenate or serial dilutions of purified enzyme in cold deionized water
- **Enzyme** 80 μ M free AFC, AMC or MNA (Catalog #, T07, T02 or T06) in DMSO
- **Fluorescence Standard**

Method:

- Add 10 μ l of enzyme sample or d.H₂O to 480 μ l of buffer. Mix by inversion and equilibrate on ice for 15 minutes.
- Add 10 μ l of substrate.
- Mix by inversion and record increase in fluorescence for approximately five minutes (record increase in fluorescence from T₀ to T_{end} where fluorescence units generated at T_{end} are significantly different from those at T₀).

Preparing Calibration Curve:

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

Calculation of FU to Units of Enzyme Activity:

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

$$\text{Time (min)} \times \text{slope} \times \text{ml enzyme sample assayed} = \text{min} \cdot \text{ml}$$

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.

Storage:

Desiccate AFC-, AMC-, or MNA050 in solid form at room temperature. Store DMSO/DMF solutions at -20° C. Material stable for at least one year, if stored as recommended.

Effective Inhibitors:

Soybean trypsin inhibitor, benzamidine

Reference:

– Derived from Barrett, A.J., et. al. (1980). *Mammalian Protease 1*: 132