Product Information

ApoBrdU Red DNA Fragmentation Kit

I. Kit Contents:

Components	K2073-60 60 assays	Cap Color	Store Temp.	Part Number
Positive Control Cells	5 ml	Brown	-20℃	K2073-C-1
Negative Control Cells	5 ml	Natural	-20℃	K2073-C-2
Wash Buffer	120 ml	Blue	+4℃	K2073-C-3
Reaction Buffer	0.6 ml	Green	+4℃	K2073-C-4
TdT Enzymes	45 µl	Yellow	-20℃	K2073-C-5
Br-dUTP	0.48 ml	Violet	-20℃	K2073-C-6
Rinse Buffer	120 ml	Red	+4℃	K2073-C-7
Anti-BrdU-Red Antibody	0.3 ml	Orange	+4℃	K2073-C-8
7-AAD/RNase Staining Buffer	30 ml	Amber bottle	+4℃	K2073-C-9

II. Introduction:

Internucleosomal DNA fragmentation is a sign of apoptosis in mammalian cells. The Apo-BrdU Red DNA Fragmentation Kit provides a simple and convenient way for detection of DNA fragmentation in fixed cell preparations or tissue sections based on fluorescence microscopy or flow cytometry method. The kit provides complete components including positive and negative control cells. The assay uses Br-dUTP (bromolated deoxyuridine triphosphate nucleotides) which is more easily incorporated into DNA strand breaks than the larger ligands such as digoxigenin, fluorescein and biotin. The greater incorporation generates brighter signal and the Br-dUTP sites can be identified by a Red fluorescein labeled anti-BrdU monoclonal antibody. The assay is suited for studying apoptosis with GFP transfected cells.

III. Apobrud Assay Protocal For Cultured Cells:

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 and resuspend in 0.5 ml of PBS.
3. Fix the cells by adding 5 ml of 4% (w/v) formaldehyde 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 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 (or overnight if you prefer) on 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. Detection by Flow Cytometry:

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) for 5 min and carefully remove the ethanol by aspiration.
2. Resuspend the 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 in 50 µl of the DNA Labeling Solution prepared as below:

DNA Labeling Solution	1 assay	10 assays
TdT Reaction Buffer (green cap)	10 µl	100 µl
TdT Enzyme (yellow cap)	0.75 µl	7.5 µl
Br-dUTP (violet 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 DNA Labeling 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 for 5 min. Remove supernatant by aspiration.

7. Repeat one time the rinsing step (step 6).

8. Resuspend cells in 0.1 ml of the Antibody Solution prepared as below:

Antibody Solution	1 assay	10 assays
Anti-BrdU-FITC Antibody (orange cap)	5 µl	50 µl
Rinse Buffer (red cap)	95 µl	950 µl

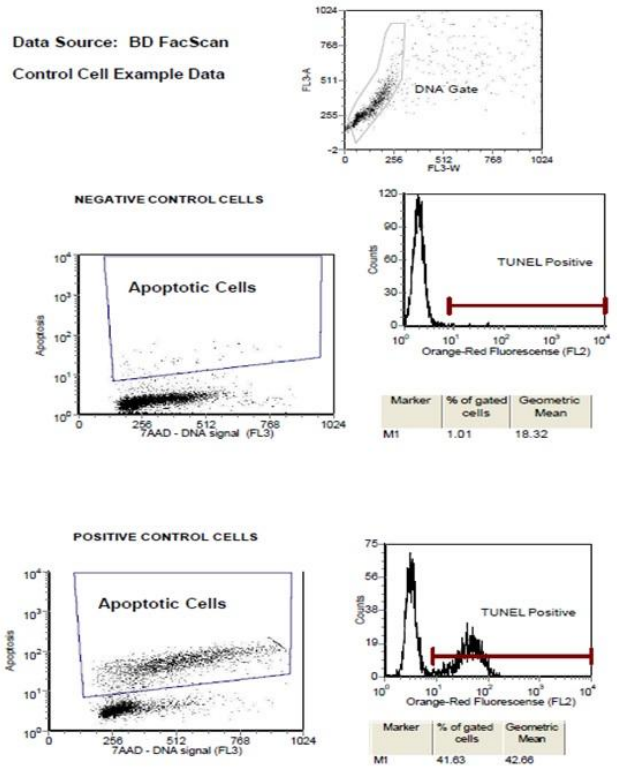
9. Incubate the cells with the Antibody Solution in the dark for 30 min at room temperature.

10. Add 0.5 ml of 7-AAD/RNase A Solution (amber bottle).

Note: If DNA cell cycle information is not necessary, add 0.5 ml PBS instead and continue with step 12.

