



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

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DOI

<https://dx.doi.org/10.17504/protocols.io.e2nbgde>

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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



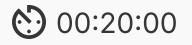
- 1 Pulse actively dividing cells with BrdU (in vitro, cell culture media can be pulsed by adding 10-40 μ M of BrdU for 1-2 hours).
- 2 Harvest cells and centrifuge for 5 minutes at 1200-1500 rpm (200-300 x g)
 00:05:00
- 3 Wash cells in Cell Staining Buffer (Cat. No. 420201) and centrifuge for 5 minutes at 1200-1500 rpm (200-300 x g). Discard supernatant
 00:05:00
- 4 Aliquot 5×10^5 - 1×10^6 cells per 12 x 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 μ l 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 μ l 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 μ g 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 μ l 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



- 14 Repeat step 6.
- 15 Stain DNA by adding 1 μ g of either 7-AAD (Cat. No. 420403) or DAPI (Cat. No. 422801). Wait for 5 minutes prior to acquiring samples on flow cytometer