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TECHNICAL INFORMATION

ENZYME SYSTEMS PRODUCTS
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Technical Data Sheet

Method for Assay of Trypsin with BOC-Gln-Ala-Arg

Materials:

– ***Buffer*** 100 mM Hepes, pH 7.3, 50mM $CaCl_2$

– ***Substrate*** 20 mM solution of BOC-Gln-Ala-Arg (Catalog # AFC-, AMC-, or MNA123)

– ***Enzyme*** Tissue homogenate or purified enzyme solution (serial dilutions of enzyme)

– ***Fluorescence Standard*** 80 µM free substrate (Catalog # T07, T02 or T06) in DMSO)

Method:

– Add 10 µl of enzyme or tissue homogenate to 490 µl of buffer. Mix. Incubate at 25° C for 20 minutes. If testing inhibitor, add it to enzyme buffer solution and incubate additional 15 minutes.
– With fluorometer adjusted to appropriate excitation and emission wavelengths (refer to ESP catalog), add 20 µl of substrate to enzyme solution.
– Record increase in fluorescence from T_0 to T_{end} where fluorescence units generated at T_{end} are significantly different from those at T_0.
– Record fluorescence units generated by 10, 20, and 30 µl free AFC, AMC or MNA in 490, 480 and 470 µl buffer solution respectively.
– Graph fluorescence units vs. micromole AFC, AMC or MNA. Use slope to convert fluorescence units generated by enzyme to activity.

Storage:

Desiccate AFC-, AMC-, or MNA123 in solid form at room temperature. Store DMSO/DMF solutions at -20° C. Material is stable for at least one year, if stored as recommended.