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CASPASE FAMILY COLOURIMETRIC SUBSTRATE SET

Code: 110-BV-1CSS ***Lot no.:*** ***Exp.:*** 1 year from date of despatch

Description: Ready-to-use colorimetric substrates for assaying activities of members of caspase family proteases. These substrates are formulated in a kit in the ready-to-use format. YVAD is the recognition sequence for caspase-1/ICE. VDVAD is the recognition sequence for caspase-2/ICH-1. DEVD is the recognition sequence for caspase-3/CPP32. WEHD is the recognition sequence for Caspase-5/ICE_{rel}III. VEID is the recognition sequence for caspase-6/Mch2. IETD is the recognition sequence for caspase-8/FLICE, and LEHD is the recognition sequence for Caspase-9/Mch6.

Quantity: 125 ul (4 mM) each of the following:

Caspase-1 Substrate, Ac-YVAD-*p*NA
Caspase-2 Substrate, Ac-VDVAD-*p*NA
Caspase-3 Substrate, Ac-DEVD-*p*NA
Caspase-5 Substrate, Ac-WEHD-*p*NA
Caspase-6 Substrate, Ac-VEID-*p*NA
Caspase-8 Substrate, Ac-IETD-*p*NA
Caspase-9 Substrate, Ac-LEHD-*p*NA

Storage: Store at –20 °C.

Assay Procedure:

1. Induce apoptosis in cells by desired method. Concurrently incubate a control culture *without* induction. Count cells and pellet 1-5 x 10^6 cells.
3. Resuspend cells in 50 ul of chilled Cell Lysis Buffer (Code.# 110-BV-1CLBB) 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.
   Assay protein concentration.
   Dilute 50-200 ug protein to 50 ul Cell Lysis Buffer for each assay.
   Add 50 ul of 2X Reaction Buffer (Code.# 110-BV-1RBA) containing 10 mM DTT (Code # 110-BV-IDTT) to each sample.
9. Add 5 ul each of the 4 mM *p*NA conjugated substrates (200 uM final conc.) individually and incubate at 37°C for 1-2 hour.
   Read samples at 400- or 405-nm in a microtitre plate reader, or spectrophotometer using a 100-ul micro quartz cuvette (Sigma), or dilute sample to 1 ml with Dilution Buffer (Code # 110-BV-1DBA) 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

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