

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)₂-Rhodamine 110 (D₂R), a reported substrate for members of caspase family proteases. The caspase substrate D₂R 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 D₂R 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:

Component	K200-25	K200-100	Cap Code	Part Number
	25 assays	100 assays		
D ₂ R Reagent	25 µl	100 µl	Amber	K200-xx(x)-1
DTT (1 M)	125 µl	400 µl	Green	K200-xx(x)-2
D ₂ R Incubation Buffer	12.5 ml	50 ml	Wide Mouth	K200-xx(x)-3

III. Assay Protocols:

General Considerations:

- After thawing, store the Incubation Buffer at 4°C.
- Protect D₂R 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 10⁵ cells.
3. Resuspend cells in 0.5 ml of D₂R Incubation Buffer.
4. Add 4 µl of the 1 M DTT (8 mM final concentration).
5. Add 1 µl of the D₂R Reagent.
6. Incubate at 37°C for 10-20 min in the dark.
7. Analyze 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.

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

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

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- Senescence Detection Kit
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- LDH-Cytotoxicity Assay Kit
- Bioluminescence Cytotoxicity Assay Kit
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- CETP & PLTP Activity Assay & Drug Discovery Kits
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