Direct Staining of Intracellular CD68: Leucoperm Accessory Reagent Method

Protocol

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Method for cell permeabilization required prior to intracellular staining using Leucoperm Accessory Reagent. The method described below produces excellent results in our labs; however, other permeabilization techniques have been published, and may also be used successfully for this application. These methods should always be used in conjunction with the product and batch specific information provided with each vial. 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

- Ammonium Chloride Red Blood Cell Lysis Solution
- Anticoagulant (**Note:** for basic staining any appropriate anticoagulant, such as heparin, EDTA, or acid citrate dextrose, may be used. In some instances specific anticoagulants may be required)
- Leucoperm Accessory Reagent (Cat. #BUF09). Includes Reagent A (cell fixation agent) and Reagent B (cell permeabilization agent). **Note:** Leucoperm Reagent B containing 10% human serum (900 µl Reagent B: 100 µl human serum)
- PBS
- PBS (#BUF036A) containing 1% bovine serum albumin (PBS/BSA)
- Serum: match to target cell type
  - Mouse Serum (#C11SA)
  - Rat Serum (#C13SC)
  - Human Serum
- 10% serum (diluted in PBS)
- VivaFix Cell Viability Assay (#1351111-8)
- Optional: 0.5% (w/v) paraformaldehyde in PBS (**Note:** dissolve on heated stirrer and cool before use)

Method

1. Lyse 10 ml blood by incubating in Ammonium Chloride Red Blood Cell Lysis Solution (or other appropriate lysis buffer) for a maximum of 10 min on a rocker at RT. Wash blood 2 times (2x) in 20-50 ml RT PBS 1% BSA. (Alternatively harvest cells from alternate source and adjust to 1x 10^7 cells/ml in PBS 1% BSA after red blood cell lysis).
2. Re-suspend blood in 10 ml of PBS 1% BSA, and set aside 100 µl per control tube, which lacks VivaFix Assay.
3. The remaining volume is washed 1x in 20-50 ml PBS.
4. Re-suspend cells in 10 ml PBS and incubate for 30 min with VivaFix Assay at the recommended dilution (1/500 dilution of the stock solution) avoiding direct light.
5. Wash cells 2x in 20-50 ml PBS 1% BSA.
6. Re-suspend in 10 ml 10% serum and incubate for 15 min before adding 100 µl to each test tube.
7. If required, perform staining of cell surface antigens using appropriate directly conjugated monoclonal antibodies at 4°C for at least 30 min, avoiding direct light.
8. Wash cells 1x in 2 ml PBS 1% BSA.
9. Optional step: as a control for surface staining, unpermeabilized samples can be re-suspended in 100 µl 10% serum until stained with CD68.
10. Re-suspend cells in 100 µl PBS 1% BSA.
11. Add 100 µl of Leucoperm Reagent A. Incubate for 15 min at RT.
12. Wash cells 1x in 2 ml PBS 1% BSA.
13. Add 100 µl of Leucoperm Reagent B containing 10% serum.
14. Add CD68 antibody at the vendor-recommended dilution and incubate for at least 30 min, avoiding direct light.
15. Wash the cells 1x in 2 ml PBS 1% BSA.
16. If required, add an appropriate secondary reagent at the vendor-recommended dilution. Mix well and incubate for at least 30 min, avoiding direct light.
17. Wash the cells 1x in 2 ml PBS 1% BSA.
18. Re-suspend in 200 µl PBS for immediate analysis or with 200 µl of 0.5% formaldehyde in PBS if required.
19. Acquire data by flow cytometry. Analyze fixed cells within 24 hr.

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Notes

The following should be considered when designing your flow cytometry experiments:

- Appropriate controls should be carried out for flow cytometry, consider including the following:
  - 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 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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