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Anti-BrdU Staining Protocols Using DNAse with Surface and Fluorescent Proteins V.3

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[1]BioLegend

1 Works for me dx.doi.org/10.17504/protocols.io.bac9iaz6

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EXTERNAL LINK

https://www.biolegend.com/protocols/anti-brdu-staining-using-dnase-with-surface-and-fluorescent-proteins/4241/

GUIDELINES

Note: We offer two protocols here depending on what your experiment requires. Ethanol treatment is usually harsher toward any other fluors or fluorescent proteins that may be present in your sample. As such, the DNAse method may be gentler under those conditions.

MATERIALS

NAME	CATALOG #	VENDOR
Cell Staining Buffer	420201	BioLegend
7-AAD Viability Staining Solution	420403, 420404	BioLegend
DAPI (46-Diamidino-2-Phenylindole Dilactate)	422801	BioLegend

1 Pulse actively dividing cells with BrdU (*in vitro*, cell culture media can be pulsed by adding 10-40µM of BrdU for 1-2 hours).

2 Harvest cells and centrifuge for 5 minutes at 1200-1500 rpm (200-300xg).

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

4 Aliquot 5 x 10^5-1 x 10^6 cells per 12 x 75mm tube

5 Optional: Stain cells for surface antigens if required, utilizing the Cell Surface Immunofluorescence Staining Protocol.

6 Wash cells by adding 1ml of Cell Staining Buffer to each tube and centrifuging for 5 minutes at 1200-1500 rpm (200-300xg). Discard supernatant.

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

8 Wash cells by repeating step 6 twiceOptional: Cells can be stored in FACS buffer at 4°C for up to 72 hrs

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

10 Wash cells by repeating step 6 twice.

11 Treat cells with 20µg 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.

12 Wash cells by repeating step 6 twice.

13 Add 50µl 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 1µg of either 7-AAD (Cat. No. 420403) or DAPI (Cat. No. 422801). Wait for 5 minutes prior to acquiring samples on flow cytometer. Note: Adding a 7-AAD or DAPI stain allows you to analyze total DNA content and provides the characteristic horseshoe flow cytometric staining pattern when compared against BrdU. This helps identify the different phases of the cell cycle.