Caspase-1/ICE Colorimetric Substrate, YVAD-*p*NA

CATALOG NO: 1104-200 200 assays (1 x 1 ml)
1104-1000 1000 assays (5 x 1 ml)

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

SHELF LIFE: 6 months under proper storage conditions

MOL. WEIGHT: 629.0

SEQUENCE: Ac-Tyr-Val-Ala-Asp-*p*NA

PURITY: >98% by HPLC analysis.

DESCRIPTION:
Ready-to-use colorimetric substrate for caspase-1/ICE and related caspases that recognize the amino acid sequence YVAD. Caspase-1 and related caspase activity can be quantified by spectrophotometric detection of free *p*NA (λ= 400 nm) after cleavage from the peptide substrate YVAD-*p*NA, using a spectrophotometer or multi-well plate reader. The ready-to-use caspase substrate provides an economic alternative for large volume users.

ASSAY PROCEDURE:

1. Induce apoptosis in cells by desired method. Concurrently incubate a control culture *without* induction.

   Note: Active recombinant human caspase-1 is available to use as a positive control (BioVision, Cat.# 1081-25, -100).
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 100-300 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.
10. Add 5 µl of the 4 mM of YVAD-*p*NA (200 µM 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-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

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

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