

Z-LEHD-ProRed™ 620

Catalog number: 13435

Unit size: 1 mg

Component	Storage	Amount
Z-LEHD-ProRed™ 620	Freeze (<-15 °C), Minimize light exposure	1 mg

OVERVIEW

ProRed™-derived protease substrates are colorless and non-fluorescent. Cleavage of blocking protease-cleavable peptide residue by caspases generates the strongly red fluorescent ProRed™ that can be monitored fluorimetrically at ~620 nm with excitation of ~530 nm. ProRed™-derived caspase substrates are the most sensitive red indicators for the fluorimetric detection of various caspase activities. This LEHD-ProRed™ substrate is specific for detecting caspase 9.

AT A GLANCEImportant 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

Example data analysis and images of this product can be found on the web at: <https://www.aatbio.com/products/z-lehd-prored-620>

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.