

## CaspSCREEN™ Flow Cytometric Apoptosis Detection Kit (Catalog #K200-25, -100; Store kit at -20°C)

### I. Introduction:

Activation of members of caspase-family proteases plays a key role in apoptosis. The CaspSCREEN™ Flow Cytometric Apoptosis Detection Kit provides a convenient means for detecting activation of caspases by flow cytometry in living cells. The assay is based on the cleavage of (aspartyl)<sub>2</sub>-Rhodamine 110 (D<sub>2</sub>R), a reported substrate for members of caspase family proteases. The caspase substrate D<sub>2</sub>R is non-fluorescent, however, upon cleavage of the substrate by cellular caspases, the released rhodamine 110 gives rise to fluorescence that can be measured at excitation of 488 nm and emission of 530 nm. As the D<sub>2</sub>R is more cell-permeable than other fluorometric caspase substrates, activation of caspases can easily be measured in intact cells by flow cytometry.

### II. Kit Contents:

Components	K200-25	K200-100
		25 assays
D <sub>2</sub> R Reagent	25 µl	100 µl
DTT (1 M)	75 µl	300 µl
D <sub>2</sub> R Incubation Buffer	7.5 ml	30 ml

### III. Assay Protocols:

#### General Considerations

- After thawing, store the Incubation Buffer at 4°C.
- Protect D<sub>2</sub>R reagent from light.

#### B. Assay Procedures

1. Induce apoptosis in cells by desired method. Concurrently incubate a control culture *without* induction.
2. Count cells and pellet 1 x 10<sup>5</sup> cells.
3. Resuspend cells in 0.3 ml of D<sub>2</sub>R Incubation Buffer.
3. Add 3 µl of the 1 M DTT (10 mM final concentration).
4. Add 1 µl of the D<sub>2</sub>R Reagent.
5. Incubate at 37°C for 10-20 min in the dark.
6. Analyzing cells by flow cytometry using FL-1 channel (Ex/Em = 488/530 nm).

### IV. Storage and Stability:

- Store kit at -20°C (Store the Incubation Buffer at 4°C after opening).
- All reagents are stable for 1 year under proper storage conditions.

### V. References:

1. Hug, H., *et al.* (1999) *Biochemistry* **38**:13906-13911.
2. Los, M., *et al.* (1999) *Methods in Mo. Biol.*, Vol. 12, Ch. 14. Human Press Inc., Totowa, NJ.

### VI. 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 Set
- Apoptosis 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