(Ac-LEHD)2-R110

Catalog number: 13427
Unit size: 1 mg

Component	Storage	Amount
(Ac-LEHD)2-R110	Freeze (<-15 °C), Minimize light exposure	1 mg

OVERVIEW

Caspase 9 is a member of the CED-3 subfamily of the caspase family of cysteine proteases that play an essential role in the execution phase of apoptosis. These enzymes share a dominant primary specificity for cleaving bonds following aspartic acid residues. "Initiator" caspases (such as caspase 8) activate "effector" caspases, such as caspases 3 and 7. The effector caspases then cleave cellular substrates ultimately leading to the morphological changes of apoptosis. (Ac-LHED)2-R110 is a selective fluorogenic substrate for caspase 9. The caspase 9-induced hydrolysis of (Ac-LHED)2-R110 results in the release of R110 fluorophore that is detected using an excitation wavelength of ~490 nm and an emission wavelengh of ~520 nm. The assay can be run in the assay buffer consisting of 50 mM MES, pH 6.5, 10% PEG 8000, 0.1% CHAPS, 5 mM DTT, and 1 mM EDTA.

AT A GLANCE

Important
Following protocol only provides a guideline, and should be modified according to your specific needs.

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

1. (Ac-LEHD)2-R110 stock solution (10X):
Prepare a 10 mM stock solution in DMSO.

PREPARATION OF WORKING SOLUTION

Add 50 µL of substrate stock solution into 100 µL of DTT (1M), 400 µL of EDTA (100 mM), and 10 mL of Tris Buffer (20 mM), pH = 7.4, to prepare a 50 µM caspase substrate assay solution (2X).

SAMPLE EXPERIMENTAL PROTOCOL

1. Mix equal volume of the caspase standards or samples with 2X caspase substrate assay solution, and incubate the solutions at room temperature for at least 1 hour.

2. Monitor the fluorescence using a fluorescence microplate reader at Ex/Em = 498/520 nm, or absorbance using an absorbance microplate reader.

EXAMPLE DATA ANALYSIS AND FIGURES

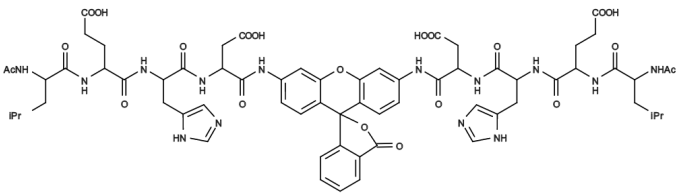

Figure 1. Chemical structure for (Ac-LEHD)2-R110

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