

## Instruction Manual

Catalog Number	PK-CA577-1102-1000
Description	Ready-to-use colorimetric substrate for caspase-5 and related caspases that recognize the amino acid sequence WEHD. Caspase-5 and related caspase activity can be quantified by spectrophotometric detection of free pNA ( $\lambda = 400 \text{ nm}$ ) after cleaved from the peptide substrate WEHD-pNA, using a spectrophotometer or multi-well plate reader. The ready-to-use caspase substrate provides an economic alternative for researchers who perform large volume of caspase assays.
Quantity	1000 assays
Sequence / Molecular Weight / Molecular Formula	Ac-Trp-Glu-His-Asp-pNA; 862 Da
Purity	>99% by HPLC analysis
Appearance / Formulation / Solubility	4 mM in DMSO
Storage & Stability	Store at $-20^{\circ}\text{C}$ , protected from light. Stable for 1 year under proper storage conditions.
Applications	<ol style="list-style-type: none"> <li>1. Induce apoptosis in cells by desired method. Concurrently incubate a control culture without induction.</li> <li>2. Count cells and pellet <math>1-5 \times 10^6</math> cells.</li> <li>3. Resuspend cells in 50 <math>\mu\text{l}</math> of chilled Cell Lysis Buffer (Cat.# PK-CA577-1067-100, PK-CA577-1067-400) and incubate cells on ice for 10 minutes.</li> <li>4. Centrifuge for 1 minute in a microcentrifuge (10,000 x g).</li> <li>5. Transfer supernatant (cytosolic extract) to a fresh tube and put on ice.</li> <li>6. Assay protein concentration.</li> <li>7. Dilute 50-200 <math>\mu\text{g}</math> protein to 50 <math>\mu\text{l}</math> Cell Lysis Buffer for each assay.</li> <li>8. Add 50 <math>\mu\text{l}</math> of 2X Reaction Buffer (Cat.# PK-CA577-1068-20, PK-CA577-1068-80) containing 10 <math>\mu\text{M}</math> DTT (Cat. # PK-CA577-1201-1) to each sample.</li> <li>9. Add 5 <math>\mu\text{l}</math> of the 4 mM of WEHD-pNA (200 <math>\mu\text{M}</math> final conc.) and incubate at <math>37^{\circ}\text{C}</math> for 1-2 hour.</li> <li>10. Read samples at 400- or 405-nm in a microtiter plate reader, or spectrophotometer using a 100-<math>\mu\text{l}</math> 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).</li> </ol> <p>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.</p>
References	NA
Caution	NA

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