CREATIVE BIOMART®
Assay Kit

Caspase-2 fluorometric assay Kit

Product Information

Cat.No. Kit-0160

Product Overview

The Caspase-2 Fluorometric Assay Kit provides a simple and convenient means for assaying the activity of caspases that recognize the sequence VDVAD. The assay is based on detection of cleavage of substrate VDVAD-AFC (AFC: 7-amino-4-trifluoromethyl coumarin). VDVAD-AFC emits blue light (λmax = 400 nm); upon cleavage of the substrate by caspase-2 or related caspases, free AFC emits a yellow-green fluorescence (λmax = 505 nm), which can be quantified using a fluorometer or fluorecence microtiter plate reader. Comparison of the fluorescence of AFC from an apoptotic sample with an uninduced control allows determination of the fold increase in Caspase-2 activity.

Storage

-20°C

Shipping

Gel Pack

Size

25 assays

Kit Components

25ml Cell Lysis Buffer,
2ml 2X Reaction Buffer,
125µl VDVAD-AFC Substrate (1mM),
100µl DTT.

Technical Notes

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). •

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

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After thawing, store the Cell Lysis Buffer and 2X Reaction Buffer at 4°C. All kit reagents are stable for 6 months •
Protect VDVAD-AFC from light. •
We recommend using a flat bottom, opaque, white or black 96-well plate for enhanced sensitivity.

Target Species

Mammalian

Detection method Fluorecence (400 nm excitation filter and 505 nm emission filter)

Assay Protocol

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 or use 50-200 µg cell lysates if protein concentration has been measured.
3. Resuspend cells in 50 µl of chilled Cell Lysis Buffer. Incubate cells on ice for 10 minutes.
4. Add 50 µl of 2X Reaction Buffer (containing 10 mM DTT) to each sample. Add 5 µl of the 1 mM VDVAD-AFC substrate (50 µM final concentration) and incubate at 37°C for 1-2 hour.
5. Read samples in a fluorometer equipped with a 400-nm excitation filter and 505-nm emission filter. For a plate-reading set-up, transfer the samples to a 96-well plate. You may also perform the entire assay directly in a 96-well plate. Fold-increase in Caspase-2 activity can be determined by comparing the results of treated samples with the level of the uninduced control.

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