

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

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## Abstract

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

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## Protocol

### Step 1.

Pulse actively dividing cells with BrdU (in vitro, cell culture media can be pulsed by adding 10-40  $\mu$ M 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°C) 70% Ethanol to 1-2 X 10<sup>7</sup> cells dropwise while slowly vortexing. Incubate at -20°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  $1 \times 10^7$  cells per/ml of staining buffer and aliquot 100  $\mu$ l 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  $\times$  g).

 DURATION

00:05:00

### Step 10.

Stain DNA by adding 1  $\mu$ g 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