11. Incubate the cells in the dark for 30 min at room temperature.

12. Analyze the cells by flow cytometry (Ex/Em = 488/576 nm for BrdU-Red and 488/655 nm for 7-AAD). Cells should be analyzed within 3 hours of staining.



Frequently Asked Questions

1. Can we use alcohol with GFP transfected cells? What alternative do we have?

Alcohol will kill GFP. Here is an alternative protocol for GFP transfected cells:1. Fix with 4% formaldehyde in PBS2. Use detergent instead of alcohol. A suggested detergent mixture is: a) Start with a PBS solution (pH 7.2 – 7.4)b) 0.1% triton X 100c) 4% FBS (fetal bovine serum) 3. Pellet the cells 4. Resuspend the cell (about 1 million cells) in 2 ml of the detergent solution from above. 5. Incubate 3 – 5 min. at RT 6. Spin at 300G for 10 min. 7. Add DNA labeling and continue from the instruction sheet.

2. Is it possible to use this kit for microscopy analysis and is there a protocol available for this analysis?

For microscopy, take the cells before you add 7-AAD and observe the under a fluorescence microscope at 488 nm. You see the green fluorescence on the broken strands.

3. Can we use this kit on GFP transfected cells?

Dealing with GFP transfected cells can be tricky because many treatments cause the loss of the GFP. In general you can follow this protocol: 1. The EtOH step can be left out. This is how we permeablilize the cells, so substituting tween 20, triton X-100 or even using a commercially available permeabilization buffer is acceptable. (I do not recommend any that are based upon Saponin, they usually do not yield reproducible results).2. Unfortunately, the paraformaldehyde step is a necessary step in the assay because it is used to cross-link the DNA fragments so that they do not leach out of the cells upon permeabilization. I have been told that the paraformaldehyde can "kill" the GFP signal. However, if it is done with 1% and for only 15 minutes, this can decrease the problem. Your customer may want to do a "trial" with just transfected cells and look at the GFP signal before and after varying times and percentages of paraformaldehyde to test the conditions.DNase should NOT be used because that will cause digestion of the DNA which can not be distinguished from the breaks caused by apoptosis

4. What type of cells were used for the controls?

They are HL60 cells.

5. Can you please give some general technical tips to perform this assay successfully?

1. For those researchers using adherent cell line systems, the cells in the supernatant have a higher probability of being apoptotic than do the adherent cells. Save cells in the supernatant for assay prior to trypsinization of the adherent cell layer.2. Cell fixation using a DNA crossing linking chemical fixative is an important step in analyzing apoptosis. Unfixed cells may lose smaller fragments of DNA that are not chemically fixed in place inside the cell during washing steps. The researcher may have to explore alternative fixation and permeablization methods to fully exploit their systems.3. A cytospin or centrifigal cytology slide can be prepared from APO-BRDUTM sample in the following manner. After completion of the Fluorescein~PRB-1 antibody staining , but prior to the Propidium Iodide/RNAse A treatment, put a drop of the stained cells on a slide, spin it and observe the sample under a fluorescence microscope.4. Surface marker staining of cellular antigens can be accomplished by first incubating the cells with the fluorescent labeled antibody and then using a comercially available fix and perm solution to rapidly fix and permeabilize the cells in preparation for the APO-BRDUTM Assay.5. To minimize cell loss during the assay, restrict the assay to the use of a single 12 X 75 mm test tube. If polystyrene plastic test tubes are used an electrostatic charge can build up on the sides of the tube. Cells will adhere to the side of the tube and the sequential use of multiple tubes can result in significant cell loss during the assay.6. Occasionally a mirror image population of cells at lower intensity is observed in the flow cytometry dual parameter display. This population arises because during the 50 µl DNA Labeling Reaction some cells have become stuck to the side of the test tube and are not fully exposed to the reaction solution. This phenomenon can be overcome by washing all the cells from side of the tube and making sure all cells are properly suspended at the beginning of the labeling reaction.7. If a low intensity of fluorescein staining is observed, try increasing the incubation time during the 50 µl DNA Labeling Reaction. Some researchers have found labeling

times of up to four hours at 37℃ may be required for certain cell systems.8. If the DNA cell cycle information is not required, it is not necessary to add the PI/RNase A solution to each tube.

6. What is the shelf life of this kit?

This kit is good for 12 months from the date of shipment in the unopened form when stored at the appropriate temperature and appropriate conditions. Please refer to the datasheet for storage information and shelf life of each of the components.

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.

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