

# BioVision

## Caspase-3 Fluorometric Substrate, DEVD-AFC

rev. 04/06

For research use only

**CATALOG #:** 1007-200 200 assays  
1007-1000 1000 assays

**LOT #:** \_\_\_\_\_

**STORAGE CONDITION:** Store at -20°C, protected from light.

**SHELF LIFE:** 6 months under proper storage conditions

**MOLECULAR WEIGHT:** 729.0

**SEQUENCE:** Ac-Asp-Glu-Val-Asp-AFC  
(AFC, 7-amino-4-trifluoromethyl coumarin)

**PURITY:** >98% by HPLC analysis.

### DESCRIPTION:

Ready-to-use fluorometric substrate for caspase-3/CPP32 ( $K_m = 9.7 \mu\text{M}$ ) and related caspases that recognize the amino acid sequence DEVD. The sequence DEVD is based on caspase-3 cleavage site in poly (ADP-ribose) polymerase (PARP). CPP32 and related caspase activity can be quantified by fluorescent detection of free AFC after cleaved from the peptide substrate DEVD-AFC at  $\text{Ex.} = 400 \text{ nm}$  and  $\text{Em.} = 505 \text{ nm}$ , using a fluorometer or a multi-well fluorescence plate reader. Alternatively, a shift in fluorescence from blue to green upon cleavage can be visualized using a hand-held long-UV lamp.

### ASSAY PROTOCOL:

1. Induce apoptosis in cells by desired method. Concurrently incubate a control culture *without* induction.
2. Count cells and pellet  $1-5 \times 10^6$  cells or use 50-200  $\mu\text{g}$  cell lysates if protein concentration has been measured.
3. Resuspend cells in 50  $\mu\text{l}$  of chilled Cell Lysis Buffer (Cat.# 1067-100).
4. Incubate cells on ice for 10 minutes.
5. Add 50  $\mu\text{l}$  of 2X Reaction Buffer (Cat.# 1068-20, -80) containing 10 mM DTT (Cat.# 1201-1) to each sample.
6. Add 5  $\mu\text{l}$  of the 1 mM DEVD-AFC (50  $\mu\text{M}$  final conc.) into each tube individually and incubate at 37°C for 1-2 hour.
7. Read samples in a fluorometer equipped with a 400-nm excitation filter and 505-nm emission filter. For a plate-reading set-up, transfer the samples to a 96-well plate. You may perform the entire assay directly in a 96-well plate.
8. Fold-increase in caspase-3 activity can be determined by comparing these results with the level of the uninduced control.

### RELATED PRODUCTS:

#### Apoptosis Detection Kits & Reagents

- Annexin V Kits & Bulk Reagents
- Caspase Assay Kits & Reagents
- Mitochondrial Apoptosis Kits & Reagents
- Nuclear Apoptosis Kits & Reagents
- Apoptosis Inducers and Set
- Apoptosis siRNA Vectors

#### Cell Fractionation System

- Mitochondria/Cytosol Fractionation Kit
- Nuclear/Cytosol Fractionation Kit
- Membrane Protein Extraction Kit
- Cytosol/Particulate Rapid Separation Kit
- Mammalian Cell Extraction Kit
- FractionPREP Fractionation System

#### Cell Proliferation & Senescence

- Quick Cell Proliferation Assay Kit
- Senescence Detection Kit
- High Throughput Apoptosis/Cell Viability Assay Kits
- LDH-Cytotoxicity Assay Kit
- Bioluminescence Cytotoxicity Assay Kit
- Live/Dead Cell Staining Kit

#### Cell Damage & Repair

- HDAC Fluorometric & Colorimetric Assays & Drug Discovery Kits
- HAT Colorimetric Assay Kit & Reagents
- DNA Damage Quantification Kit
- Glutathione & Nitric Oxide Fluorometric & Colorimetric Assay Kits

#### Signal Transduction

- cAMP & cGMP Assay Kits
- Akt & JNK Activity Assay Kits
- Beta-Secretase Activity Assay Kit

#### Adipocyte & Lipid Transfer

- Recombinant Adiponectin, Survivin, & Leptin
- CETP Activity Assay & Drug Discovery Kits
- PLTP Activity Assay & Drug Discovery Kits
- Total Cholesterol Quantification Kit

#### Molecular Biology & Reporter Assays

- siRNA Vectors
- Cloning Insert Quick Screening Kit
- Mitochondrial & Genomic DNA Isolation Kits
- 5 Minutes DNA Ligation Kit
- 20 Minutes Gel Staining/Destaining Kit
- $\beta$ -Galactosidase Staining Kit & Luciferase Reporter Assay Kit

#### Growth Factors and Cytokines

#### Monoclonal and Polyclonal Antibodies

BioVision Research Products

980 Linda Vista Avenue, Mountain View, CA 94043 USA

Tel: 650-428-0236 • Fax: 650-428-0336

[www.biovision.com](http://www.biovision.com) • Email: tech@biovision.com