CREATIVE BIOMART®
Assay Kit

Caspase-1 colorimetric assay Kit

Product Information

Common Name

Caspase

Cat.No. Kit-0154

Product Overview

Based on the spectrophotometric detection of the chromophore pNA (p-nitroaniline) after cleavage from the labelled substrate YVAD-pNA.

Description

The Caspase-1/ICE Colorimetric Protease Assay Kits provide a simple and convenient means for assaying the activity of caspases that recognize the sequence YVAD.

Storage

Store kit at –20°C (Store Cell Lysis Buffer, 2X Reaction Buffer, and Dilution Buffer at 4°C after opening). All reagents are stable for at least 6 months.

Size

25 tests

Handling

Aliquot enough 2X Reaction Buffer for the number of assays to be performed. Add DTT to the 2X Reaction Buffer immediately before use (10 mM final concentration: add 10 µl of 1.0 M DTT stock per 1 ml of 2X Reaction Buffer). •
After thawing, store Cell Lysis Buffer, 2X Reaction Buffer, and Dilution Buffer at 4°C. •
Protect YVAD-pNA from light.

Kit Components

25ml Cell Lysis Buffer,
2ml 2X Reaction Buffer,
125µl YVAD-pNA (1mM),
100µl DTT (1M),

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25ml Dilution Buffer.

Assay Protocol

1. Induce apoptosis or treat cells by desired method. Concurrently incubate a control culture without treatment.
Note: Active Recombinant Caspase-1 can be used as a positive control for the caspase-1 activity assays.
2. Pellet 2-5 x 10^6 cells or use 100-200 µg cell lysates if protein concentration has been measured.
3. Resuspend in 50 µl of chilled Cell Lysis Buffer and incubate on ice for 10 min.
4. Centrifuge for 1 min in a microcentrifuge (10,000 x g).
5. Transfer supernatant (cytosolic extract) to a fresh tube and keep on ice.
6. Assay protein concentration.
7. Dilute 100-200 µg protein to 50 µl Cell Lysis Buffer for each assay.
8. Add 50 µl of 2X Reaction Buffer (containing 10 mM DTT) to each sample. Add 5 µl of the 4 mM YVAD-pNA substrate (200 µM final conc.). Incubate at 37°C for 1-2 hours.
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 and use 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-1 activity can be determined by comparing the results of treated samples with the level of the untreated control.
Note: Background reading from cell lysates and buffers should be subtracted from the readings of both treated and the untreated samples before calculating fold increase in Caspase-1 activity.

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