

## Caspase-Family Colorimetric Substrate Set II

**CATALOG #:** K134-9-25  
**LOT #:** \_\_\_\_\_  
**STORAGE CONDITIONS:** Store at -20°C.  
**SHELF LIFE:** 6-12 months under proper storage conditions

### DESCRIPTION:

Ready-to-use colorimetric substrates for members of caspase family proteases. All substrates were provided in liquid ready-to-use form.

### KIT CONTENTS:

125 µl (4 mM) each of the following substrates dissolved in DMSO:

Caspase-1 Substrate, Ac-YVAD-pNA  
Caspase-2 Substrate, Ac-VDVAD-pNA  
Caspase-3 Substrate, Ac-DEVD-pNA  
Caspase-4 Substrate, Ac-LEVD-pNA  
Caspase-5 Substrate, Ac-WEHD-pNA  
Caspase-6 Substrate, Ac-VEID-pNA  
Caspase-8 Substrate, Ac-IETD-pNA  
Caspase-9 Substrate, Ac-LEHD-pNA  
Caspase-10 Substrate, Ac-AEVD-pNA

### 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 µl 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 to a fresh tube and assay protein Concentration.
6. Dilute 200-300 µg protein to 50 µl Cell Lysis Buffer for each assay.
7. Add 50 µl of 2X Reaction Buffer (Cat.# 1068-20) containing 10 mM DTT (Cat.# 1201-1) to each sample.
8. Add 5 µl of the 4 mM pNA conjugated substrates (200 µM final conc.) into each tube individually 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-µl 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).

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

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

### 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
- β-Galactosidase Staining Kit & Luciferase Reporter Assay Kit

#### Growth Factors and Cytokines

#### Monoclonal and Polyclonal Antibodies