BrdU Staining of Cells for Cell Cycle Analysis and Apoptosis

Protocol

FC14

BrdU is an analogue of thymidine readily incorporated into DNA during DNA synthesis and is an accurate method to monitor proliferation and apoptosis. The anti-BrdU antibody mouse monoclonal MCA2483 (clone Bu20a) and rat monoclonal MCA2060 (clone BU1/75 (ICR1)) are suitable for flow cytometry. The following methods were used and provide a useful guide for using anti-BrdU antibodies.

Reagents:

- Phosphate buffered saline (PBS) (BUF036A) containing 1% bovine serum albumin (PBS/BSA).
- PBS
- 2 M HCl containing 0.5% Triton X-100
- 0.05% (v/v) Tween -20 in PBS
- Propidium iodide
- 0.1 M $Na_2B_4O_7$, pH 8.5

Method:

1. Add BrdU to the cell suspension in culture medium to a final concentration of 10 µM and incubate for at least 30 minutes at 37°C in a CO_2 incubator.
2. Wash cells twice with PBS/BSA, at 500 x g for 10 minutes at room temperature, decant supernatant.
3. Resuspend in 2-5 ml cold (4°C) 70% ethanol. Add drop wise to cell pellet while vortexing. Fix for at least 30 minutes on ice.
4. Centrifuge at 500 x g for 10 minutes, decant supernatant.
5. Resuspend the pellet in 2 ml of 2 M HCl containing 0.5% Triton X-100. Incubate for 30 minutes at room temperature (preferably on a rocking platform).
6. Centrifuge at 500 x g for 10 minutes, decant supernatant. Resuspend in 3 ml of 0.1 M $Na_2B_4O_7$, pH 8.5 for 2 minutes at room temperature.
7. Centrifuge at 500 x g for 10 minutes, decant supernatant, and resuspend in room temperature 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 room temperature PBS/BSA. Centrifuge at 500 x g for 10 minutes at room temperature.
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 minutes at 4°C.
12. Wash with 2 ml of PBS/BSA, centrifuge at 500 x g for 10 minutes.
13. Re-suspend cells in 1 ml of PBS. Add propidium iodide e.g. 1-2 drops of ReadiDrop™ propidium iodide (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 DNAsel may be applicable if this is required.

- Appropriate controls should always be carried out, for flow cytometry the following should be considered for inclusion;
  - A known positive sample
  - Isotype controls used to determine if the staining is specific
  - Unstained cells should always be included in the experimental set-up to monitor autofluorescence.
- For all multi-color flow cytometry experiments it is advisable to include compensation controls and Fluorescence Minus One (FMO) controls, which assist with identifying gating boundaries.

For more information on flow cytometry resources visit **bio-rad-antibodies.com/applications**

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