Active Caspase-8 Staining Kit (Fluorometric)

LS-K373-25, -100 (25, 100 Tests) • **Store at -20°C**

Introduction

Activation of caspases plays a central role in apoptosis. The Active Caspase-8 Staining Kit provides a convenient means for detecting activated caspase-8 in living cells. The assay utilizes a caspase-8 inhibitor IETD-FMK conjugated to sulfo-rhodamine (Red-IETD-FMK) as the fluorescent marker. Red-IETD-FMK is cell permeable, nontoxic, and irreversibly binds to activated caspase-8 in apoptotic cells. The fluorescence label allows detection of activated caspase-8 in apoptotic cells directly by fluorescence microscopy, flow cytometry, or fluorescence plate reader.

Components

Component	K373-25	K373-100
	25 Tests	100 Tests
Red-IETD-FMK	25 µl	100 µl
Wash Buffer	50 ml	2 x 100 ml
Z-VAD-FMK	10 µl	10 µl

Assay Procedure

A. Staining Procedure

1. Induce apoptosis in cells (1 x 10^6/ml) by desired method. Concurrently incubate a control culture without induction. An additional negative control can be prepared by adding the caspase inhibitor Z-VAD-FMK at 1 µl/ml to an induced culture to inhibit caspase activation.
2. Aliquot 300 µl each of the induced and control cultures into Eppendorf tubes.
3. Add 1 µl of Red-IETD-FMK into each tube and incubate for 0.5-1 hour at 37°C incubator with 5% CO_2.
4. Centrifuge cells at 3000 rpm for 5 minutes and remove supernatant.
5. Resuspend cells in 0.5 ml of Wash Buffer, and centrifuge again.
6. Repeat Step 5.

   Proceed to B, C, or D depending on methods of analysis.

B. Quantification by Flow Cytometry

For flow cytometric analysis, resuspend cells in 300 µl of Wash buffer. Put samples on ice. Analyzing samples by flow cytometry using the FL-2 channel.

C. Detection by Fluorescence Microscopy

For fluorescence microscopic analysis, resuspend cells in 100 µl Wash buffer. Put one drop of the cell suspension onto a microslide and cover with a coverslip. Observe cells under a fluorescence microscope using rhodamine filter. Caspase-8 positive cells appear to have brighter red signals, whereas caspase-8 negative control cells show much weaker signal.

D. Analysis by Fluorescence Plate Reader

For analysis with fluorescence plate reader, resuspend cells in 100 µl Wash Buffer and transfer the cell suspension to each well of the black microtiter plate. Measure the fluorescence intensity at Ex/Em = 540/570 nm (Note: Ex/Em=488/570 nm will also work, although it's not an optimal wavelength). For control, use wells containing unlabeled cells.

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