



## PRODUCT DATA SHEET

**Product:** Biotin-VAD-FMK (Pan-Caspase Inhibitor)

**Cat. No.:** AB-014 (1 mg)

**Chemical Name:**

Biotin-Val-Ala-Asp(OMe)-CH<sub>2</sub>F

**Molecular Weight:**

672

**Form:**

Brown solid

**Description:**

Biotinylated peptide-fluoromethyl ketone inhibitor of caspases.

The FMK (fluoromethyl ketone, CH<sub>2</sub>F) inhibitor has several advantages over other types of derivatives: Penetrates cell membranes, Not toxic to cells, Irreversible inhibition.

**Specificity:**

Broad-spectrum caspase inhibitor.

**Protocol:**

Dissolve the caspase inhibitor in high purity DMSO (>99.9%) before use to make a stock solution of 20 mM.

**Method for assay of Biotin-VAD-FMK Inhibitor:**

1. Grow and treat  $1 \times 10^6$  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 1/1,000<sup>th</sup> of the original media volume (10  $\mu$ L for 10 mL media).
3. Add 20  $\mu$ L of 2X Biotin-VAD-FMK in MGD buffer. (Final Biotin-VAD-FMK concentration = 10  $\mu$ M.)
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  $\mu$ L 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) for four hours. Develop by ECL.

**MGD Buffer:**

50 mM NaCl  
2 mM MgCl<sub>2</sub>  
5 mM EDTA  
10 mM HEPES pH 7.0  
1 mM DTT  
Protease Inhibitor

**IMPORTANT NOTE for *in vitro* use:** Our peptide inhibitors are synthesized as methyl esters to enhance cell permeability. In intact cells, the methyl groups are removed by endogenous enzymes. For *in vitro* experiments with purified enzymes, however, the methyl groups must first be removed by treating the inhibitor with esterase. A procedure is available upon request.

**Storage:**

Biotin-VAD-FMK has a shelf-life of up to 1 year if stored desiccated at room temperature. However, for long term storage, desiccated at 4°C is recommended. DMSO stock solutions have a shelf-life of 6-8 months at -20°C if care is taken by choosing DMSO with maximum dryness (>99.9%). Keep sealed after removing from the freezer until the temperature of the vial equilibrates with room temperature.

**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.