Green Caspase-2 Staining Kit

Instruction Manual

Catalog Number	PK-CA577-K182-25
Description	Activation of caspases plays a central role in apoptosis. The PromoKine Green 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. FITC-VDVAD-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 using a fluorescence plate reader.
Quantity	25 assays
Kit Components	Components – Quantity FITC-VDVAD-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-2 activation. 2. Aliquot 300 µl each of the induced and control cultures into eppendorf tubes. 3. Add 1 µl of FITC-VDVAD-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 the FITC filter. Caspase positive cells appear to have brighter green signals, whereas caspase negative control cells show much weaker signal. D. Analysis by Fluorescence Plate Reader: For analysis with a 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.

PromoCell GmbH
Sickingenstr. 63/65
69126 Heidelberg
Germany

North America 1 – 866 – 251 – 2860 (toll free)
Deutschland 0800 – 776 66 23 (gebührenfrei)
France 0800 90 93 32 (ligne verte)
United Kingdom 0800 – 96 03 33 (toll free)
Other Countries +49 6221 – 649 34 0

Email: info@promokine.info
www.promokine.info