Flow Cytometric Apoptosis Detection Kit

(Catalog #BN00471 -25, -100 Store kit at –20°C)

FOR RESEARCH USE ONLY! Not to be used on humans.

I. Introduction:

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 (D_2R), a reported substrate for members of caspase family proteases. The caspase substrate D_2R 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_2R 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	BN00471 25 assays	BN00471 100 assays	Cap Code	Part Number
D_2R Reagent	25 µl	100 µl	Amber	BN00471-1
DTT (1 M)	125 µl	400 µl	Green	BN00471-2
D_2R Incubation Buffer	12.5 ml	50 ml	Wide Mouth	BN00471-3

III. Assay Protocols:

General Considerations:

- After thawing, store the Incubation Buffer at 4°C.
- Protect D_2R 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 10^5 cells.
3. Resuspend cells in 0.5 ml of D_2R Incubation Buffer.
4. Add 4 µl of the 1 M DTT (8 mM final concentration).
5. Add 1 µl of the D_2R 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.