BrdU Staining of Cells for Cell Cycle Analysis and Apoptosis

Protocol

FC14

BrdU is an analog of thymidine readily incorporated into DNA during DNA synthesis. It provides an accurate method of monitoring proliferation and apoptosis. The Mouse Anti-BrdU Antibody, clone Bu20a, is suitable for flow cytometry. The following methods were used and provide a useful guide for using anti-BrdU antibodies.

Reagents:

- 0.05% (v/v) Tween 20 in phosphate buffered saline (PBS)
- 0.1 M $Na_2B_4O_7$, pH 8.5
- 2 M HCl containing 0.5% Triton X-100
- PBS
- PBS containing 1% bovine serum albumin (PBS/BSA)
- Propidium iodide

Method:

1. Add BrdU to the cell suspension in culture medium to a final concentration of 10 µM and incubate for at least 30 min at 37°C in a CO_2 incubator.
2. Wash cells twice with PBS/BSA, at 500 x g for 10 min at RT, decant supernatant.
3. Resuspend in 2-5 ml cold (4°C) 70% ethanol. Add dropwise to cell pellet while vortexing. Fix for at least 30 min on ice.
4. Centrifuge at 500 x g for 10 min, decant supernatant.
5. Resuspend the pellet in 2 ml of 2 M HCl containing 0.5% Triton X-100. Incubate for 30 min at RT (preferably on a rocking platform).
6. Centrifuge at 500 x g for 10 min, decant supernatant. Resuspend in 3 ml of 0.1 M $Na_2B_4O_7$, pH 8.5 for 2 min at RT.
7. Centrifuge at 500 x g for 10 min, decant supernatant, and resuspend in RT PBS/BSA + 0.05% Tween 20. Adjust cell concentration to 1 × 10^7 cells/ml.
8. Aliquot 100 µl of the cell suspension into required number of FACS tubes.
9. Incubate with antibody at the recommended vendor dilution overnight at 4°C avoiding direct light.
10. Resuspend in 2 ml of RT PBS/BSA. Centrifuge at 500 x g for 10 min at RT.
11. If a secondary antibody is required, then decant the supernatant, add 100 µl of PBS/BSA and incubate with the secondary antibody at the vendor recommended dilution for at least 30 min at 4°C.
12. Wash with 2 ml of PBS/BSA, centrifuge at 500 x g for 10 min.
13. Re-suspend cells in 1 ml of PBS. Add propidium iodide e.g. 1-2 drops of ReadiDrop™ Propidium Iodide (cat. #1351101).
14. Analyze by flow cytometry. The propidium iodide should be read on the appropriate channel in the linear scale. Doublets should be gated out using the Area vs Height or Width depending on your instrument.

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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