Green Caspase-9 Staining Kit

PromoKine

Instruction Manual

Catalog Number	PK-CA577-K189-25
Description	Activation of caspases plays a central role in apoptosis. The PromoKine Green Caspase-9 Staining Kit provides a convenient means for sensitive detection of activated Caspase-9 in living cells. The assay utilizes the Caspase-9 inhibitor LEHD-FMK conjugated to FITC (FITC-LEHD-FMK) as a fluorescent marker. FITC-LEHD-FMK is cell permeable, nontoxic, and irreversibly binds to activated Caspase-9 in apoptotic cells. The FITC label allows detection of activated caspases in apoptotic cells directly by fluorescence microscopy, flow cytometry, or using a fluorescence plate reader.
Quantity	25 assays
Kit Components	Components – Quantity FITC-LEHD-FMK – 25 µl Wash Buffer – 50 ml Z-VAD-FMK – 10 µl
Applications / Assay Protocol	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-9 activation. 2. Aliquot 300 µl each of the induced and control cultures into eppendorf tubes. 3. Add 1 µl of FITC-LEHD-FMK into each tube and incubate for 0.5-1 hour at 37°C incubator with 5% CO_2. 4. Centrifuge cells at 3,000 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. Keep samples on ice. Analyzing samples by flow cytometry using the FL-1 channel. C. 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 a FITC filter. Caspase-9 positive cells appear to have brighter green signals, whereas Caspase-9 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 then transfer the cell suspension into each well of a black microtiter plate. Measure the fluorescence intensity at Ex. = 485 nm and Em. = 535 nm. For control, use wells containing unlabeled cells.
Storage & Stability	Store kit at –20°C upon arrival. Store individual reagents as indicated on the respective labels.

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