Caspase-9 Substrate LEHD-AFC

PromoKine

Instruction Manual

Catalog Number	PK-CA577-1075-1000
Description	Ready-to-use fluorometric substrate for caspase-9/Mch-6 and related caspases that recognize the amino acid sequence LEHD. Caspase activity can be quantified by fluorescent detection of free AFC after cleavage from the peptide substrate LEHD-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. The ready-to-use caspase substrate provides an economic alternative for researchers who perform large amount of caspase assays. Cell Lysis Buffer (Cat. # PK-CA577-1067-100, -400) and 2X Reaction Buffer (Cat. # PK-CA577-1068-20, -80) and DTT (Cat.# PK-CA577-1201-1) for caspase assays are also available separately.
Quantity	1000 assays
Sequence / Molecular Weight / Molecular Formula	Ac-Leu-Glu-His-Asp-AFC ; 765 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	ASSAY PROTOCOL: 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-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 LEHD-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 LEHD-dependent activity can be determined by comparing these results with the level of the uninduced control.
References	NA
Caution	NA

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