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CASPASE FAMILY FLUOROMETRIC SUBSTRATE SET

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

Description: These fluorometric substrates are for assaying activities of members of the caspase family proteases. These substrates are formulated in a kit in a 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 (1 mM) of each of the following substrates in DMSO:

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

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 or use 50-200 ug cell lysates if protein concentration has been measured.
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. Add 50 ul of 2X Reaction Buffer (Code.# 110-BV-1RBA) containing 10 mM DTT (Code # 110-BV-IDTT) to each sample.
6. Add 5 ul of the 1 mM AFC conjugated substrates (50 uM final conc.) into each tube individually and incubate at 37°C for 1-2 hours.
7. Read samples in a fluorometer with a 400nm excitation filter and a 505nm emission filter. For a plate reading set-up, transfer the samples to a 96 well plate. Alternatively you may 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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