

Instruction Manual

Catalog Number	PK-CA577-1110-1000
Description	Ready-to-use colorimetric substrate for caspase-4 and related caspases that recognize the amino acid sequence LEVD. Caspase-4 and related caspase activity can be quantified by spectrophotometric detection of free pNA ($\lambda = 400$ nm) after cleavage from the peptide substrate LEVD-pNA, using a spectrophotometer or multi-well plate reader. The ready-to-use caspase substrate provides an economic alternative for large volume users.
Quantity	1000 assays
Sequence / Molecular Weight / Molecular Formula	Ac- Leu-Glu-Val-Asp-pNA; 629 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 under proper storage conditions.
Applications	<p>1. Induce apoptosis in cells by desired method. Concurrently incubate a control culture without induction.</p> <p>Note: Active recombinant human Caspase-4 is available to use as a positive control (Cat.# PK-RP577-1084-25, PK-RP577-1084-100).</p> <p>2. Count cells and pellet $1-5 \times 10^6$ cells.</p> <p>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.</p> <p>4. Centrifuge for 1 minute in a microcentrifuge (10,000 x g).</p> <p>5. Transfer supernatant (cytosolic extract) to a fresh tube and put on ice.</p> <p>6. Assay protein concentration.</p> <p>7. Dilute 100-300 μg protein to 50 μl Cell Lysis Buffer for each assay.</p> <p>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.</p> <p>9. Add 5 μl of the 4 mM of LEVD-pNA (200 μM final conc.) and incubate at 37°C for 1-2 hour.</p> <p>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> <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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