Caspase-9 Colorimetric Substrate, LEHD-*p*NA

CATALOG NO: 1076-200 200 assays (1 x 1 ml)
1076-1000 1000 assays (5 x 1 ml)

STORAGE: Store at –20° C, protected from light. Stable for 6 months

MOL. WEIGHT: 675

SEQUENCE: Ac-Leu-Glu-His-Asp-*p*NA

PURITY: >98% by HPLC analysis.

DESCRIPTION:
Ready-to-use colorimetric substrate for caspase-9/Mch6 and related caspases that recognize the amino acid sequence LEHD. Caspase-9 and related caspase activity can be quantified by spectrophotometric detection of free *p*NA (λ= 400 nm) after cleavage from the peptide substrate LEHD-*p*NA, using a spectrophotometer or multi-well plate reader.

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 10^6 cells.
3. Resuspend cells in 50 µl of chilled Cell Lysis Buffer (Cat.# 1067-100, -400) 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 µg protein to 50 µl Cell Lysis Buffer for each assay.
8. Add 50 µl of 2X Reaction Buffer (Cat.# 1068-20, -80) containing 10 mM DTT (Cat.# 1201-1) to each sample.
9. Add 5 µl of the 4 mM substrate LEHD-*p*NA (200 µM final conc.) into each tube and incubate at 37° C for 1-2 hour.
10. Read samples at 400 or 405-nm in a microtiter plate reader, or spectrophotometer using a 100-µl micro quartz cuvette (Sigma), or dilute sample to 1 ml with Dilution Buffer (Cat.# 1066-100, -500) and using regular cuvette (note: Dilution of the samples proportionally decreases the reading).

    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.

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

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- cAMP & cGMP Assay Kits
- Akt & JNK Activity Assay Kits
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- 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
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- 5 Minutes DNA Ligation Kit
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