APExBIO
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Product Information

Apo-BrdU DNA Fragmentation Assay Kit

I. Kit Contents:

Components	K2070-60 60 assays	Cap Color	Store Temp.	Part Number
Positive Control Cells	5 ml	Brown	-20℃	K2070-C-1
Negative Control Cells	5 ml	Natural	-20℃	K2070-C-2
Wash Buffer	120 ml	Blue	+4℃	K2070-C-3
Reaction Buffer	0.6 ml	Green	+4℃	K2070-C-4
TdT Enzymes	45 µl	Yellow	-20℃	K2070-C-5
Br-dUTP	0.48 ml	Violet	-20℃	K2070-C-6
Rinse Buffer	120 ml	Red	+4℃	K2070-C-7
Anti-BrdU-FITC Antibody	0.3 ml	Orange	+4℃	K2070-C-8
PI/RNase Staining Buffer	30 ml	Amber bottle	+4℃	K2070-C-9

II. Introduction:

Internucleosomal DNA fragmentation is a sign of apoptosis in mammalian cells. The BrdU In Situ 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 assay uses Br-dUTP (bromolated deoxyuridine triphosphate nucleotides) which is more easily incorporated into DNA strand breaks than other larger ligands such as digoxigenin, fluorescein and biotin. The greater incorporation generates brighter signal and the Br-dUTP sites can be identified by a fluorescein labeled anti-BrdU monoclonal antibody.

III. Assay Protocol 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 10^6 cells 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 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 and Fluorescence Microscopy:

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 Propidium Iodide/RNase A Solution (amber bottle).
11. Incubate the cells in the dark for 30 min at room temperature.
12. Analyze the cells by fluorescence microscopy using FITC and rhodamine filters (apoptotic cells show green staining over an orange-red PI counter-staining) or flow cytometry (Ex/Em = 488/520 nm for FITC, and 488/623 nm for PI). Cells should be analyzed within 3 hours of staining.

IV. Assay Protocol For Tissue Section:

A. Tissue Section Preparations:

The protocol describes the preparation of formalin-fixed, paraffin-embedded tissue section mounted on glass slides. For information on fixing and embedding techniques, see Ben-Sasson et al.,. Most steps are performed in Coplin Jars.

Note: If using fresh-frozen tissue sections, proceed directly to step 7.

1. Remove paraffin by immersing slides in a Coplin jar containing fresh xylene. Incubate at room temperature for 5 minutes.
2. Repeat in a second Coplin jar containing fresh xylene.
3. Immerse the slides in a Coplin Jar containing 100% ethanol and incubate at room temperature for 5 min.
4. Rehydrate the slides by sequential 3-min, room temperature incubations in Coplin jars containing:
   100% ethanol
   95% ethanol
   85% ethanol
   70% ethanol
   50% ethanol
5. Immerse the slides in a Coplin jar containing 0.85% NaCl and incubate at room temperature for 5 min.
6. Immerse the slides in a Coplin jar containing PBS and incubate at room temperature for 5 minutes.
7. Fix the slides by immersing them in a Coplin jar containing fresh 4% formaldehyde/PBS, and incubate at room temperature for 15 min.
8. Wash the slides by immersing them in a Coplin jar containing PBS, and incubate at room temp. for 5 min.
9. Transfer to another Coplin jar containing PBS, and incubate at room temperature for 5 min.
10. Allow the liquid to drain thoroughly and place slides on a flat surface.

11. Prepare 20 µg/ml of Proteinase K Solution (combine 2 µl of 10 mg/ml Protease K and 998 µl of 100 mM Tris-HCl, pH 8.0, 50 mM EDTA) and cover each section with 100 µl of it. Incubate at room temperature for 5 min.
12. Immerse the slides in Coplin jar containing PBS, and incubate at room temperature for 5 min.
13. Transfer the slides to a Coplin jar containing 4% formaldehyde/PBS and incubate at room temperature for 5 minutes.
14. Wash the slides by immersion in Coplin jar containing PBS, and incubate at room temperature for 5 min.

B. Detection by Fluorescence Microscopy:

1. Remove slides from PBS and tap gently to remove excess liquid. Cover the cells in 100 µl of Wash buffer (blue cap).
2. Use forceps, gently place a piece of plastic coverslip on top of the cells to evenly spread the liquid, incubate for 5 min. Remove plastic coverslip and gently tap the slides to remove excess liquid.
3. Repeat step 2. Carefully blot dry around the edges with tissue paper.
4. Gently place 50 µl of the DNA Labeling Solution (prepared as in Section IIIB, Step 4) on the cells.
5. Use forceps, gently place a piece of plastic coverslip on top of the cells to evenly spread the liquid.
6. Place the slides in a dark, humidified 37℃ incubator for 60 minutes.
   Note: Ensure high humidity by placing wet paper towels in the bottom of the dry incubator.
7. Using forceps, remove the plastic coverslips. Rinse the slides to a fresh Coplin jar filled with PBS for 5 min.
8. Repeat step 7. Carefully blot dry around the edges with tissue paper.
9. Place 100 µl of the Antibody Solution (Prepared as in Section IIIB, step 8).
10. Use forceps, gently place a piece of plastic coverslip on top of the cells to evenly spread the liquid.
11. Incubate the cells with the antibody solution in a humidified incubator for 30 min at room temperature.
12. Carefully remove the solution from slides. Add 100 µl of Propidium Iodide/Rnase A solution (amber bottle).
13. Use forceps, gently place a piece of plastic coverslip on top of the cells to evenly spread the liquid.
14. Incubate the slides in the dark in a humidified incubator for 30 min at room temperature.
15. Wash the cells by transferring the slides to a fresh Coplin jar filled with ddH2O and incubate at room temperature for 5 min.
16. Repeat Step 15.
17. [Optional] Add a drop of anti-Fade solution and cover the treated portion of the slide with a glass coverslip.
18. [Optional] Seal the edges of the coverslip with rubber cement or clear nail polish.
19. View slides as soon as possible using FITC and rhodamine filters. Apoptotic cells will exhibit strong nuclear green fluorescence. All cells should be stained with PI and exhibit strong red counter staining.

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Our promise

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