

## Instruction Manual

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| Catalog Number                                  | PK-CA577-1071-1000   |
| Description                                     | Ready-to-use fluorometric substrate for caspases that recognize the amino acid sequence VDVAD. Caspase activity can be quantified by fluorescent detection of free AFC after cleaved from the peptide substrate VDVAD-AFC at Ex/Em = 400/505 nm using a fluorometer or a fluorescence plate reader. Alternatively, a shift in fluorescence from blue to green upon cleavage can be visualized using a hand-held long-UV lamp. The ready-to-use caspase substrate provides an economic alternative for researchers who perform large amount of caspase assays. Cell Lysis Buffer (PK-CA577-1067-100, -400), 2X Reaction Buffer (PK-CA577-1068-20, -80), DTT (Cat. # PK-CA577-1201-1), and other reagents used for caspase activity assays are also available separately.  |
| Quantity  | 1000 assays  |
| Sequence / Molecular Weight / Molecular Formula | Ac-Val-Asp-Val-Ala-Asp-AFC (AFC, 7-amino-4-trifluoromethyl coumarin); 771 Da   |
| Purity  | >98% by HPLC analysis  |
| Appearance / Formulation / Solubility           | 1 mM in DMSO   |
| Storage & Stability                             | Store at -20°C, protected from light. Stable for 6 months  |
| 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 or use 50-200 <math>\mu\text{g}</math> cell lysates if protein concentration has been measured.</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).</li> <li>4. Incubate cells on ice for 10 minutes.</li> <li>5. Add 50 <math>\mu\text{l}</math> of 2X Reaction Buffer (Cat. # PK-CA577-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>6. Add 5 <math>\mu\text{l}</math> of the 1 mM VDVAD-AFC (50 <math>\mu\text{M}</math> 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 VDVAD-dependent caspase activity can be determined by comparing these results with the level of the uninduced control.</p> |
| References                                      | NA   |
| Caution   | NA   |

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