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Cell Preparation for Flow Cytometry

Research Use Only

Introduction

These are the standard protocols used at eBioscience for the preparation of various cell types to be used for flow cytometric analysis. This information is to serve as a guide as individual investigators may need to optimize protocols for their particular cell type.

General Notes

1. Single cell suspensions are required for optimal staining of samples for flow cytometry.
2. The narrow bores of the sample injection needle and tubing on a flow cytometer will be easily clogged by aggregated cells and debris.
3. Preparation of single cell suspensions from solid tissue requires mechanical dissociation and/or enzymatic digestion for optimal recovery of cells from the tissue. Conditions and enzyme requirements for digestion of the tissue of interest will need to be determined empirically.

Useful websites

Worthington Tissue Dissociation Guide (http://www.tissuedissociation.com)
The Worthington Tissue Dissociation Guide provides a useful summary and guide of the various methods that can be used for tissue dissociation.

Protocol A: Cell Preparation of Tissue Culture Cells

Materials

- Accutase™ Enzyme Cell Detachment Medium (Cat. No. 00-4555) or trypsin or EDTA
- eBioscience® Flow Cytometry Staining Buffer (Cat. No. 00-4222)
- 15 or 50 mL conical centrifuge tubes

Experimental Procedure

1. For cells that grow in suspension, decant the cells into a conical centrifuge tube and perform a cell count and viability analysis. Proceed to Step 4.
2. For adherent cells lines, detach your cells from the plate. The most common method is scraping but can result in cell clumps which can clog the cytometer. Trypsin is another option, but it can destroy the epitope of the protein you may be interested in staining. You will need to test this empirically. EDTA (10 mM in PBS) will detach cells and will have minimal effect on protein staining (except where the epitope is modified by the removal of calcium ions). Lastly, Accutase™ Enzyme Cell Detachment Medium can also be used, but can alter some cell surface epitopes; the effect will need to be determined empirically for the epitopes being evaluated.

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3. Place cells into a conical centrifuge tube and perform a cell count and viability analysis.
4. Centrifuge cells and resuspend in an appropriate volume of Flow Cytometry Staining Buffer so that the final cell concentration is $1x10^7$ cells/mL.

Protocol B: Cell Preparation from Lymphoid Tissue

Mechanical disruption of lymphoid tissue is generally sufficient to release cells to a single cell suspension.

Materials

- 60 mm x 15 mm tissue culture dish
- 3 mL syringe
- Cell strainer (nylon mesh)
- eBioscience® Flow Cytometry Staining Buffer (Cat. No. 00-4222)
- 15 or 50 mL conical centrifuge tubes

Experimental Procedure

1. Harvest tissue (spleen, lymph nodes, thymus) into a tissue culture dish and tease it apart into a single cell suspension by pressing with the plunger of a 3 ml syringe (Alternatively, mash tissue between two frosted microscope slides using 10 mL of Flow Cytometry Staining Buffer).
2. Collect cells in 10 mL of Flow Cytometry Staining Buffer and pass cell suspension through a cell strainer to eliminate clumps and debris. Collect cell suspension in a conical tube.
3. Centrifuge cell suspension 4-5 minutes (300-400x*g*) at 4°C, discard supernatant.
4. Resuspend the cell pellet and perform a cell count and viability analysis.
5. Centrifuge cells as in Step 3 and resuspend in appropriate volume of Flow Cytometry Staining Buffer so that the final cell concentration is $1x10^7$ cells/mL.

Protocol C: Cell Preparation from Non-Lymphoid Tissue

Materials

- Scissors or scalpel blade
- PBS or other suitable physiologic buffer
- 60 mm x 15 mm tissue culture dish
- 3 mL syringe
- Cell strainer (nylon mesh)
- eBioscience® Flow Cytometry Staining Buffer (Cat. No. 00-4222)
- 15 or 50 mL conical centrifuge tubes

Experimental Procedure

1. Harvest tissue and mince into 2-4 mm pieces using scissors or scalpel blade.

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2. Add appropriate amount of enzyme(s) diluted in PBS and incubate at the optimal temperature for the appropriate amount of time according to enzyme manufacturer instructions.
   Note: The appropriate enzyme(s) to use will vary depending on the tissue; http://www.tissuedissociation.com is a useful site to determine a starting point when the optimal enzyme(s) for a particular tissue are not known.
3. Disperse cells by gentle pipeting and filter through a cell strainer to eliminate clumps and debris. Collect cell suspension in a conical tube.
4. Centrifuge cell suspension 4-5 minutes (300-400x*g*) at 4°C, discard supernatant.
5. Resuspend the cell pellet in PBS to remove excess enzyme solution
6. Centrifuge cells as in Step 4.
7. Repeat steps 5 and 6.
8. Resuspend the cell pellet in Flow Cytometry Staining Buffer and perform a cell count and viability analysis.
9. Centrifuge cells as in Step 4 and resuspend in appropriate volume of Flow Cytometry Staining Buffer so that the final cell concentration is $1x10^7$ cells/mL.

Protocol D: Isolation of PBMC from whole blood

Materials

- PBS
- Ficoll® Paque or other density separation medium
- eBioscience® Flow Cytometry Staining Buffer (Cat. No. 00-4222)
- 15 or 50 mL conical centrifuge tubes

Experimental Procedure

1. Dilute blood sample at least 1:1 with PBS in a conical tube.
2. Underlay the diluted sample with a volume of Ficoll® that is equal to the original sample volume.
3. Centrifuge for 20 minutes (400x*g*) with the brake OFF.
4. Harvest PBMC located at the interface of the PBS and Ficoll® layers into a fresh tube.
5. Fill the tube with PBS to wash the cells.
6. Centrifuge cell suspension 4-5 minutes (300-400x*g*) at 4°C, discard supernatant.
7. Resuspend the cell pellet in Flow Cytometry Staining Buffer and perform a cell count and viability analysis.
8. Centrifuge cells as in Step 6, then resuspend cells in an appropriate volume of Flow Cytometry Staining Buffer so that the final cell concentration is $1x10^7$ cells/mL.

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