

## 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:

Components	K199-25	K199-100
	25 assays	100 assays
Red-LEHD-FMK	25 µl	100 µl
Wash Buffer	50 ml	2 x 100 ml
Z-VAD-FMK	10 µl	10 µl

### III. Caspase-9 Assay Procedure:

#### A. Staining Procedure:

1. Induce apoptosis in cells ( $1 \times 10^6$ /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<sub>2</sub>.
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.

### IV. Related Products:

#### Apoptosis Detection Kits & Reagents

- Annexin V Kits & Bulk Reagents
- Caspase Assay Kits & Reagents
- Mitochondrial Apoptosis Kits & Reagents
- Nuclear Apoptosis Kits & Reagents
- Apoptosis Inducers and siRNA Vectors

#### Cell Fractionation System

- Mitochondria/Cytosol Fractionation Kit
- Nuclear/Cytosol Fractionation Kit
- Membrane Protein Extraction Kit
- Cytosol/Particulate Rapid Separation Kit
- Mammalian Cell Extraction Kit
- FractionPREP Fractionation System

#### Cell Proliferation & Senescence

- Quick Cell Proliferation Assay Kit
- Senescence Detection Kit
- High Throughput Apoptosis/Cell Viability Assay Kits
- LDH-Cytotoxicity Assay Kit
- Bioluminescence Cytotoxicity Assay Kit
- Live/Dead Cell Staining Kit

#### Cell Damage & Repair

- HDAC & HAT Fluorometric & Colorimetric Assays & Drug Discovery Kits
- DNA Damage Quantification Kit
- Glutathione & Nitric Oxide Fluorometric & Colorimetric Assay Kits

#### Signal Transduction

- cAMP & cGMP Assay Kits
- Akt & JNK Activity Assay Kits
- Beta-Secretase Activity Assay Kit

#### Adipocyte & Lipid Transfer

- Recombinant Adiponectin, Survivin, & Leptin
- CETP & PLTP Activity Assay & Drug Discovery Kits
- Total Cholesterol Quantification Kit

#### Molecular Biology & Reporter Assays

- siRNA Expression Vectors
- Cloning Insert Quick Screening Kit
- Mitochondrial & Genomic DNA Isolation Kits
- 5 Minutes DNA Ligation Kit
- 20 Minutes Gel Staining/Destaining Kit
- β-Galactosidase Staining Kit
- Luciferase Reporter Assay Kit

#### Growth Factors and Cytokines

#### Monoclonal and Polyclonal Antibodies