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

Kelsey Miller

Abstract

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

Citation: Kelsey Miller Anti-BrdU Staining Using 70% Ethanol and 2N HCl. **protocols.io**
dx.doi.org/10.17504/protocols.io.e2pbgdn
Published: 03 Jun 2016

Protocol

Step 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).

Step 2.

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

DURATION
00:05:00

Step 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

DURATION
00:05:00

Step 4.

Dislodge cell pellet and add 5 ml of ice-cold (-20^{0}C) 70% Ethanol to 1-2 X 107 cells dropwise while slowly vortexing. Incubate at -20^{0}C for at least 2 hours. Cells may be stored for several days.

DURATION
02:00:00

Step 5.

Repeat step 3 twice

Step 6.

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

DURATION
00:20:00

Step 7.

Repeat step 3.

Step 8.

Resuspend cells at a concentration of 1 x 107 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

DURATION
00:20:00

Step 9.

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

DURATION
00:05:00

Step 10.

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

DURATION
00:05:00