Introduction and Background

A. Overview

Activation of caspases plays a central role in apoptosis. The Fluorescein Active Caspase-2 Staining Kit provides a convenient means for sensitive detection of activated caspase-2 in living cells. The assay utilizes the caspase-2 inhibitor, VDVAD-FMK, conjugated to FITC (FITC-VDVAD-FMK) as a marker. FITCVDVAD-FMK is cell permeable, nontoxic, and irreversibly binds to activated caspase-2 in apoptotic cells. The FITC label allows for direct detection of activated caspases in apoptotic cells by fluorescence microscopy, flow cytometry, or fluorescence plate reader.

B. Notice for Application of Kit

✓ This kit has been configured for research use only and is not for diagnostic and clinical use.

Material and Method

A. List of component

1. FITC-VDVAD-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 negative control can be prepared by adding the caspase inhibitor Z-VAD-FMK at 1 µl/ml to an induced culture to inhibit caspase-2 activation.
   b. Aliquot 300 µl each of the induced and control cultures into eppendorf tubes.
   c. Add 1 µl of FITC-VDVAD-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. Keep samples on ice. Analyzing samples by flow cytometry using the FL-1 channel.

3. Detection by Fluorescence Microscopy:

   For fluorescence microscopic analysis, resuspend cells in 100 µl Wash Buffer. Transfer one drop of the cell suspension onto a microslide and cover with a coverslip. Observe cells under a fluorescence microscope using FITC 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 into each well in the black microtiter plate. Measure the fluorescence intensity at Ex. = 485 nm and Em. = 535 nm. For control, use wells containing unlabeled cells.