Caspase Colorimetric Substrate Set II Plus

PromoKine

Instruction Manual

Catalog Number	PK-CA577-K138
Description	Contains 9 ready-to-use caspase-1/-2/-3/-4/-5/-6/-8/-9/-10 pNA-labeled substrates and all buffers for performing caspase assays. All substrates are provided in liquid ready-to-use form.
Kit Components	

Concentration	Description	Volume
4 mM	Caspase-1 Substrate, Ac-YVAD-pNA	125 µl
4 mM	Caspase-2 Substrate, Ac-VDVAD-pNA	125 µl
4 mM	Caspase-3/7 Substrate, Ac-DEVD-pNA	125 µl
4 mM	Caspase-4 Substrate, Ac-LEVD-pNA	125 µl
4 mM	Caspase-5 Substrate, Ac-WEHD-pNA	125 µl
4 mM	Caspase-6 Substrate, Ac-VEID-pNA	125 µl
4 mM	Caspase-8 Substrate, Ac-IETD-pNA	125 µl
4 mM	Caspase-9 Substrate, Ac-LEHD-pNA	125 µl
4 mM	Caspase-10 Substrate, Ac-AEVD-pNA	125 µl
N/A	Cell Lysis Buffer	100 ml
N/A	Dilution Buffer	200 ml
N/A	2X Reaction Buffer	20 ml
1 M	DTT	0.4 ml

Quantity	9 x 25 assays
Sequence / Molecular Weight / Molecular Formula	see data sheets of individual caspase substrates
Purity	see data sheets of individual caspase substrates
Appearance / Formulation / Solubility	Solution in DMSO
Storage & Stability	Store at -20°C. Stable for 6 months under proper storage conditions.
Applications	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. 3. Resuspend cells in 50 µl of chilled Cell Lysis Buffer (Cat.# PK-CA577-1067-100) and incubate cells on ice for 10 minutes. 4. Centrifuge for 1 minute in a microcentrifuge (10,000 x g). 5. Transfer supernatant to a fresh tube and assay protein concentration. 6. Dilute 100-300 µg protein to 50 µl Cell Lysis Buffer for each assay. 7. Add 50 µl of 2X Reaction Buffer (Cat.# PK-CA577-1068-20) containing 10 mM DTT (Cat.# PK-CA577-1201-1) to each sample. 8. Add 5 µl of the 4 mM pNA conjugated substrates (200 µM final conc.) into each tube individually and incubate at 37°C for 1-2 hour. 9. Read samples at 400- or 405-nm in a microtiter plate reader, or spectrophotometer using a 100-µl micro quartz cuvet (Sigma), or dilute sample to 1 ml with Dilution Buffer (Cat.# PK-CA577-1066-100, PK-CA577-1066-500) and using regular cuvet (note: Dilution of the samples proportionally decreases the reading). 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.
References	NA
Caution	NA

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