Z-IETD-ProRed™ 620

Catalog number: 13434
Unit size: 1 mg

Component	Storage	Amount
Z-IETD-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 IETD-ProRed™ substrate is specific for detecting caspase 8.

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% CO_2 incubator for at least1 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

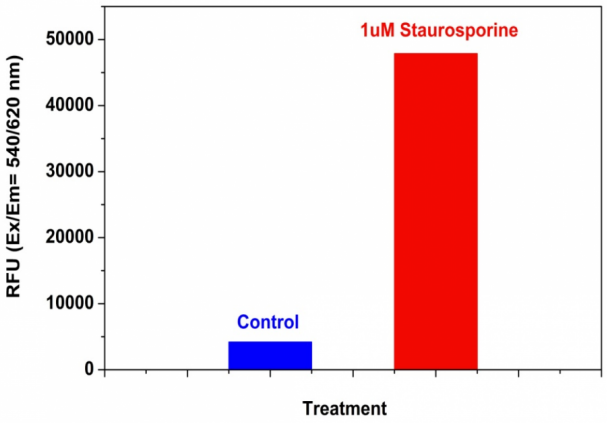

Figure 1. Detection of Caspase 8 Activity in Jurkat cells with Z-IETD-ProRed™ 620. Jurkat cells were seeded on the same day at 200,000 cells/90 µL/well in a Costar black wall/clear bottom 96-well plate. The cells were treated with staurosporine at the final concentration of 1 µM for 5 hours while the untreated cells were used as control. The caspase 8 assay solution (100 µL/well) was added and incubated at room temperature for 1 hour. The fluorescence intensity was measured at Ex/Em = 540/620 nm with a FlexStation™ microplate reader (Molecular Devices).

DISCLAIMER