



🔒 Anti-BrdU Staining Using 70% Ethanol and 2N HCl V.3

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Abstract


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

Troubleshooting




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

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

 00:05:00


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

 00:05:00


4 Dislodge cell pellet and add 5 ml of ice-cold (-20°C) 70% Ethanol to $1-2 \times 10^7$ 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).

 02:00:00


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

 00:20:00

7 Repeat step 3.

8 Dislodge cell pellet and add 2ml of 0.1M $\text{Na}_2\text{B}_4\text{O}_7$ for 10 minutes at room temperature.


 00:10:00

9 Repeat step 3.


10 Resuspend cells at a concentration of 1×10^7 cells per/ml of staining buffer and aliquot 100 μ l per tube. Add anti-BrdU antibody at appropriate concentration and incubate for 20



minutes at room temperature.

 00:20:00

- 11 Wash cells in Cell Staining Buffer (Cat. No. **420201**) and centrifuge for 5 minutes at 1200-1500 rpm (200-300xg).

 00:05:00

- 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.