Caspase-8 Substrate IETD-pNA

PromoKine

Instruction Manual

Catalog Number	PK-CA577-1063-1000
Description	Ready-to-use colorimetric substrate for FLICE/caspase-8 and related caspases that recognize the amino acid sequence IETD. The sequence IETD is based on caspase-8 cleavage site in CPP32/Caspase-3 proenzyme. FLICE and related caspase activity can be quantified by spectrophotometric detection of free pNA (λ= 400 nm) after cleavage from the peptide substrate IETD-pNA, using a spectrophotometer or multi-well plate reader.
Quantity	1000 assays
Sequence / Molecular Weight / Molecular Formula	Ac-Ile-Glu-Thr-Asp-pNA (pNA, p-nitroanilide); 639 Da
Purity	>95% by HPLC analysis.
Appearance / Formulation / Solubility	4 mM in DMSO
Storage & Stability	Store at -20°C, protected from light. 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 (PK-CA577-1067-100, PK-CA577-1067-400) and incubate cells on ice for 10 minutes. 4. Centrifuge for 1 minute 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 50-200 µg protein to 50 µl Cell Lysis Buffer for each assay. 8. Add 50 µl of 2X Reaction Buffer (Cat. # PK-CA577-PK-CA577-1068-20, PK-CA577-1068-80) containing 10 µM DTT (Cat. # PK-CA577-1201-1) to each sample. 9. 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. 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-CA577-1066-400) and using regular cuvet (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.
References	NA
Caution	NA

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