

Instruction Manual

Catalog Number	PK-CA577-1076-200
Description	Ready-to-use colorimetric substrate for caspase-9/Mch6 and related caspases that recognize the amino acid sequence LEHD. Caspase-9 and related caspase activity can be quantified by spectrophotometric detection of free pNA ($\lambda = 400$ nm) after cleavage from the peptide substrate LEHD-pNA, using a spectrophotometer or multi-well plate reader.
Quantity	200 assays (2 x 0.5 ml)
Sequence / Molecular Weight / Molecular Formula	Ac-Leu-Glu-His-Asp-pNA; 675 Da
Purity	>98% by HPLC analysis
Appearance / Formulation / Solubility	4 mM in DMSO
Storage & Stability	Store at -20°C , protected from light. Stable for 6 months
Applications	<ol style="list-style-type: none"> 1. Induce apoptosis in cells by desired method. Concurrently incubate a control culture without induction. 2. Count cells and pellet $1-5 \times 10^6$ cells. 3. Resuspend cells in 50 μl of chilled Cell Lysis Buffer (Cat.# 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-CA577-1068-20, PK-CA577-1068-80) containing 10 μM DTT (Cat. # PK-CA577-1201-1) to each sample. 9. Add 5 μl of the 4 mM substrate LEHD-pNA (200 μM final conc.) into each tube and incubate at 37°C for 1-2 hour. 10. 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). <p>You may also perform the entire assay directly in a 96-well plate.</p> <p>Fold-increase in caspase activity can be determined by comparing these results with the level of the uninduced control.</p> <p>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.</p>
References	NA
Caution	NA

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