CaspGLOW™ Green Active Caspase-3/7 Staining Kit

(Catalog# K2030-25, -100; Store at -20°C)

I. Introduction:

Activation of caspases plays a central role in apoptosis. The **CaspGLOW™ Active Caspase-3/7 Staining Kit** provides a convenient means for detecting activated caspase-3/7 in living cells. The assay utilizes the caspase inhibitor DEVD-FMK conjugated to FITC (Green-DEVD-FMK) as the fluorescent *in situ* marker. Green-DEVD-FMK is cell permeable, nontoxic, and irreversibly binds to the activated caspase-3/7 in apoptotic cells. The green fluorescence label allows the direct detection of activated caspase-3/7 in apoptotic cells by fluorescence microscopy, flow cytometry, or fluorescence plate reader. The caspase inhibitor, Z-VAD-FMK, is included in the kit as an additional control. It can be used to inhibit caspase activation in an induced culture.

II. Kit Contents:

Component	K2030-25 25 assays	K2030-100 100 assays	Cap Code	Part Number
Green-DEVD-FMK	25 µl	100 µl	Green	K2030-xx(x)-1
Wash Buffer	50 ml	2 x 100 ml	NM	K2030-xx(x)-2
Z-VAD-FMK	10 µl	10 µl	Amber	K2030-xx(x)-3

III. Caspase Assay Procedure:

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 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 the eppendorf tubes.
3. Add 1 µl of Green-DEVD-FMK into each tube and incubate for 0.5-1 hr at 37°C incubator with 5% CO_2.
4. Centrifuge cells at *300 x g* and RT for 5 min and remove the supernatant.
5. Resuspend cells in 0.5 ml of Wash Buffer and centrifuge at *300 x g* and RT for 5 min.
6. Repeat step 5 twice for a total of three washes.
7. Resuspend cells in Wash Buffer using the volume listed in the desired analysis method below.
8. Proceed to B, C, or D depending on the method of analysis.

B. Quantification by Flow Cytometry:

For Flow Cytometric analysis, resuspend cells in 300 µl of Wash buffer. Put samples on ice. Analyze samples by Flow Cytometry using the FL-1 channel.

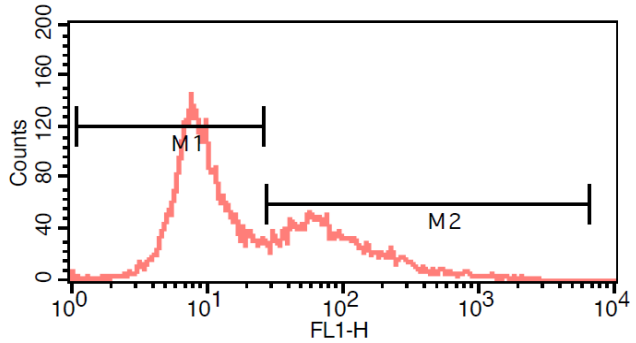
C. Detection by Fluorescence Microscopy:

For Fluorescence Microscopic analysis, resuspend cells in 100 µl Wash buffer. Put 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 a 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 to each well of the black microtiter plate. Measure the fluorescence intensity at Ex/Em = 488/530 nm. For control, use wells containing unlabeled cells.

Sample Flow Cytometry Histogram for Camptothecin treated Jurkat Cells:



Marker	Events	% Total	Mean
All	20000	100.00	53.89
M1	12529	62.64	9.62
M2	7363	36.81	129.66

Related Products:

- CaspGLOW™ Fluorescein Active Caspase Staining Kit (**Cat. No. K180-25, -100**)
- CaspGLOW™ Fluorescein Active Caspase-2 Staining Kit (**Cat. No. K182-25, -100**)
- CaspGLOW™ Fluorescein Active Caspase-3 Staining Kit (**Cat. No. K183-25, -100**)
- CaspGLOW™ Fluorescein Active Caspase-8 Staining Kit (**Cat. No. K188-25, -100**)
- CaspGLOW™ Fluorescein Active Caspase-9 Staining Kit (**Cat. No. K189-25, -100**)
- CaspGLOW™ Red Active Caspase Staining Kit (**Cat. No. K190-25, -100**)
- CaspGLOW™ Red Active Caspase-3 Staining Kit (**Cat. No. K193-25, -100**)
- CaspGLOW™ Red Active Caspase-8 Staining Kit (**Cat. No. K198-25, -100**)
- CaspGLOW™ Red Active Caspase-9 Staining Kit (**Cat. No. K199-25, -100**)

FOR RESEARCH USE ONLY! Not to be used on humans.

GENERAL TROUBLESHOOTING GUIDE FOR CaspGLOW BASED ASSAYS:

Problems	Cause(s)	Solution
High background	• Cell density is higher than recommended	• Refer to datasheet and use the suggested cell number
	• Cells were not washed well with wash buffer after staining	• Use the wash buffer provided, and as instructed in the datasheet
	• Cells were Incubated for extended period of time	• Refer to datasheets for proper incubation time
	• Use of extremely confluent cells	• Perform assay when cells are at 70-95% confluency
	• Cells were contaminated	• Check for bacteria/ yeast/ mycoplasma contamination
Lower signal level	• Cells did not initiate apoptosis	• Determine the optimal time and dose for apoptosis induction (time-course experiment)
	• Very few cells were used for analysis	• Refer to data sheet for appropriate cell number
	• Incorrect setting of the equipment or wavelength used to read samples	• Refer to datasheet and use the recommended filter setting
	• Use of expired kit or improperly stored reagents	• Always check the expiry date and store the components appropriately
Erratic results	• Old (unhealthy) cells used	• Seed healthy cells and make sure cells are healthy prior to induction of apoptosis
	• Adherent cells were dislodged and washed away prior to assaying	• Collect all cells (both attached and dislodged) after induction for accurate results
	• Incorrect incubation times or temperatures	• Refer to datasheet & verify correct incubation times and temperatures
	• Incorrect volumes used	• Use calibrated pipettes and aliquot correctly
Note: The most probable cause is listed under each section. Causes may overlap with other sections.		

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