BrdU Staining of Cells with Anti-BrdU Antibody (Clone Bu20a) by Flow Cytometry

Protocol

FC19

For use with flow cytometry tested Mouse Anti-BrdU Antibody, clone Bu20a. This method provides a general procedure for use with the majority of Bio-Rad reagents. In some cases specific recommendations are provided on product datasheets, and these methods should always be used in conjunction with product and batch specific information provided with each vial. Note that a certain level of technical skill and immunological knowledge is required for the successful design and implementation of these techniques - these are guidelines only and may need to be adjusted for particular applications.

Reagents

- DNA stains/cell viability dyes, such as propidium iodide
- 2 M hydrochloric acid (HCl) containing 0.5% Triton X-100
- Mouse Anti-BrdU Antibody (clone Bu20a, catalog #MCA2483)
- 0.1 M $Na_2B_4O_7$, pH 8.5
- PBS
- PBS containing 1% bovine serum albumin (PBS/BSA)
- 0.05% (v/v) Tween 20 in PBS

Method

1. Label cells with BrdU. Add BrdU to the cell suspension in culture medium to a final concentration of 10 µM and incubate for 30 min in a CO_2 incubator at 37°C.
2. Wash cells 2 times (2x) with PBS/BSA, centrifuge cells at 500 x g for 10 min, decant supernatant, and resuspend in a minimum volume of PBS.
3. Add cells slowly into 5 ml 70% ethanol chilled to –20°C, mix continuously by vortexing.
4. Incubate on ice for 30 min.
5. Centrifuge at 500 x g for 10 min, and decant supernatant.
6. Add 2 ml of 2 M HCl containing 0.5% Triton X-100, and incubate the cells for 30 min at RT on a rocking platform set to 15 rpm. Denaturation of the DNA by HCl is critical for successful BrdU staining. Centrifuge at 500 x g for 10 min, decant supernatant, and resuspend in 1 ml of 0.1 M $Na_2B_4O_7$, pH 8.5.
7. Centrifuge at 500 x g for 10 min, decant supernatant, and resuspend the cells in PBS/BSA plus 0.05% Tween 20 to a concentration of 1×10^7 cells/ml.
8. Aliquot 100 µl of the cell suspension into the required number of FACS tubes.
9. Incubate cells with Mouse Anti-BrdU Antibody (clone Bu20a) at the recommended dilution for 1 hr at RT. Alternatively, incubation can be performed overnight at 4°C.
10. To wash, add 2 ml of PBS/BSA and centrifuge the cells at 500 x g for 5 min.
    (Optional) When using an unconjugated Bu20a antibody format perform incubation with a secondary antibody. For this purpose decant the supernatant and perform secondary antibody incubation for 45 min at RT (refer to the respective datasheet about the recommended dilution). Wash cells by repeating step 10.
11. Decant the supernatant and add 0.5 ml of sheath fluid.
12. For total DNA staining, add DNA stains such as ReadiDrop™ Propidium Iodide (#1351101), ReadiDrop 7-AAD (#1351102), or PureBlu™ Hoechst 33342 (#1351304), to each tube.
13. Analyze cells by flow cytometry following the manufacturer's instructions. The DNA stain should be read on the appropriate channel set to the height and linear scale and not log scale.

Notes

The acid treatment to unwind the DNA may affect surface immunophenotyping. Staining of cells with BrdU using DNAse I may be applicable if this is required.

- Appropriate controls should be carried out for flow cytometry, consider including the following:
  - A known positive sample
  - Isotype controls (to determine if the staining is specific)
  - Unstained cells (should always be included to monitor autofluorescence)
- For all multicolor flow cytometry experiments include compensation controls and fluorescence minus one (FMO) controls, which assist with identifying gating boundaries.

Visit **bio-rad-antibodies.com/applications** for more information about flow cytometry.

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