

## Instruction Manual

<b>Catalog Number</b>	PK-CA577-1062-1000
<b>Description</b>	Ready-to-use fluorometric substrate for FLICE/caspase-8 and related caspases that recognize the amino acid sequence IETD. The sequence IETD is based on caspase-8 cleavage site in CPP32/caspase-3 proenzyme. FLICE and related caspase activity can be quantified by fluorescent detection of free AFC after cleavage from the peptide substrate IETD-AFC at Ex. = 400 nm and Em. = 505 nm, using a fluorometer or multi-well fluorescence plate reader. Alternatively, a shift in fluorescence from blue to green upon cleavage can be visualized using a hand-held long-UV lamp.
<b>Quantity</b>	1000 assays
<b>Sequence / Molecular Weight / Molecular Formula</b>	Ac-Ile-Glu-Thr-Asp-AFC (AFC, 7-amino-4-trifluoromethyl coumarin); 729 Da
<b>Purity</b>	>95% by HPLC analysis.
<b>Appearance / Formulation / Solubility</b>	2 mM in DMSO
<b>Storage &amp; Stability</b>	Store at -20°C, protected from light. Stable for 1 year under proper storage conditions.
<b>Applications</b>	<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 or use 50-200 µg cell lysates if protein concentration has been measured.</li> <li>3. Resuspend cells in 50 µl of chilled Cell Lysis Buffer (Cat.# PK-CA577-1067-100, PK-CA577-1067-400).</li> <li>4. Incubate cells on ice for 10 minutes.</li> <li>5. Add 50 µl of 2X Reaction Buffer (Cat. # PK-CA577-PK-CA577-1068-20, PK-CA577-1068-80) containing 10 µM DTT (Cat. # PK-CA577-1201-1) to each sample.</li> <li>6. Add 5 µl of the 1 mM IETD-AFC (50 µM final conc.) into each tube individually and incubate at 37°C for 1-2 hour.</li> <li>7. Read samples in a fluorometer equipped with a 400-nm excitation filter and 505-nm emission filter. For a plate-reading set-up, transfer the samples to a 96-well plate. You may perform the entire assay directly in a 96-well plate.</li> </ol> <p>Fold-increase in IETD-dependent Caspase-8 activity can be determined by comparing these results with the level of the uninduced control.</p>
<b>References</b>	NA
<b>Caution</b>	NA

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