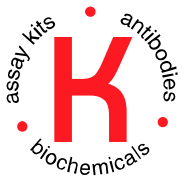

KAMIYA BIOMEDICAL COMPANY

Apo-BrdU *In Situ* DNA Fragmentation Assay

For conveniently detecting DNA fragmentation by fluorescence microscopy or flow cytometry

Cat. No. KT-216

For Research Use Only. Not for Use in Diagnostic Procedures.

PRODUCT INFORMATION

Apo-BrdU *In Situ* DNA Fragmentation Assay

Cat. No. KT-216

INTRODUCTION:

Internucleosomal DNA fragmentation is a hallmark of apoptosis in mammalian cells. The **K-ASSAY®** Apo-BrdU *In Situ* DNA Fragmentation Assay provides complete components, including positive and negative control cells, for conveniently detecting DNA fragmentation by fluorescence microscopy or flow cytometry. The kit utilizes Br-dUTP (bromolated deoxyuridine triphosphate nucleotides) which is more readily incorporated into DNA strand breaks than other larger ligands (e. g., fluorescein, biotin or digoxigenin). The greater incorporation gives rise to a brighter signal when the Br-dUTP sites are identified by a fluorescence labeled anti-BrdU monoclonal antibody. The assay is suitable for studying apoptosis with GFP transfected cells.

COMPONENTS

• Positive Control Cells	5 mL	(Brown cap)	store at -20°C
• Negative Control Cells	5 mL	(Natural cap)	store at -20°C
• Wash Buffer	120 mL	(Blue cap)	store at 4°C
• Reaction Buffer	0.6 mL	(Green cap)	store at 4°C
• TdT Enzymes	45 µL	(Yellow cap)	store at -20°C
• Br-dUTP	0.48 mL	(Violet cap)	store at -20°C
• Rinse Buffer	120 mL	(Red cap)	store at 4°C
• Anti-BrdU Antibody	0.3 mL	(Orange cap)	store at 4°C
• 7-AAD/RNase Staining Buffer	30 mL	(Amber cap)	store at 4°C

PROTOCOLS

Apo-BrdU 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 1-5X10^6 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 minutes at 300X g and discard the supernatant.
5. Wash cells in 5 mL of PBS and pellet the cells by centrifugation. Repeat the wash and centrifugation steps one more time.
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 minutes (or overnight if you prefer) on ice or in the freezer.
8. Cells can be stored in 70% (v/v) ethanol at -20°C for several days prior to use.

B. Detection by Flow Cytometry and Fluorescence Microscopy

The procedure can be used for both control cells and cells being tested,

1. Resuspend the fixed cells by swirling the vials. Remove 1 mL aliquots of the cell suspension (~1 X 10^6) cells and place in 12 X 75 mm tubes. Centrifuge (300X g) for 5 min. and carefully remove the ethanol by filtration.
2. Resuspend the cells with 1 mL of Wash Buffer (Blue cap). Centrifuge as before and remove supernatant carefully by aspiration.
3. Repeat Step 2 as before once.
4. Resuspend in 50 µL of the DNA Labeling Solution prepared below.

DNA Labeling Solution	1 assay	10 assay
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°C. 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 the rinsing step one time.
8. Resuspend the cells in 0.1 mL of the Antibody Solution prepared as below.

Antibody Solution	1 assay	10 assay
Anti-BrdU 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 (RT).
10. Add 0.5 mL of 7-AAD/RNase A Solution (Amber bottle).
11. Incubate the cells in the dark for 30 min. at RT.
12. Analyze the cells by fluorescence microscopy (apoptotic cells show bright red staining over blue counter staining) or flow cytometry. Cells should be analyzed within 3 hours of staining.

APO-BrdU Assay Protocol For Tissue Sections:

A. Tissue Section Preparations

The protocol describes the preparation of formalin-fixed, paraffin-embedded tissue sections mounted on glass slides. Most steps are performed in Coplin jars.

NOTE: If you are using fresh-frozen sections, proceed directly to Step 7.

1. Remove paraffin by immersing slides in a Coplin jar containing fresh xylene. Incubate at RT for 5 min.
2. Repeat wash in a second Coplin jar containing fresh xylene.
3. Immerse the slides in a Coplin jar containing 100% ethanol and incubate at RT for 5 min.
4. Rehydrate the slides by sequential 3 min. RT incubations in Coplin jars containing:

    a. 100% ethanol
    b. 95% ethanol
    c. 85% ethanol
    d. 70% ethanol
    e. 50% ethanol

5. Immerse the slides in a Coplin jar containing 0.85% NaCl and incubate at RT for 5 min.
6. Immerse the slides in a Coplin jar containing PBS and incubate at RT for 5 min.
7. Fix the slides by immersing them in a Coplin jar containing 4% formaldehyde/PBS and incubate at RT for 15 min.
8. Wash the slides by immersing them in a Coplin jar containing PBS and incubate at RT for 5 min.
9. Transfer to another Coplin jar containing PBS and incubate at RT for 5 min.
10. Allow the liquid to drain thoroughly and place slides on a flat surface.
11. Prepare 20 µg/mL of Protease 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 the solution. Incubate at RT for 5 min. (NOTE: Protease K is not provided)
12. Immerse the slides in a Coplin jar containing PBS and incubate at RT for 5 min.
13. Transfer the slides to a Coplin jar containing 4% formaldehyde/PBS and incubate at RT for 5 min.
14. Wash the slides by immersing them in a Coplin jar containing PBS and incubate at RT 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. Using forceps, gently place a piece of plastic coverslip on top of the cells to evenly spread out 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 described above) on the cells.
5. Using forceps, gently place a piece of plastic coverslip on top of the cells to evenly spread out the liquid.
6. Place the slides in a dark, humidified 37°C incubator for 60 min. Note: Ensure high humidity by placing wet paper towels in the bottom of a dry incubator.
7. Using forceps, remove the plastic coverslip. Rinse the slides in a fresh Coplin jar filled with PBS for 5 min.
8. Repeat the rinse in step 7. Carefully blot dry around the edges with tissue paper.
9. Place 100 µL of the Antibody solution on the cells (Prepared as described above).
10 Using forceps, gently place a piece of plastic coverslip on top of the cells to evenly spread out the liquid.
11 Incubate the cells with the antibody solution in a humidified incubator at RT for 30 min.
12. Carefully remove the solution from the slides. Add 100 µL of 7-AAD/RNase A solution (Amber bottle) on to the cells.
13. Using forceps, gently place a piece of plastic coverslip on top of the cells to evenly spread out the liquid.
14. Incubate the slides in the dark in a humidified incubator at RT for 30 min.
15. Wash the cells by transferring the slides to a new Coplin jar filled with ddH_2O and incubate at RH 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. Apoptotic cells will exhibit strong nuclear red fluorescence. All cells should be stained with 7-AAD and exhibit strong blue counter staining.

STORAGE

Note some components are stored at 4°C while some require -20°C. The kit is stable until the expiration date shown on the label when stored properly.

FOR RESEARCH USE ONLY

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