Anti-BrdU Staining Using 70% Ethanol and 2N HCl V.4

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Abstract

Note: We offer two protocols here depending on what your experiment requires. Ethanol treatment is usually harsher toward any other fluors or fluorescent proteins that may be present in your sample. As such, the DNAse method may be gentler under those conditions

Materials

MATERIALS

- PBS 10x Concentrate (Previously Covance catalog# SIG-31020) **BioLegend Catalog #**926201
- 7-AAD Viability Staining Solution **BioLegend Catalog #**420403, 420404
- Cell Staining Buffer **BioLegend Catalog #**420201
- DAPI (46-Diamidino-2-Phenylindole Dilactate) **BioLegend Catalog #**422801

Troubleshooting

1 Pulse actively dividing cells with BrdU (in vitro, cell culture media can be pulsed by adding 10-40µM of BrdU for 1-2 hours).

2 Harvest cells and centrifuge for 5 minutes at 1200-1500 rpm (200-300xg).

3 Wash cells in 1x PBS (PBS, 10x Concentrate, Cat. No. **926201**) and centrifuge for 5 minutes at 1200-1500 rpm (200-300xg). 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 5ml 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.
Note: For ethanol permeabilization, we recommend this step last for at least 2 hours as this improves permeabilization and overall staining. If you're still having difficulty, you can attempt to extend this to an overnight incubation (assuming you aren't also staining for cell surface markers that could be lost).

5 Repeat step 3 twice.

6 Dislodge cell pellet and add 2ml of 2 N HCl and incubate for 20 minutes at room temperature.

7 Repeat step 3.

8 Dislodge cell pellet and add 2ml 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 × 10^7 cells per/ml of staining buffer and aliquot 100µl 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 (200-300xg).

12 Stain DNA by adding 1µg of either 7-AAD (Cat. No. **420403**) or DAPI (Cat. No. **422801**). Wait for 5 minutes prior to acquiring samples on flow cytometer.Note: Adding a 7-AAD or DAPI stain allows you to analyze total DNA content and provides the characteristic horseshoe flow cytometric staining pattern when compared against BrdU. This helps identify the different phases of the cell cycle.