



***CaspSCREEN™ FLOW  
CYTOMETRIC APOPTOSIS  
DETECTION KIT***

*Kit for 25 Assays*

***Catalog No. APT105***

**FOR RESEARCH USE ONLY  
Not for use in diagnostic procedures**

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## Application

Activation of ICE-family proteases/caspases initiates apoptosis in mammalian cells. The CaspSCREEN™ Flow Cytometric Apoptosis Detection Kit provides a simple and convenient means for detecting activation of caspases by flow cytometry in intact cells. The assay utilizes a substrate that contains two aspartate residues linked to rhodamine 110 (D<sub>2</sub>R), a reported substrate for members of caspase family proteases. The rhodamine 110 conjugated substrate is non-fluorescent, however, upon cleavage of the substrate by cellular caspases or apoptosis-related proteases, 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 substrate is more cell-permeable than other fluorometric caspase substrates, apoptosis can easily be measured in intact cells by flow cytometry.

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## Kit Components

1. **D<sub>2</sub>R** - 25 µL
2. **DTT (1 M)**: 75 µL
3. **Incubation Buffer**: 7.5 mL

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## Materials Not Supplied

- Microcentrifuge and 1.5 mL Microcentrifuge tubes
- 37°C water bath or incubator
- Flow Cytometer

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## Storage

We recommend storing reagents at -20°C upon arrival. (Store Incubation Buffer at 4°C after opening). The performance of this product is guaranteed for 1 year under proper storage conditions.

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## Preparation of Reagents

### General Considerations:

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

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## Assay Instructions

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 Incubation Buffer pre-warmed to 37°C.
4. Add 3 µL of the 1M DTT (10 mM final concentration)
5. Add 1 µL of the D<sub>2</sub>R reagent.
6. Incubate at 37°C for 10-20 minutes in the dark.
7. Analyze cells by flow cytometry using FITC channel (Ex = 488 nm; Em = 530 nm).

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## References

1. Hug, H. et al. (1999). *Biochemistry* **38**: 13906-13911.

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## **Warranty**

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