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TECHNICAL INFORMATION

ENZYME SYSTEMS PRODUCTS
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Technical Data Sheet

Method for Assay of Granzyme A with Z-Gly-Pro-Arg

Materials:

– ***Buffer***
Of choice, refer to literature

– ***Substrate***
20 mM solution of Z-Gly-Pro-Arg (Catalog # AFC-, AMC-, or MNA061) in DMSO

– ***Enzyme***
Cell lysate or purified enzyme solution (~15 nanograms enzyme)

– ***Fluorescent Standard***
80 µM free AFC, AMC or MNA (Catalog # T07, T02 or T06) in DMSO

Method:

– Add 10 µl of enzyme to 490 µl of buffer. Mix. Incubate at 30° C for 30 minutes.
– With fluorometer, adjust to 400nm excitation, 505 emission, 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 substrate in 490, 480, and 470 µl buffer solution, respectively.
– Graph fluorescence units vs. micromole AFC. Use slope to convert fluorescence units generated by enzyme to activity.

Storage:

Desiccate AFC-, AMC-, or MNA061 in solid form at room temperature. Store DMSO/DMF solution at -20° C. Material is stable for at least one year, if stored as recommended.

References:

– Smyth, M.J. et.al. (1992). Purification and cloning of a novel serine protease, RMK-Mer-1, form the granules of a Rat Natural Killer Cell Leukemia. *Journal of Biological Chemistry* **267(34)**: 24418-24425
– Velotti, F., et.al. (1992). Differential Expression of Granzyme A and Granzyme B Protease and their secretion by Fresh Rat Natural Killer Cells (NK) and Lymphokine-activated Killer Cells with NK Phenotype (LAK-NK). *European Journal of Immunology* **22**: 1049-1053