Caspase-3 Staining Kit (Red)

Catalog Number KA0744

96 assays

Version: 03

Intended for research use only

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Introduction and Background

A. Overview

Activation of caspases plays a central role in apoptosis. The Red Active Caspase Staining Kit provides a convenient means for detecting activated caspases-3 in living cells. The assay utilizes a caspase-3 inhibitor DEVD-FMK conjugated to sulfo-rhodamine (Red-DEVD-FMK) as the fluorescent in situ marker. Red-DEVD-FMK is cell permeable, nontoxic, and irreversibly binds to activated caspases-3 in apoptotic cells. The red fluorescence label allows for direct detection of activated caspases-3 in apoptotic cells by fluorescence microscopy, flow cytometry, or fluorescence plate reader.

Material and Method

A. List of component

1. Red-DEVD-FMK 100 µl.
2. Wash Buffer 2 x 100 ml.
3. Z-VAD-FMK 10 µl.

B. Stability and storage

Store kit at -20℃.

C. Protocol

1. Staining:
   a. Induce apoptosis in cells (1 x 10^6/ml) by desired method. Concurrently incubate a control culture without induction. An additional control can be prepared by adding the caspase inhibitor Z-VAD-FMK at 1 µl/ml to an induced culture to inhibit caspase activation.
   b. Aliquot 300 µl each of the induced and control cultures into eppendorf tubes.
   c. Add 1 µl of Red-DEVD-FMK into each tube and incubate for 0.5-1 hour at 37℃ incubator with 5% CO_2.
   d. Centrifuge cells at 3000 rpm for 5 minutes and remove supernatant.
   e. Resuspend cells in 0.5 ml of Wash Buffer, and centrifuge again.
   f. Repeat Step e.
   g. Proceed to 2, 3, or 4 depending on methods of analysis.
2. 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.
3. 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 positive cells appear to have brighter green signals, whereas caspase negative control cells show much weaker signal.

4. Analysis by Fluorescence Plate Reader:

   For analysis with fluorescence plate reader, resuspend cells in 100 µl Wash Buffer and then 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.