CaspGLOW™ Red Active Caspase-9 Staining Kit

(Catalog# K199-25, -100; Store kit at –20° C)

I. Introduction:

Activation of caspases plays a central role in apoptosis. The **CaspGLOW™ Red Active Caspase-9 Staining Kit** provides a convenient means for detecting activated caspase-9 in living cells. The assay utilizes a caspase-9 inhibitor LEHD-FMK conjugated to sulfo-rhodamine (Red-LEHD-FMK) as the fluorescent marker. Red-LEHD-FMK is cell permeable, nontoxic, and irreversibly binds to activated caspase-9 in apoptotic cells. The fluorescence label allows detection of activated caspase-9 in apoptotic cells by fluorescence microscopy, flow cytometry, or fluorescence plate reader.

II. Kit Contents:

Component	K199-25 25 assays	K199-100 100 assays	Part Number
Red-LEHD-FMK	25 µl	100 µl	K199-xx(x)-1
Wash Buffer	50 ml	2 x 100 ml	K199-xx(x)-2
Z-VAD-FMK	10µl	10 µl	K199-xx(x)-3

III. Caspase-9 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 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 Red-LEHD-FMK into each tube and incubate for 0.5-1 hour at 37° C incubator with 5% CO_2.
4. Centrifuge cells at 3000 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. Put samples on ice. Analyzing samples by flow cytometry using the FL-2 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 rhodamine filter. Caspase-9 positive cells appear to have brighter red 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 to each well of the black microtiter plate. Measure the fluorescence intensity at Ex/Em = 540/570 nm (Note: Ex/Em=488/570 nm will also work, although it's not an optimal wavelength). For control, use wells containing unlabeled cells.

RELATED PRODUCTS:

- CaspGLOW Fluorescein Active Caspase Staining Kit (**Cat. No. K180-25, 100)**
- CaspGLOW Fluorescein Active Caspase-12 Staining Kit (**Cat. No. K172-25, 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)**

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

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GENERAL TROUBLESHOOTING GUIDE FOR CaspGLOW BASED ASSAYS:

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