Protocol

BrdU Staining of Cells with Anti-BrdU Antibody

Flow Cytometry

For use with flow cytometry tested Anti-BrdU Antibody (clone RF04-2 (MCA6143), clone AbD33758kg (HCA320), clone AbD33761kd (HCA323), AbD33761kg (HCA321), and AbD33758kd (HCA322)). 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

- Anti-BrdU Antibody
- 100 mM BrdU stock solution
- FACS buffer
- 2M hydrochloric acid (HCl)
- Leucoperm Reagent (Cat. #BUF09B)
- ReadiDrop Propidium Iodide (#1351101)
- Secondary Antibody

Method

1. Seed Jurkat cells at a density of 2 x 10^6 cells/well in RPIM-1640 medium containing 10% FBS into a 6-well plate.
2. Add BrdU stock solution to each well of the 6-well plate so that you have a final concentration of 100 µM BrdU (for instance for 3 ml of media, add 3 µl of the 100 mM stock solution of BrdU). Incubate for 1 hr at 37°C.
3. Collect the treated cells in a 15 ml falcon tube. Wash cells twice with 15 ml FACS buffer, centrifuging the cells at 1,300 rpm for 5 min at room temperature (RT) between each wash and decanting the supernatant.
4. Resuspend the cells in 100 µl Leucoperm Reagent A (#BUF09B). Incubate for 15 min at RT.
5. Wash cells twice with 15 ml FACS buffer, centrifuging the cells at 1700 rpm for 5 min at RT between each wash, and decant the supernatant.
6. Resuspend the cells in 100 µl Leucoperm Reagent B (BUF09B). Incubate for 5 min at RT.
7. Wash cells twice with 1 ml FACS buffer, centrifuging the cells at 1700 rpm for 5 min at RT between each wash, and decant the supernatant..
8. Resuspend the pellet in 0.5 ml of 2 M HCl. Incubate for 30 min at RT (preferably on a rocking platform).
9. Wash cells twice with 1 ml FACS buffer, centrifuging the cells at 1,700 rpm for 5 min at RT between each wash, and decant the supernatant.
10. Resuspend the pellet in FACS buffer to a final density of 5 × 10^6 cells/ml.
11. Add 100 µl of cell suspension to a 5 ml FACS tube. Centrifuge the cells, discard the supernatant, and add the anti-BrdU antibody of choice at the required dilution. Incubate for 1 hr at RT or 4°C overnight, avoiding direct light.
12. Wash cells with 1 ml FACS buffer, centrifuging the cells at 1,500 rpm for 5 min at RT between each wash, and decant the supernatant.
13. Incubate with a secondary antibody for 30 min at RT, avoiding direct light.
14. Wash cells twice with 1 ml FACS buffer, centrifuging the cells at 1,500 rpm for 5 min at RT between each wash and decanting the supernatant.
15. Prepare FACS buffer with ReadiDrop Propidium Iodide (#1351101) by adding 1 drop of ReadiDrop Propidium Iodide per 500 µl of FACS buffer.
16. Add 200 µl of FACS buffer with ReadiDrop Propidium Iodide to the cells. Analyze by flow cytometry.

Notes

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.

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