MURINE CELL PREPARATION PROTOCOL FOR FLOW CYTOMETRY

Important notes

1. Ensure you observe all proper animal husbandry practices and ethics for any mouse work.
2. Antibody and dye titrations should always be performed when using any new antibody and or encounter a new lot/batch.

Abstract

This guidance is designed to outline the steps needed to obtain a single cell suspension of mouse-derived cells for analysis on a conventional flow cytometer. It will touch on the standard methods used to disaggregate tissue and its treatment, and the harvesting of peripheral blood cells that are alive and intact. Subsequent downstream processing for flow cytometry staining is described briefly.

Process

1. Perform cull or harvest according to institutional OHS and ethics guidelines.
2. Harvest organs or blood and place in labelled containers.
3. To maintain cell integrity and viability store harvested sample in appropriate media or suitable isotonic buffer as required.
4. To obtain a single cell suspension from tissue or blood, follow the brief guide below.
    a. For biopsies of organs or tissue or whole organs

    Briefly, mince the tissue into small pieces (between 3-5mm^3) in a tissue culture dish containing HBSS or other medium. Dissociate the tissue via mechanical, enzymatic or chemical means to obtain a cellular suspension. Perform a series of wash steps to remove debris, dead cells and or inactivate enzymes or chemicals. Proceed with 4b if the sample contains unwanted red blood cells.

    b. For whole blood

    Perform red blood cell lysis if required (if downstream work requires analysis of smaller cells like RBC or platelets, or subcellular particles; fractionate or perform density gradient enrichment steps as required). Red blood cell lysis is considered essential to minimise analysis of coincident events that are below the cytometer's electronic threshold, and can be performed using hypotonic lysis using distilled water, buffers like NH_4Cl or commercially-available kits. Wash cells by centrifugation to remove residual lysis buffer/solution.

5. Wash cells by adding appropriate buffer and pellet by centrifugation at 400 *×g* for 5 minutes. Decant supernatant and repeat a second time, and resuspend in a nominal small volume to obtain a single cell suspension.
6. Perform a viable cell count (if downstream studies aren't required, skip to step 10).
7. Prepare cells accordingly for any functional studies, and if appropriate incubate to rest cells before manipulation.
8. Halt any functional experimental work.

9. Wash and centrifuge cells before performing a cell count.
10. Resuspend cells in cell staining buffer in an appropriately small volume to allow efficient staining.
11. Perform any blocking steps as required.
12. If using a Fixable Live/Dead dye, perform this stain first, according to manufacturer's recommended instructions for use (ensure that no BSA or FBS etc. is present, as it will interfere with staining).
13. Prepare antibody cocktail or stain.
14. Stain the cells for extracellular antigens/markers appropriately (having performed any titrations necessary) as reagents commonly used for fixation and permeabilisation tend to decrease surface antigen availability. Incubate for a minimum of 30 minutes at RT or 4°C, and in the dark.
15. Wash the cells by centrifugation at 400 *×g* for 5 minutes.
16. Aspirate or decant supernatant being careful not to disturb the cell pellet. Resuspend with cell staining buffer and repeat wash a further two times to wash unbound antibody.
17. Fixation of cells is required for samples which are not being analysed for any functional or metabolic activity. Fix to a final concentration of 0.5-2% PFA for 15-30 minutes, either at RT or 4°C, and in the dark (fixative choice is dependent on the assay and cell physiology).
18. Wash cells and centrifuge to remove residual fixative.
19. If permeabilisation is required for intracellular staining, prepare reagents and again predetermine which fixation and permeabilisation method/s suits your work.
20. Stain for intracellular antigens, using the same permeabilisation buffer for the staining procedure to maintain the pore structures.
21. Wash unbound antibody/stain as described previously.
22. Resuspend cells in appropriate volume of buffer for acquisition on the cytometer. Ensure samples are in correct format for acquisition on the instruments (correct tubes etc.).
23. If fixation is not required and space in your panel exists for viability staining with a DNA intercalator, add the dye prior to acquisition on the cytometer.
24. Acquisition on the cytometer should only be performed by trained individuals.

Other Resources

Further information regarding common laboratory protocols can be found:

- Holmes, K et al: Preparation of Cells and Reagents for Flow Cytometry. Current Protocols in Immunology; (2001); 5.3.1-5.3.24.
- Kay, J *et al*: Preparing Mouse Organs for Flow Cytometry. MIT Department of Biological Engineering; Cambridge, MA.

Further dissociation guidance can be found:

- AMREPFlow cell dissociation guide
- http://www.worthington-biochem.com/tissuedissociation/