

Instruction Manual

Catalog Number	PK-CA577-K188-25	
Description	Activation of caspases plays a central role in apoptosis. The PromoKine Green Caspase-8 Staining Kit provides a convenient means for sensitive detection of activated Caspase-8 in living cells. The assay utilizes the Caspase-8 inhibitor, IETD-FMK, conjugated to FITC (FITC-IETD-FMK) as a fluorescent in situ marker. FITC-IETD-FMK is cell permeable, nontoxic, and binds irreversibly to activated Caspase-8 in apoptotic cells. The FITC label allows detection of activated Caspase-8 in apoptotic cells directly by fluorescence microscopy, flow cytometry or using a fluorescence plate reader.	
Quantity	25 assays	
Kit Components	Components	Quantity
	FITC-IETD-FMK	25 µl
	Wash Buffer	50 ml
	Z-VAD-FMK	10 µl
Applications / Assay Protocol	<p>A. Staining Procedure:</p> <ol style="list-style-type: none"> 1. Induce apoptosis in cells (1×10^6/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 FITC-IETD-FMK into each tube and incubate for 0.5-1 hour at 37°C incubator with 5% CO₂. 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. <p>Proceed to B, C, or D depending on methods of analysis.</p> <p>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-1 channel.</p> <p>C. Detection by Fluorescence Microscopy: For fluorescence microscopic analysis, resuspend cells in 100 µl Wash Buffer. Add 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-8 positive cells appear to have brighter green signals, whereas Caspase-8 negative control cells show much weaker signals.</p> <p>D. Analysis by Fluorescence Plate Reader: For analysis using a fluorescence plate reader, resuspend cells in 100 µl Wash Buffer. Then, transfer the cell suspension to the respective 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.</p>	
Storage & Stability	Store kit at -20°C upon arrival. Store individual reagents as indicated on the respective labels.	

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