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Flow Cytometry ICS Nuclear Antigens V.2

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Human Islet Research Network

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ABSTRACT

High-parameter flow cytometry enables identification and characterization of a wide range of cell populations within a biological sample. A combined analysis of extracellular epitope staining (ECS) and intra-cellular epitope staining (ICS) using a collection of fluorophore-labeled antibodies sufficiently identifies discrete cell populations and their respective phenotypes. Importantly, ECS/ICS 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/ICS procedures for cell-surface and intra-cellular epitope labeling.

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MATERIALS TEXT

Materials Required

1x phosphate saline buffer

1. (PBS) **Corning Catalog #21-031-CM**

Bovine serum albumin (BSA) **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)

11.

e-Bioscience Foxp3 / Transcription Factor Staining Buffer Set **Invitrogen - Thermo**

Fisher Catalog # 00-5523-00

Paraformaldehyde (PFA) **Electron Microscopy**

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

13. Fluorophore-labelled antibodies of choice

14. Hemocytometer

Procedure

1 Thawing and Resting

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

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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.

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 ($2x10^6$ cells) 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. Add **5 µl** of 1:60 Aqua viability dye directly to cell pellet and resuspend cells.

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

e. 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).

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

3 Fixation, Permeabilization, and ICS Staining

a. Fix cells with **100 µl** of 1xFixation/permeabilization buffer (1 part concentrate + 3 parts diluent) for 30 minutes at RT in the dark.
Note: Make ICS abs here

b. Add **100 µl** of 1x perm/wash buffer and centrifuge 800 xg for 5 min.

c. Remove supernatant and add **100 µl** ICS cocktail (made in perm/wash buffer, made with diH_2O) to the cells.

d. Incubate for 1h at RT in the dark.

e. Add **100 µl** perm/wash buffer. Centrifuge at 800 xg for 5 min.

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f. Discard supernatant and resuspend pellet in **200 µl** 1% PFA and transfer to FACS tubes.

g. Wrap in aluminum foil and store at **4 °C** until flow cytometry.

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