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TECHNICAL INFORMATION

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Technical Data Sheet

Method for Assay of Z-Asp(OMe)-Glu(OMe)-Val-Asp(OMe)-FMK Inhibitor
Description:
Z-Asp(OMe)-Glu(OMe)-Val-Asp(OMe)-FMK is an irreversible and cell permeable inhibitor of CPP32/Apopain, a member of the CIE/CED-3 family of cysteine proteases.
Background:
Members of the caspase family play key roles in inflammation and mammalian apoptosis (reviewed in 1). Z-DEVD-FMK is an irreversible and cell permeable specific inhibitor of caspase-3. The peptide is O-methylated in the P1 position on aspartic acid providing enhanced stability and increased cell permeability. The inhibitor can be used to inhibit primarily caspase-3 activity and to study events downsteam of caspase-3 activation.(2,3)
Storage:
Store sealed reagent at -20° C upon arrival. Lyophilized samples are stable for 1 year, desiccated, at RT. Upon reconstitution in 100% DMSO, stock concentrations of 10 - 20 mM are stable for 6 months at ≤ -20°C. Avoid repeated freeze/thaw cycles by aliquoting the stock solution prior to freezing.
Method:

1.Dissolve 1mg of Z-Asp(OMe)-Glu(OMe)-Val-Asp(OMe)-FMK in:

– 75 µl DMSO = 20 mM
– 150 µl DMSO = 10 mM
– 300 µl DMSO = 5 mM, etc

NOTE: DMF may also be used

2.Add 2 µl of above stock solutions to 1ml of culture medium containing cells to give final DMSO concentration of 0.2%. Levels of DMSO above this may cause some cellular toxicity thus masking the effect of the ICE-protease inhibitors. 2 µl of 10mM stock solution in 1ml medium = 20 µM final Z-Asp(OMe)-Glu(OMe)-Val-Asp(OMe)-FMK concentration.

NOTE: For in-vitro or in-vivo experiments extending for 12 to 48 hours, fresh inhibitor may have to be added (injected) due to inactivity by reaction with cysteine proteases.

PLEASE NOTE:
This inhibitor is designed as a methyl ester to facilitate cell permeability. If the intended use is on purified or recombinant enzymes, esterase should be added to generate the free carboxyl groups. Please contact us for more details. Levels of DMSO above 0.2% may cause some cellular toxicity in culture medium, thus masking the effect of the inhibitor.
REFERENCES
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3. Schrantz, N., D.A. Blanchard, M.T. Affredou, S. Sharma, G. Leca, and A. Vazquez. 1999. Role of caspases and possible involvement of retinoblastoma protein during TGFbeta-mediated apoptosis of human B lymphocytes. *Oncogene* 18:3511-3519.