Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

For research use only

# Apo-BrdU-Red™ *In Situ* DNA Fragmentation Assay Kit

**(Catalog #K404-60; 60 assays; Store Components Separately-see below)**

## I. INTRODUCTION:

Internucleosomal DNA fragmentation is a hallmark of apoptosis in mammalian cells. BioVision's **Apo-BrdU-Red *In Situ* 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 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 brighter signal when the Br-dUTP sites are identified by a Red fluorescence labeled anti-BrdU monoclonal antibody. The assay is suitable for studying apoptosis with GFP transfected cells.

## II. KIT CONTENTS:

| Components | Cap Color | Volume | Store Temp. | Part# |
|---|---|---|---|---|
| Positive Control Cells | brown | 5 ml | -20˚C | K404-60-1 |
| Negative Control Cells | natural | 5 ml | -20˚C | K404-60-2 |
| Wash Buffer | blue | 120 ml | +4˚C | K404-60-3 |
| Reaction Buffer | green | 0.6 ml | +4˚C | K404-60-4 |
| TdT Enzymes | yellow | 45 µl | -20˚C | K404-60-5 |
| Br-dUTP | violet | 0.48 ml | -20˚C | K404-60-6 |
| Rinse Buffer | red | 120 ml | +4˚C | K404-60-7 |
| Anti-BrdU-Red Antibody | orange | 0.3 ml | +4˚C | K404-60-8 |
| 7-AAD/RNase Staining Buffer | amber bottle | 30 ml | +4˚C | K404-60-9 |

## III. APOBRDU 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 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˚C until use. Cells can be stored at –20˚C for several days before use.

### B. Detection by Flow Cytometry:

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

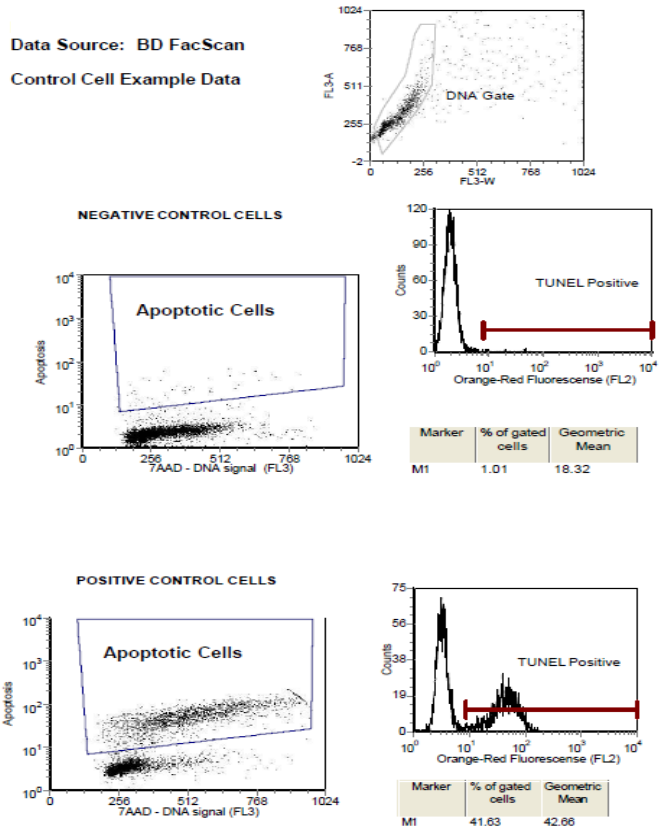
1. Resuspend the fixed cells by swirling the vials. Remove 1 ml aliquots of the cell suspension (~1 x $10^6$ cells per ml) and place in 12 x 75 mm tubes. Centrifuge (300 x g) for 5 min and carefully remove the ethanol by aspiration.
2. Resuspend the cells with 1 ml of **Wash Buffer** (blue cap). Centrifuge 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 |
| $ddH_20$ | 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 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-Red 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.




**RELATED PRODUCTS:**

Apoptosis Detection Kits & Reagents

- Annexin V Kits & Bulk Reagents
- Caspase Assay Kits & Reagents
- Mitochondrial Apoptosis Kits & Reagents
- Nuclear Apoptosis Kits & Reagents
- Apoptosis Inducers and Set
- Apoptotic Cell Isolation Kit
- Apoptosis siRNA Expression Vectors

Cell Proliferation & Senescence

- Quick Cell Proliferation Assay Kit
- Senescence Detection Kit
- LDH-Cytotoxicity Assay Kit
- Bioluminescence Cytotoxicity Assay Kit
- Live/Dead Cell Staining Kit

Metabolism Assays

- Ascorbic acid, Lactate, Pyruvate Assay Kits
- Glucose, sucrose, Galactose, Lactose, Maltose Assay Kits
- Choline, Ethanol, Uric acid, L-Amino acid assay Kits
- ADP/ATP, NAD/NADH, NADP/NADPH Quantitation Kits