TUNEL Assay Kit (Direct In Situ) (BN00655)

(Catalog BN00655; Store at –20°C)

I. Introduction:

Internucleosomal DNA fragmentation is a hallmark of apoptosis in mammalian cells. Assay Genie's **DNA Fragmentation Assay Kit** provides complete components including positive and negative control cells for conveniently detecting DNA fragmentation by fluorescence microscopy or flow cytometry. The TUNEL-based detection kit utilizes terminal deoxynucleotidyl transferase (TdT) to catalyze incorporation of fluorescein-12-dUTP at the free 3'-hydroxyl ends of the fragmented DNA. The fluorescein-labeled DNA can then be observed by fluorescence microscopy or analyzed by flow cytometry.

II. Kit Contents:

Component	Color Code	Volume	Store Temp.
Positive Control Cells	Brown	5 ml	-20° C
Negative Control Cells	natural	5 ml	-20° C
Wash Buffer	blue	100 ml	+4° C
Reaction Buffer	green	0.5	+4° C
TdT Enzymes	yellow	38 µl	-20° C
FITC-dUTP	orange	0.40 ml	-20° C
Rinse Buffer	red	100 ml	+4° C
PI/RNase Staining Buffer	amber	25 ml	+4° C

III. Storage Condition:

Kit components should be stored separately as indicated above. Shelf life is 1 year from the date of the product shipment, under proper storage conditions.

IV. Assay Protocol:

A. Cell Fixation

1. Induce apoptosis in cells by desired method. Concurrently incubate a control culture without induction.
2. Pellet 1-5 x 10^6 cells at 300 x g and resuspend in 0.5 ml of PBS.
3. Fix the cells by adding 5 ml of 1% (w/v) paraformaldehyde in PBS and place on ice for 15 minutes.
4. Centrifuge the cells for 5 min at 300 x g and discard the supernatant.
5. Wash the cells in 5 ml of PBS and pellet the cells by centrifugation. Repeat one time the wash and centrifugation step.
6. Resuspend the cells in 0.5 ml of PBS.
7. Add the cells to 5 ml of ice-cold 70% (v/v) ethanol. Let cells stand for a minimum of 30 min in ice or in the freezer.
8. Store the cells in 70% (v/v) ethanol at –20° C until use. Cells can be stored at –20° C for several days before use.

B. Apo-DIRECT Assay Protocol:

The procedures can be used for both control cells and your testing cells.

1. Resuspend the fixed cells by swirling the vials. Remove 1 ml aliquots of the cell suspension (~1 x 10^6 cells per ml) and place in 12 x 75 mm tubes. Centrifuge (300 x g) cells for 5 min and carefully remove the ethanol by aspiration.
2. Resuspend each tube of cells with 1 ml of **Wash Buffer** (blue cap). Centrifuge as before and remove supernatant carefully by aspiration.
3. Repeat one time the washing step (step 2).
4. Resuspend each tube of the cells in 50 µl of the **Staining Solution** prepared as below:

Staining Solution	1 assay	10 assays
TdT Reaction Buffer (green cap)	10 µl	100 µl
TdT Enzyme (yellow cap)	0.75 µl	7.5 µl
FITC-dUTP (orange cap)	8 µl	80 µl
ddH_20	32.25 µl	322.5 µl
Total Volume	51 µl	510 µl

5. Incubate the cells in the **Staining Solution** for 60 min at 37° C. Shake cells every 15 min to resuspend.
6. Add 1 ml of **Rinse Buffer** (red cap) to each tube and centrifuge (300 x g) for 5 min. Remove supernatant by aspiration.
7. Repeat the rinsing step (step 6).
8. Resuspend the cell pellet in 0.5 ml of **Propidium Iodide/RNase A Solution** (amber bottle).
9. Incubate the cells in the dark for 30 min at room temperature.
10. Analyze the cells by fluorescence microscopy (apoptotic cells show green staining over an orange-red PI counter-staining) or flow cytometry. Cells should be analyzed within 3 hours of staining.