Product Information

ApoDIRECT DNA Fragmentation Assay Kit

I. Kit Contents:

Components	K2071-50 50 assays	Cap Color	Store Temp.	Part Number
Positive Control Cells	5 ml	Brown	-20℃	K2071-C-1
Negative Control Cells	5 ml	Natural	-20℃	K2071-C-2
Wash Buffer	100 ml	Blue	+4℃	K2071-C-3
Reaction Buffer	0.5 ml	Green	+4℃	K2071-C-4
TdT Enzymes	38 µl	Yellow	-20℃	K2071-C-5
FITC-dUTP	0.40 ml	Orange	-20℃	K2071-C-6
Rinse Buffer	100 ml	Red	+4℃	K2071-C-7
PI/RNase Staining Buffer	25 ml	Amber bottle	+4℃	K2071-C-8

II. Introduction:

Internucleosomal DNA fragmentation is a sign of apoptosis in mammalian cells. The ApoDIRECT DNA Fragmentation Assay Kit provides a simple and convenient way for detection of DNA fragmentation in biological samples based on fluorescence microscopy or flow cytometry method. The kit provides complete components including positive and negative control cells. The TUNEL-based assay uses terminal deoxynucleotidyl transferase (TdT) to catalyze fluorescein-12-dUTP incorporate into the free 3'-hydroxyl ends of DNA fragmentation. The fluorescein-labeled DNA can then be easily analyzed by flow cytometry or observed by fluorescence microscopy.

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. Apo-DIRECT Tissue Section:

A. Cell Fixation

1. Induce apoptosis in cells by desired method. Concurrently incubate a control culture without induction.
2. Pellet 1-5 x 106 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℃ until use. Cells can be stored at –20℃ 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_2O	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℃. 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.

For research use only! Not to be used in humans.

Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

Tel: +1-(832)696-8203
Fax: +1-832-641-3177
Email: sales@apexbt.com