PRODUCT DATA SHEET

Product: Biotin-DEVD-FMK (Irreversible caspase-1, 3, 4, 7, 8, 10 inhibitor)

Cat. No.: AD-003 (1 mg)

Chemical Name:
Biotin-Asp(OMe)-Glu(OMe)-Val-Asp(OMe)-CH_2F

Molecular Weight:
873

Form:
Brown solid

Description:
Biotinylated peptide-fluoromethyl ketone inhibitor of Caspases-3/CPP32, -7, -1, -4, -8, and -10. The CH_2F (fluoromethyl ketone) inhibitor has several advantages over other types of derivatives: penetrates cell membranes, not toxic to cells, irreversible inhibition.

Introduction:
Caspase-3/CPP32 is a member of the cysteine proteases family involved in apoptosis induction. All apoptotic pathways studied to date involve proteolytic activation of Caspase-3/CPP32 as a central event in the progression of cell death. Although the death-inducing consequences of Caspase-3/CPP32 activation have not been conclusively established, several crucial substrates for the protease have been identified in vitro, including DNA-dependent protein kinase, Poly(ADP-ribose) Polymerase (PARP), Replication factor C, and Gelsolin. These substrates are involved in the later stages of apoptosis, strongly suggesting that Caspase-3/CPP32 has a key role in promoting the final processes leading to cell death.

Specificity:
Binds to and inhibits Caspases-3/CPP32, -7, -1, -4, -8, and -10. Strong inhibition of Caspase-3 and Caspase-7 but no inhibition of Caspase-2. Moderate inhibition of Caspase-1 and Caspase-4. Very weak inhibition of Caspase-6.

Applications:
Detecting and quantitating Caspases-3/CPP32, -7, -1, -4, -8, and -10 protein molecules.

Protocol:
Dissolve Caspase-3/CPP32 Inhibitor/Biotin Tag at 20 mM in DMSO (high purity 99.9%) before use.

Method:
1. Grow and treat 1 x 10^6 cells at the appropriate dose and time to obtain 50-60% apoptosis.
2. Collect cells by centrifugation, remove supernatant and re-suspend cell pellet in an initial volume of 1/1,000th of the original media volume (10 µL for 10 mL media).
3. Add 20 µL of 2X Biotin-DEVD-FMK in MGD buffer (Final Biotin-DEVD-FMK concentration = 20 µM.) MGD buffer: 50 mM NaCl
   2 mM $MgCl_2$
   5 mM EDTA
   10 mM HEPES 7.0
   1 mM DTT
   Protease Inhibitor
4. Freeze/thaw 3X to lyse cells.
5. Incubate at 37°C for 15 minutes. Spin two minutes at 14K to remove cell debris.
6. Transfer supernatant to new tube, add 13 µL of 4X SDS-PAGE buffer and run all on 10% SDS-PAGE gel and transfer to PVDF membrane.
7. Block one hour and incubate with streptavidin-HRP (1/1,000) four hours. Develop by ECL.

NOTE: The Carboxyl groups are OMe ester form to enhance cell permeability. If purified enzyme is used, esterase should be added for the hydrolysis of the ester groups.

For extended use *in vivo* or *in vitro*:
For experiments extending 12 to 48 hours, fresh inhibitor may have to be added (injected) due to inactivation of the inhibitor by endogenous cysteine proteases.

Storage and Stability:
Store in a desiccator at room temperature or 4°C. DMSO stock solutions have a shelf life of 6-8 months if stored at -20°C.

Limitations:
For research use only. Not for use in diagnostics or in humans.

Warranty:
No warranties, expressed or implied, are made regarding the use of this product. KAMIYA BIOMEDICAL COMPANY is not liable for any damage, personal injury, or economic loss caused by this product.