Caspase-8 Substrate IETD-AFC

PromoKine

Instruction Manual

Catalog Number	PK-CA577-1062-200
Description	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.
Quantity	200 assays
Sequence / Molecular Weight / Molecular Formula	Ac-Ile-Glu-Thr-Asp-AFC (AFC, 7-amino-4-trifluoromethyl coumarin); 729 Da
Purity	>95% by HPLC analysis.
Appearance / Formulation / Solubility	2 mM in DMSO
Storage & Stability	Store at -20°C, protected from light. Stable for 1 year under proper storage conditions.
Applications	1. Induce apoptosis in cells by desired method. Concurrently incubate a control culture without induction. 2. Count cells and pellet 1-5 x 10^6 cells or use 50-200 µg cell lysates if protein concentration has been measured. 3. Resuspend cells in 50 µl of chilled Cell Lysis Buffer (Cat.# PK-CA577-1067-100, PK-CA577-1067-400). 4. Incubate cells on ice for 10 minutes. 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. 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. 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. Fold-increase in IETD-dependent Caspase-8 activity can be determined by comparing these results with the level of the uninduced control.
References	NA
Caution	NA

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