



PRODUCT DATA SHEET

Product: Biotin-FA-FMK (Cathepsin B Inhibitor)

Cat. No.: AD-012 (5 mg)

Chemical Name:

Biotin-Phe-Ala-CH₂F

Molecular Weight:

478

Description:

Trifluoroacetic acid salt of the fluoromethyl ketone biotinylated peptide inhibitor of Cathepsin B (Caspase inhibitor negative control).

The CH₂F (fluoromethyl ketone) inhibitor has several advantages over other types of derivatives:

- Penetrates cell membranes
- Not toxic to cells
- Irreversible inhibition

Introduction:

The ten known caspases are involved in the proteolytic cascade that results in apoptosis and in the maturation process of IL-1 β necessary for inflammation. The use of biotinylated FMK-type inhibitors for various caspases requires a biotinylated FMK-type inhibitor that does not affect caspases as a negative control. The Biotin-FA-FMK does not inhibit the activity of caspases in intact cells and does not block the induction of apoptosis as caspase inhibitors do. It can, therefore, be used as a negative control for the FMK moiety of the biotinylated caspase inhibitor, both *in vivo* and *in vitro*.

Specificity:

Specifically binds to and inhibits Cathepsin B. Does not bind to or inhibit any of the caspases.

Form:

Brown solid.

Applications:

Irreversible binding to and inhibition of Cathepsin B. The second order rate constant (k_2) of inactivation for human liver Cathepsin B is $1,220 \text{ M}^{-1}\text{s}^{-1}$, determined from the observed rate of inactivation (k) divided by the inhibitor concentration (I): $k_2 = k/I$.

Serves as a negative control in studies using the biotinylated FMK-containing caspase inhibitors.

Protocol:

Dissolve Biotin-FA-FMK in high purity (>99.9%) DMSO before use.

For use on intact cells:

1. Prepare desired concentrated stock solution in DMSO as follows:
 - Dissolve 1 mg Biotin-FA-FMK in 68 μL DMSO = 25 mM
 - in 85 μL DMSO = 20 mM
 - in 169 μL DMSO = 10 mM, etc.
2. Add 2 μL of the stock solution to 1 mL culture medium containing cells such that the final DMSO concentration is 0.2%. Levels of DMSO above this may cause some cellular toxicity, thus masking the true effects of the inhibitor. The recommended final concentration of Biotin-FA-FMK for use as a caspase inhibitor negative control is 50 μM (2 μL of 25 mM stock solution in 1 mL cell culture).
3. Detect bound Biotin-FA-FMK by any of several avidin/streptavidin methods.

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 Biotin-FA-FMK in a desiccator at room temperature (RT) or 4°C. Biotin-FA-FMK has a shelf-life of >2 years if stored desiccated at -20°C. For long term, storage at -20°C is recommended.

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.