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Flow Cytometry ECS Surface Antigens

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ABSTRACT

High-parameter flow cytometry enables identification and characterization of a wide range of cell populations within a biological sample. Analysis of cell-surface epitopes using a collection of fluorophore-labeled antibodies, or extracellular stain (ECS), sufficiently labels population-identifying markers for further analysis with flow cytometry. Importantly, ECS can be applied to cryo-preserved cell suspensions recovered in tissue culture media, enabling samples to be conveniently analyzed after collection and proper cryopreservation. However, consistent cryopreserved sample recovery and ECS procedures are critical to data comparison across multiple experiments. Herein, we describe a standardized protocol for cryopreserved sample recovery and ECS procedures for cell-surface epitope labeling.

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MATERIALS TEXT

Materials Required

1x phosphate saline buffer (PBS) Corning 21-031-

1. CM **Corning Catalog #21-031-CM**

Bovine serum albumin (BSA) Gemini 700-101P **Gemini**

2. **Bioproducts Catalog #700-101P**

Sodium Azide **Fisher**

3. **Scientific Catalog #S2271-500**

4. FACS buffer (1xPBS, 10g/L BSA, 1 g/L sodium azide)

5. RPMI **Corning Catalog #10-040-CM**

Fetal calf serum (FCS) **Gemini**

6. **Bioproducts Catalog # 900-108**

Penicillin/streptomycin 10000 U/mL penicillin 10000 µg/mL

7. streptomycin **Lonza Catalog #17-602E**

Lonza (RRID:SCR_000377)

8. "R10" medium (RPMI, 10% FCS, 1% penicillin/streptomycin)

DNAse

9. I **Roche Catalog #04716728001**

Live/Dead fixable Aqua Dead Cell Stain kit **Invitrogen - Thermo**

10. **Fisher Catalog #L34966**

diluted 1:60 in 1x PBS (prepare fresh each day from DMSO stock)

Paraformaldehyde (PFA) **Electron Microscopy**

11. **Sciences Catalog #15712-S** diluted to 1% in PBS

12. Fluorophore-labelled antibodies of choice

13. Hemocytometer

Procedure

1 Thawing and Resting

a. Pre-warm R10 media in a **37 °C** water bath.

b. Thaw samples in-vial using a **37 °C** water bath.

c. Add thawed cells to **14 mL** of R10, then spin cells at 500 xg for 5 min.

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d. Resuspend cell pellet in **3 mL** of R10 and count cells.

e. Rest cells at least 3 hours (up to overnight) at 2x10^6 cells/mL in R10 medium + 1 µL/mL DNAse I at **37 °C** , 5% CO_2.

Note: during resting, prepare antibody cocktail master mix. Adjust volume of ECS for **50 µl** per test with FACS buffer.

f. After resting, add PBS up to **15 mL** or **50 mL** (whichever is closer, rounding up) to cells and transfer to **15 mL** or **50 mL** conical tube.

g. Spin cells at 500 xg for 5 minutes at room temperature (RT).

h. Resuspend cells in PBS to 1x10^7 cells/mL and count. If cells are too dilute, re-spin cells and resuspend at 1x10^7 cells/mL. Transfer **200 mL** of cells (2x10^6cells) into each well of a V-bottom 96 well plate.

2 Viability and extracellular staining (ECS)

a. Spin plate at 500 xg for 5 minutes at RT

b. Using a multichannel pipette, carefully remove the supernatant.

c. Using a multichannel pipette, add **45 µl** of PBS to well.

d. Add **5 µl** of 1:60 Aqua viability dye directly to cell pellet and resuspend cells.

e. Incubate for 10 minutes at RT in the dark.

f. Add **50 µl** of ECS antibody cocktail to cells and incubate for 20 minutes at RT in the dark (prepare 1% PFA fixation buffer in the meantime).

g. Add **100 µl** of FACS buffer to each well and spin plate at 500 xg for 5 minutes.

h. Discard supernatant and fix cells with **200 µl** of 1% PFA.

i. Run samples on flow cytometer.

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