Caspase-5 Colorimetric Substrate, WEHD-*p*NA

CATALOG NO: 1102-200 200 assays (1 x 1 ml)
1102-1000 1000 assays (5 x 1 ml)

STORAGE: Store at –20° C, protected from light.

SHELF LIFE: 1 year under proper storage conditions

MOL. WEIGHT: 747.7

SEQUENCE: Ac-Trp-Glu-His-Asp-pNA

PURITY: >99% by HPLC analysis.

DESCRIPTION:
Ready-to-use colorimetric substrate for caspase-5 and related caspases that recognize the amino acid sequence WEHD. Caspase-5 and related caspase activity can be quantified by spectrophotometric detection of free pNA (λ = 400 nm) after cleaved from the peptide substrate WEHD-pNA, using a spectrophotometer or multi-well plate reader. The ready-to-use caspase substrate provides an economic alternative for researchers who perform large volume 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 10^6 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 (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 of WEHD-*p*NA (200 µM final conc.) 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 cuvet (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.

FOR RESEARCH USE ONLY! Not to be used in humans.

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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

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- 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

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