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Caspase-3/7 Apoptosis Assay Kit

Cat. #: C0046 Size: 100 rxns

Introduction

Caspase-3/7 Apoptosis Assay Kit allows the detection of apoptosis by assaying for increases in caspase-3 and other DEVD-specific protease activities (e.g., caspase-7). The basis for the assay is rhodamine 110 bis-(N-CBZ-L-aspartyl-L-glutamyl-L-valyl-L-aspartic acid amide) (Z-DEVD–R110). This substrate is a bisamide derivative of rhodamine 110 (R110) containing DEVD peptides covalently linked to each of R110's amino groups, thereby suppressing the dye's visible absorption and its fluorescence. Upon enzymatic cleavage, the nonfluorescent bisamide substrate is converted in a two-step process first to the fluorescent monoamide and then to the even more fluorescent R110. Both of these hydrolysis products exhibit spectral properties similar to those of fluorescein, with peak excitation and emission wavelengths of 496 nm and 520 nm, respectively. The substrate can be used to continuously monitor the activity of caspase-3 and closely related proteases in cell extract using a fluorescence microplate reader or fluorometer. In addition to the Z-DEVD-R110 substrate, the kit contains the reversible aldehyde inhibitor Ac-DEVD-CHO, as well as the reference standard R110. The Ac-DEVD-CHO inhibitor can be used to confirm that the observed fluorescence signal in both induced and control cells is due to the activity of caspase-3–like proteases. The reference standard is included to allow quantitation of the amount of R110 released in the reaction.

Package Information

Components	C0046
Cell Lysis Buffer	10 ml
Cell Assay Buffer	10 ml
Enzyme Substrate Z-DEVD-R110	250 µl, 1 mM in DMSO
Enzyme Inhibitor Ac-DEVD-CHO	20 µl, 5 mM in DMSO
Standard R110	1 ml, 80 µM in H_2O

Approximate fluorescence excitation/emission maxima, in nm: R110: 496/520.

Storage

Store at -20°C and protect from light.

Protocols

Note: The following protocol is designed for use in 96-well plates with a total assay volume of 100 µl per well. Volumes can be scaled proportionally as needed. Three control reactions are recommended: 1) Negative control using untreated cells; 2) Positive control using cells treated with an apoptosis inducing agent; 3) Inhibitor control using induced cells and Caspase-3 inhibitor.

1. Plate adherent cells in black 96-well plates. Suspension cells can be plated in flasks or plates.

2. Induce apoptosis in cells by desired methods. Remember to include untreated wells as controls.

3. Cell lysis:

For suspension cells:

a) Aliquot equal numbers of cells into microcentrifuge tubes or wells of a black 96-well plate. For best results, we recommend using at least 100,000-1,000,000 cells for each reaction.

b) Centrifuge cells at 400× g for 5 minutes and aspirate supernatant.

c) Resuspend the cell pellets in 50 µl of chilled Cell Lysis Buffer.

For adherent cells:

a) Aspirate culture medium from each well of the 96-well plate.

b) Add 50 µl chilled Cell Lysis Buffer per well.

4. Incubate cells in Lysis Buffer on ice for 30 minutes.

5. Centrifuge cell lysates in a microcentrifuge tube at maximum speed for 5 minutes at 4°C to pellet insoluble cell debris. Transfer the supernatants to new microcentrifuge tubes.

6. (Optional) To verify the signal detected by the kit is due to Caspase-3 activity, incubate an induced sample with caspase-3 inhibitor before adding substrate. Add 1 µl of 5 mM Enzyme Inhibitor Ac-DEVD-CHO to selected induced samples. Incubate at room temperature for 10 minutes. The remaining samples (without inhibitor) should be stored on ice during this time.

7. Prepare a substrate working solution by mixing 50 µl of 1 mM Enzyme Substrate Z-DEVD-R110 with 950 µl of Assay Buffer.

8. Add 50 µl of the substrate working solution to each sample and mix well. Incubate samples at 37°C for 30-60 minutes (up to 3 hours maximum).

9. Measure fluorescence with 470 nm excitation and 520 nm emission.

(Optional) R110 Reference Standard Curve

1. Dilute R110 (80 µM) to 20 µM in Cell Lysis Buffer. Perform 1:2 serial dilutions to obtain concentrations of 10, 5, 2.5, 1.25, 0.625, 0.313, and 0.156 µM R110. Use Cell Lysis Buffer for the 0 µM (blank) sample. Add 100 µl/ well of the serially diluted R110 solutions from 20 µM to 0 µM into a 96-well plate.

2. Measure the fluorescence intensity of the standards at Ex/Em=470 nm/520 nm. Subtract the fluorescence reading from the blank (0 µM R110) from each fluorescence value to calculate relative fluorescence units (RFU).

3. Plot RFU versus R110 concentration to generate a standard curve.

Note: The kinetics of fluorescence generation due to substrate cleavage is not linear because the two-step cleavage of the substrate generates an intermediate and an end-product with different fluorescence intensities. Therefore, the R110 standard can be used to quantitate the amount of R110 generated at the endpoint of the assay, but cannot be used for kinetic studies.