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TECHNICAL INFORMATION

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Technical Data Sheet

Method for Assay of Caspase 2 with Ac-Val-Asp-Val-Ala-Asp-AFC

Materials:

– ***Buffer***
100 mM HEPES, pH 7.5, 20% (v/v) glycerol, 5 mM DTT, 0.5 mM EDTA

– ***Substrate***
20 mM stock solution of Ac-Val-Asp-Val-Ala-Asp-AFC in DMSO

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

– ***Fluorescence Standard***
80 µM free AFC (Catalog # T07) in DMSO

Method:

– Add 10 µl of enzyme to 490 µl buffer. Mix. Incubate at 30° C for 30 minutes.
– With fluorometer adjusted to 400nm excitation and 505nm emission, add 20 µl of substrate to enzyme solution.
– Record increase in fluorescence from T_0 to T_{end} where fluorescence generated at T_{end} are significantly different from those at T_0.
– Record fluorescence units generated by 10, 20, and 30 µl free AFC 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 AFC142 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.