Anti-BrdU Staining Protocols

Anti-BrdU Staining Using DNAse with Surface and Fluorescent Proteins

1. Pulse actively dividing cells with BrdU (*in vitro*, cell culture media can be pulsed by adding 10-40 µM of BrdU for 1-2 hours).
2. Harvest cells and centrifuge for 5 minutes at 1200-1500 rpm (200-300 x *g*).
3. Wash cells in Cell Staining Buffer (Cat. No. 420201) and centrifuge for 5 minutes at 1200-1500 rpm (200-300 x *g*). Discard supernatant.
4. Aliquot 5 x 10^5- 1 x 10^6 cells per 12 x 75 mm tube.
5. Optional: Stain cells for surface antigens if required, utilizing the Cell Surface Immunofluorescence Staining Protocol.
6. Wash cells by adding 1 ml of Cell Staining Buffer to each tube and centrifuging for 5 minutes at 1200-1500 rpm (200-300 x *g*). Discard supernatant.
7. Fix cells by adding 100 µl of 4% paraformaldehyde at room temperature for 20-30 minutes.
8. Wash cells by repeating step 6 twice (Optional: Cells can be stored in FACS buffer at 4°C for up to 72 hrs).
9. Permeabilize cells by adding 500 µl of 0.5% Triton-X 100 in PBS for 15 minutes at room temperature.
10. Wash cells by repeating step 6 twice.
11. Treat cells with 20 µg of DNAse (Cat. No. D4513, Sigma-Aldrich) diluted in DPBS with calcium and magnesium to each tube and incubate at 37°C for 1 hour.
12. Wash cells by repeating step 6 twice.
13. Add 50 µl of Cell Staining Buffer to each tube then add the recommended concentration of anti-BrdU antibody to each tube. Incubate for 20 minutes at room temperature in the dark.
14. Repeat step 6.
15. Stain DNA by adding 1 µg of either 7-AAD (Cat. No. 420403) or DAPI (Cat. No. 422801). Wait for 5 minutes prior to acquiring samples on flow cytometer.

Anti-BrdU Staining Using 70% Ethanol and 2N HCl

1. Pulse actively dividing cells with BrdU (*in vitro*, cell culture media can be pulsed by adding 10-40 µM of BrdU for 1-2 hours).
2. Harvest cells and centrifuge for 5 minutes at 1200-1500 rpm (200-300 x *g*).
3. Wash cells in 1x PBS (PBS, 10x Concentrate, Cat. No. 926201) and centrifuge for 5 minutes at 1200-1500 rpm (200-300 x *g*). Discard supernatant.
   NOTE: The combined presence of proteins and HCl in downstream steps may cause aggregation. As such, it is highly recommended that wash steps utilize PBS without any protein additive until otherwise indicated.

4. Dislodge cell pellet and add 5 ml of ice-cold (-20°C) 70% Ethanol to 1-2 X 10^7 cells dropwise while slowly vortexing. Incubate at -20°C for at least 2 hours. Cells may be stored for several days.
5. Repeat step 3 twice.
6. Dislodge cell pellet and add 2 ml of 2 N HCl and incubate for 20 minutes at room temperature.
7. Repeat step 3.
8. Dislodge cell pellet and add 2 ml of 0.1M $Na_2B_4O_7$ for 10 minutes at room temperature.
9. Repeat step 3.
10. Resuspend cells at a concentration of 1 x 10^7 cells per/ml of staining buffer and aliquot 100 µl per tube. Add anti-BrdU antibody at appropriate concentration and incubate for 20 minutes at room temperature.
11. Wash cells in Cell Staining Buffer (Cat. No. 420201) and centrifuge for 5 minutes at 1200-1500 rpm (200-300 x *g*).
12. Stain DNA by adding 1 µg of either 7-AAD (Cat. No. 420403) or DAPI (Cat. No. 422801). Wait for 5 minutes prior to acquiring samples on flow cytometer.