CREATIVE BIOMART®
Assay Kit

Caspase-6 Colorimetric Assay Kit

Product Information

Common Name

Caspase

Cat.No. Kit-0170

Description

Activation of ICE-family proteases/caspases initiates apoptosis in mammalian cells. The Caspase-6 Colorimetric Assay Kit provides a simple and convenient means for assaying the activity of caspases that recognize the sequence VEID. The assay is based on spectrophotometric detection of the chromophore p-nitroanilide (pNA) after cleavage from the labeled substrate VEID-pNA. The pNA light emission can be quantified using a spectrophotometer or a microtiter plate reader at 400-or 405-nm.

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 VEID-pNA from light.

Kit Components

25ml Cell Lysis Buffer,
2ml 2X Reaction Buffer,
125µl VEID-pNA (4 mM),

Tel: 1-631-559-9269 1-516-512-3133
Email:info@creative-biomart.com
Fax:1-631-938-8127
45-1 Ramsey Road, Shirley, NY 11967, USA

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100µl DTT (1M),
25ml Dilution Buffer.

Assay Protocol

1. Induce apoptosis in cells by desired method. Concurrently incubate a control culture without induction.
2. Count cells and pellet 2-5 x 10^6 cells.
3. Resuspend cells in 50 µl of chilled Cell Lysis Buffer 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 100-250 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 VEID-pNA substrate (200 µM final conc.). Incubate at 37°C for 1-2 hour (or longer time if desired).
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 using regular cuvette (note: Dilution of the samples proportionally decreases the reading).

You may also perform the entire assay in a 96-well plate. Fold-increase in Mch2 activity can be determined by comparing the results of treated samples 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-6 activity.

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