

Z-LETD-FMK

 Catalog number: 13307
 Unit size: 1 mg

Component	Storage	Amount
Z-LETD-FMK	Freeze (< -15 °C), Minimize light exposure	1 mg

OVERVIEW

Z-LETD-FMK is a cell permeable, irreversible caspase protease 8 inhibitor that binds to the catalytic site of caspase- 8, which plays an important role in the induction of apoptosis. The binding of Z-LETD-FMK to caspase-8 inhibits the activity of this protease, enabling it to inhibit apoptotic events associated with caspase-8 activation. For inhibition of apoptosis, Z-LETD-FMK should be added at the same time that apoptosis is induced. Fluoromethyl ketone (FMK)-derivatized peptides act as effective irreversible inhibitors with no added cytotoxic effects.

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.

Z-LETD-FMK stock solution

Add appropriate amount of DMSO into Z-LETD-FMK to make 2-5 mM Z-LETD-FMK stock solution.

Note Store the unused Z-LETD-FMK stock solution at -20 °C in single use aliquots

SAMPLE EXPERIMENTAL PROTOCOL

The following protocol can be used as a guideline and should be optimized according to your needs.

1. Treat your samples as desired to induce apoptosis.
2. Add Z-LETD-FMK along with the treatment of your choice.

Note We recommend using 10-100 µM of final concentration of Z-LETD-FMK to inhibit caspase-8.

Note Optimal time and concentration for incubation needs to be determined experimentally.

3. Stain samples as you desired.
4. Monitor the fluorescence intensity with your choice of instrumentation.

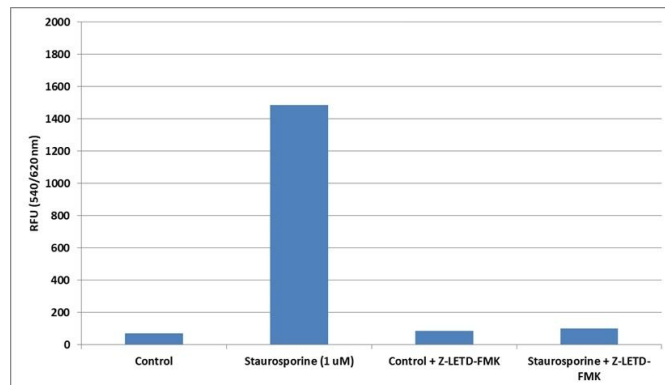
EXAMPLE DATA ANALYSIS AND FIGURES


Figure 1. Inhibition of caspase 8 Activities in Jurkat cells with caspase 8 inhibitor Z-LETD-FMK (Cat.# 13307). 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 or without 1 µM of staurosporine for 5 hours. The Z-LETD-FMK was added and co-incubated with staurosporine. The caspase-8 activity was detected with Cell Meter™ Caspase 8 Activity Apoptosis Assay Kit (Cat.# 22816). The fluorescence intensity was measured at Ex/Em = 540/620 nm (Cutoff = 610 nm) with Flexstation 3 fluorescence microplate reader (Molecular Devices).

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