Protocol III: Cell labeling for flow cytometry using fresh whole blood

Introduction

This protocol describes the procedure for labeling of cells using fresh whole blood. Keep in mind that in addition to antibodies detecting the cell markers of interest, matching isotype and FMO (fluorescence minus one) controls need to be included in the experiment. Before starting your experiment, always make sure that your cytometer is equipped with the adequate set of lasers and filters to analyze the fluorophores you wish to use.

This protocol describes the use of 100 ul blood per sample which is a sufficient amount in most cases. Optimal cell number depends on frequency of cells of interest and may vary in other experimental settings. Wear proper protection when handling blood and discard waste according to the relevant regulations. **Human blood must always be treated as potential infectious material.**

Procedure

1. Add 100µl of blood per 5ml tube to be used.
2. Add recommended test volume of each antibody to be tested to each tube. Respective mouse immunoglobulin isotype control staining (negative control) should be used in separate tube. If using a panel of several antibodies, the use of a mastermix cocktail is highly recommended. (Important note: In case of antibodies directed against antigens such as CD55, CD59, kappa or lambda light chain which have binding affinity to RBC or soluble serum antibodies, RBCs need to be lysed first before staining can be performed).
3. Incubate at room temperature (RT) for 30 minutes. Protect from light.
4. Prepare 1x Caprico RBC lysing buffer by diluting 10x stock solution (cat# 3101) with ddH_2O at room temperature.
5. Add 1ml of 1x Caprico RBC lysing buffer to each 5ml tube and vortex to mix well.
6. Incubate samples for 10 minutes at RT. Protect from light.
7. Spin samples down at 400 – 450 x g for 5 minutes at RT (20-25°C).

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8. Carefully aspirate the RBC lysed supernatant without disturbing the pellet, vortex and resuspend in 1ml of staining buffer. Recommended staining buffer 1x PBS, 4% FBS, 0.09% NaN3.
9. Centrifuge at 400 – 450 x g for 5 minutes at RT (20-25°C).
10. Carefully aspirate the supernatant without disturbing the pellet.
11. Resuspend the cells in 300µl staining buffer per tube and place on ice until running samples in the flow cytometer. Protect from light.
12. **Optional:** In case of purified or Biotin conjugated primary antibodies, prepare dilution of appropriate secondary antibody or SA-PE in staining buffer. Typical dilution for SA-PE is 1:200, while secondary antibody dilution can be expected between 1:1000 and 1:3000 depending on the antibody.
13. Add 100µl secondary antibody dilution in each tube having PBMC stained with primary antibodies instead of staining buffer in step 11.
14. Incubate on ice for 20 minutes, protect from light.
15. Add 1ml staining buffer and mix well by mild vortex. Centrifuge at 400 – 450 x g for 5 minutes at RT (20-25°C).
16. Carefully aspirate the supernatant without disturbing the pellet, vortex and resuspend in 1ml of staining buffer.
17. Centrifuge at 400 – 450 x g for 5 minutes at RT (20-25°C).
18. Carefully aspirate the supernatant without disturbing the pellet.
19. Resuspend the cells in 300µl staining buffer per tube and place on ice until running samples in the flow cytometer. Protect from light.
20. Run samples in your flow cytometer. Always control your instrument settings to obtain reliable data.
21. Analyze the data using appropriate software.

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