Anti-BrdU Staining Protocols Using DNAse with Surface and Fluorescent Proteins V.1

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Kelsey Miller[1]

[1]BioLegend

BioLegend
Tech. support email: tech@biolegend.com

Sam Li
BioLegend

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External link: http://www.biolegend.com/media_assets/support_protocol/BrdU_Staining_Protocols_05102016.pdf

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Abstract

Anti-BrdU Staining Protocol using DNAse with surface and fluorescent proteins

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).

2 Harvest cells and centrifuge for 5 minutes at 1200-1500 rpm (200-300 x g)
00:05:00

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
00:05:00

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

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

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

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
00:05:00

7 Fix cells by adding 100 µl of 4% paraformaldehyde at room temperature for 20-30 minutes
00:30:00

8 Wash cells by repeating step 6 twice.

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

9 Permeabilize cells by adding 500 µl of 0.5% Triton-X 100 in PBS for 15 minutes at room temperature
00:15:00

10 Wash cells by repeating step 6 twice

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°C for 1 hour
01:00:00

12 Wash cells by repeating step 6 twice

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

00:20:00

14 Repeat step 6.

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