

## Instruction Manual

Catalog Number	PK-CA577-K134
Description	Contains ready-to-use caspase-1/-2/-3/-4/-5/-6/-8/-9/-10 pNA-labeled substrates. All substrates are provided in liquid ready-to-use form.
Quantity	9 x 25 assays
Sequence / Molecular Weight / Molecular Formula	NA
Purity	see data sheets of individual caspase substrates
Appearance / Formulation / Solubility	Solution in DMSO
Storage & Stability	Store at -20°C. Stable for 6-12 months under proper storage conditions.
Applications	<p>1. Induce apoptosis in cells by desired method. Concurrently incubate a control culture without induction.</p> <p>2. Count cells and pellet <math>1-5 \times 10^8</math> cells.</p> <p>3. Resuspend cells in 50 <math>\mu</math>l of chilled Cell Lysis Buffer (Cat.# PK-CA577-1067-100) 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 to a fresh tube and assay protein concentration.</p> <p>6. Dilute 200-300 <math>\mu</math>g protein to 50 <math>\mu</math>l Cell Lysis Buffer for each assay.</p> <p>7. Add 50 <math>\mu</math>l of 2X Reaction Buffer (Cat.# PK-CA577-1068-20) containing 10 mM DTT (Cat.# PK-CA577-1201-1) to each sample.</p> <p>8. Add 5 <math>\mu</math>l of the 4 mM pNA conjugated substrates (200 <math>\mu</math>M final conc.) into each tube individually and incubate at 37°C for 1-2 hour.</p> <p>9. 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-CA577-1066-500) and using regular cuvet (note: Dilution of the samples proportionally decreases the reading).</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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