Introduction and Background

A. Overview

Activation of members of caspase-family proteases plays a key role in apoptosis. The 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 fluorometriccaspase substrates, activation of caspases can easily be measured in intact cells by flow cytometry.

B. Notice for Application of Kit

✓ This kit has been configured for research use only and is not for diagnostic and clinical use.

Material and Method

A. List of component

1. D_2R Reagent: 100 µl.
2. DTT (1 M): 300 µl.
3. D_2R Incubation Buffer: 30 ml.

B. Stability and storage

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

C. General Considerations

- After thawing, store the Incubation Buffer at 4℃.
- Protect D2R reagent from light.

D. Protocol

1. Induce apoptosis in cells by desired method. Concurrently incubate a control culture without induction.
2. Count cells and pellet 1 x 10^5 cells.
3. Resuspend cells in 0.3 ml of D2R Incubation Buffer.
4. Add 3 µl of the 1 M DTT (10 mM final concentration).
5. Add 1 µl of the D2R Reagent.
6. Incubate at 37℃ for 10-20 min in the dark.
7. Analyzing cells by flow cytometry using FL-1 channel (Ex/Em = 488/530 nm).