Detection of BrdU Incorporation in DNA Synthesizing Cells
(From Becton-Dickinson, 5/2004)

**Equipment:**

- 12x75mm Tubes
- Centrifuge
- Pipettors and tips
- Vacuum aspirator

**Reagents:**

- Washing Solution: PBS containing 0.5% BSA
- Denaturing Solution: 2M HCl
- Dilution Buffer: PBS containing 0.5% Tween-20, 0.5% BSA
- 0.1M Sodium Borate (Na$_2$B$_4$O$_7$), pH 8.5
- 70% Ethanol (cold)
- Propidium Iodide (10µg/ml in PBS)

**Method:**

**Note:** Bromodeoxyuridine is a known carcinogen. Propidium iodine (PI) is also known to be toxic and carcinogenic.

1) Pulse actively growing cells in a tissue culture flask for one hour with 10 µM BrdU (Sigma, Cat. No. B5002).

2) Pour contents of tissue culture flask into a centrifuge tube. Centrifuge 10 minutes at 400g (all centrifugation steps are performed at 400g, at room temperature (RT)). Aspirate supernatant. Loosen pellet by tapping tube.

3) Resuspend cells in 1ml of tissue culture media and count cells. Centrifuge 5 minutes. Aspirate supernatant.

4) While vortexing, add ice cold 70% ethanol to cells, dropwise, to a final concentration of 1x10$^6$ cells/100µl. Incubate 20 minutes at RT.

5) Aliquot 100µl of ethanol fixed cells into each test tube (12x75mm). Wash with 1ml wash buffer. Centrifuge 5 minutes. Aspirate supernatant. Loosen pellet.

6) Resuspend pellet in denaturing solution. Mix well. Incubate 20 minutes at RT.
   - **Note:** Denaturing solution must be made fresh.

7) Add 1ml wash buffer. Mix well. Centrifuge 5 minutes. Aspirate supernatant.

8) Resuspend pellet in 0.5ml of 0.1M sodium borate (Na$_2$B$_4$O$_7$), pH 8.5, to neutralize any residual acid. Incubate 2 minutes at RT.

9) Add 1ml wash buffer. Mix well. Centrifuge 5 minutes. Aspirate supernatant.

10) Add primary antibody: Dilute anti-BrdU monoclonal antibody (Pharmingen #555627) in dilution buffer, such that 50 µl contains the optimal concentration. Resuspend cell pellet in 50µl of the diluted antibody. Incubate 20 minutes at RT.

   - **Note:** Skip step 11 when using FITC-conjugated anti-BrdU (Pharmingen # 556028)
12) Add secondary antibody: Dilute FITC-conjugated goat anti-mouse Ig (PharMingen #555988) in dilution buffer, such that 50 µl contains the optimal concentration. Resuspend cell pellet in 50µl of the diluted antibody. Incubate 20 minutes at RT.

13) Add 1ml wash buffer. Mix well. Centrifuge 5 minutes. Aspirate supernatant.

14) Resuspend pellet in 0.5ml propidium iodide (10µg/ml in PBS). Incubate 30 minutes at RT, protected from light.

15) Analyze the cells by flow cytometry.