

## Instruction Manual

Catalog Number	PK-CA577-1008-1000
Description	Ready-to-use colorimetric substrate for CPP32/caspase-3 and related caspases that recognize the amino acid sequence DEVD. The sequence DEVD is based on caspase-3 cleavage site in poly (ADP-ribose) polymerase (PARP). CPP32 and related caspase activity can be quantified by spectrophotometric detection of free pNA ( $\lambda = 400$ nm) after cleavage from the peptide substrate DEVD-pNA, using a spectrophotometer or multi-well plate reader. The ready-to-use caspase substrate provides an economic alternative for researchers who perform large amounts of caspase assays.
Quantity	1000 assays
Sequence / Molecular Weight / Molecular Formula	Ac-Asp-Glu-Val-Asp-pNA; 637 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	<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</math>l of chilled Cell Lysis Buffer (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</math>g protein to 50 <math>\mu</math>l Cell Lysis Buffer for each assay.</li> <li>8. Add 50 <math>\mu</math>l of 2X Reaction Buffer (Cat. # PK-CA577-1068-20, PK-CA577-1068-80) containing 10 <math>\mu</math>M DTT (Cat. # PK-CA577-1201-1) to each sample.</li> <li>9. Add 5 <math>\mu</math>l of the 4 mM of DEVD-pNA (200 <math>\mu</math>M final conc.) 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-<math>\mu</math>l 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.</li> </ol> <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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