

Caspase-1/ICE Colorimetric Substrate, YVAD-pNA

CATALOG #: 1104-200 200 assays (2 x 0.5 ml)
1104-1000 1000 assays (5 x 1 ml)

LOT #: _____

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-pNA

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 pNA ($\lambda = 400$ nm) after cleavage from the peptide substrate YVAD-pNA, 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 $1-5 \times 10^6$ cells.
3. Resuspend cells in 50 μ 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 100-300 μ g protein to 50 μ l Cell Lysis Buffer for each assay.
8. Add 50 μ l of 2X Reaction Buffer (Cat.# 1068-20, -80) containing 10 mM DTT (Cat.# 1201-1) to each sample.
10. Add 5 μ l of the 4 mM of YVAD-pNA (200 μ 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- μ 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).

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
- β -Galactosidase Staining Kit & Luciferase Reporter Assay Kit

Growth Factors and Cytokines

Monoclonal and Polyclonal Antibodies