

Ac-LEHD-AMC *CAS 292633-16-0*

Catalog number: 13426

Unit size: 5 mg

Component	Storage	Amount
Ac-LEHD-AMC *CAS 292633-16-0*	Freeze (<-15 °C), Minimize light exposure	5 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-AMC is a selective fluorogenic substrate for caspase 9. The caspase 9-induced hydrolysis of Ac-LHED-AMC results in the release of AMC fluorophore that is detected using an excitation wavelength of ~365 nm and an emission wavelength of ~450 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 notes

It is important to store at <-15 °C and should be stored in cool, dark place.

It can be used within 12 months from the date of receipt.

SAMPLE EXPERIMENTAL PROTOCOL

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

General Solution Caspase Assays Using AMC, AFC, pNA, R110 and ProRed Substrates

1. Prepare a 10 mM stock solution in DMSO.
2. Prepare a 2X caspase substrate (50 μM) assay solution as the following: 50 μL substrate stock solution, 100 μL DTT (1M), 400 μL EDTA (100 mM), 10 mL Tris Buffer (20 mM), pH =7.4.
3. 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.
4. Monitor the fluorescence using a fluorescence microplate reader, or absorbance using an absorbance microplate reader.

Cell Caspase Assays Using Cell-Permeable FMK Caspase Probes

1. Prepare a 2-5 mM stock solution in DMSO.
2. Treat cells as desired.
3. Prepare a 2X permeable caspase substrate (20 μM) assay solution by diluting the DMSO stock solution (from Step 2.1) in Hanks with 20 mM Hepes buffer (HHBS).
4. Mix equal volume of the treated cells with 2X caspase substrate assay solution (from Step 2.3), and incubate the cells in a 37°C, 5% CO₂ incubator for at least 1 hour.
5. Wash the cells with HHBS for at least once.
6. Monitor the fluorescence intensity by a flow cytometer, a fluorescence microscope or a fluorescence microplate reader.

Cell Caspase Assays Using Cell-Permeable FMK Caspase Probes (For #13470-13476 only)

1. Prepare a 250X stock solution by adding 50 μL DMSO into the vial.
2. Treat cells as desired.
3. Add 250 X DMSO stock solution into the cell solution at a 1:250 ratio (such as 2 μL to 500 μL cells), and incubate the cells in a 37°C, 5% CO₂ incubator for 1 hour.
4. Wash the cells with HHBS for at least once.
5. Monitor the fluorescence intensity by flow cytometer, fluorescence microscopy or fluorescent microplate reader.

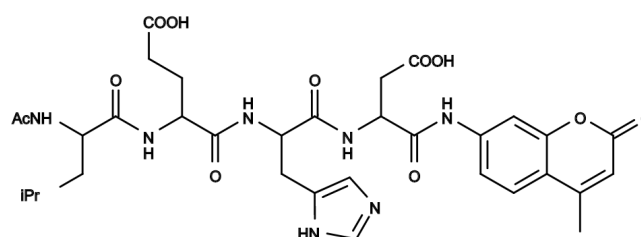
EXAMPLE DATA ANALYSIS AND FIGURES


Figure 1. Chemical structure for Ac-LEHD-AMC *CAS 292633-16-0*

DISCLAIMER

AAT Bioquest provides high-quality reagents and materials for research use only. For proper handling of potentially hazardous chemicals, please consult the Safety Data Sheet (SDS) provided for the product. Chemical analysis and/or reverse engineering of any kit or its components is strictly prohibited without written permission from AAT Bioquest. Please call 408-733-1055 or email info@aatbio.com if you have any questions.