Anti-BrdU Staining Protocols Using DNAse with Surface and Fluorescent Proteins

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Abstract

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

DURATION
00:05:00

Step 4.

Aliquot 5 x 105- 1 x 106 cells per 12 x 75 mm tube

Step 5.

Optional: Stain cells for surface antigens if required, utilizing the Cell Surface Immunofluorescence Staining Protocol (see link)

LINK:
http://www.biolegend.com/media_assets/support_protocol/BioLegend_Surface_Staining_Flow_Protocol_060215.pdf

Step 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

DURATION
00:05:00

Step 7.

Fix cells by adding 100 µl of 4% paraformaldehyde at room temperature for 20-30 minutes

DURATION
00:30:00

Step 8.

Wash cells by repeating step 6 twice.

(Optional: Cells can be stored in FACS buffer at 4^{0}C for up to 72 hrs).

Step 9.

Permeabilize cells by adding 500 µl of 0.5% Triton-X 100 in PBS for 15 minutes at room temperature

DURATION
00:15:00

Step 10.

Wash cells by repeating step 6 twice

Step 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^{0}C for 1 hour

DURATION
01:00:00

Step 12.

Wash cells by repeating step 6 twice

Step 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

DURATION

00:20:00

Step 14.

Repeat step 6.

Step 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