

## Caspase-3 Colorimetric Substrate, DEVD-pNA

**CATALOG #:** 1008-200 200 assays  
1008-1000 1000 assays

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

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

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

**MOLECULAR WEIGHT:** 637.0

**SEQUENCE:** Ac-Asp-Glu-Val-Asp-pNA

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

### DESCRIPTION:

Ready-to-use colorimetric substrate for CPP32/caspase-3 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 spectrophotometric detection of free pNA ( $\lambda = 400$  nm) after cleavage from the peptide substrate DEVD-pNA, using a spectrophotometer or multi-well plate reader. The ready-to-use caspase substrate provides an economic alternative for researchers who perform large amounts of caspase assays.

### ASSAY PROCEDURE:

1. Induce apoptosis in cells by desired method. Concurrently incubate a control culture *without* induction.
2. Count cells and pellet 1-5 x 10<sup>6</sup> cells.
3. Resuspend cells in 50  $\mu$ l of chilled Cell Lysis Buffer (Cat.# 1067-100) and incubate cells on ice for 10 minutes.
4. Centrifuge for 1 min in a microcentrifuge (10,000 x g).
5. Transfer supernatant (cytosolic extract) to a fresh tube and put on ice.
6. Assay protein concentration.
7. Dilute 50-200  $\mu$ g protein to 50  $\mu$ l Cell Lysis Buffer for each assay.
8. Add 50  $\mu$ l of 2X Reaction Buffer (Cat.# 1068-20, -80) containing 10 mM DTT (Cat.# 1201-1) to each sample.
9. Add 5  $\mu$ l of the 4 mM of DEVD-pNA (200  $\mu$ M final conc.) and incubate at 37°C for 1-2 hour.
9. Read samples at 400- or 405-nm in a microtiter plate reader, or spectrophotometer using a 100- $\mu$ l micro quartz cuvet (Sigma), or dilute sample to 1 ml with Dilution Buffer (Cat.# 1066-100, -500) and using regular cuvet (note: Dilution of the samples proportionally decreases the reading).

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For research use only

You may also perform the entire assay directly in a 96-well plate.

Fold-increase in caspase activity can be determined by comparing these results with the level of the uninduced control.

Note: Background reading from cell lysates and buffers should be subtracted from the readings of both induced and the uninduced samples before calculating fold increase in caspase activity.

**FOR RESEARCH USE ONLY! Not to be used in human.**

### 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