Apr 20, 2024 Version 3

Flow Cytometry ICS Nuclear Antigens V.3

DOI
https://dx.doi.org/10.17504/protocols.io.rm7vz3qdxgx1/v3

Michael Betts[1], Gregory Golden[1]
[1]University of Pennsylvania

Human Islet Research N...

Sandy Beshir
City of Hope

DOI: https://dx.doi.org/10.17504/protocols.io.rm7vz3qdxgx1/v3

Protocol Citation: Michael Betts, Gregory Golden 2024. Flow Cytometry ICS Nuclear Antigens. **protocols.io** https://dx.doi.org/10.17504/protocols.io.rm7vz3qdxgx1/v3Version created by **Sandy Beshir**

Protocol status: Working
We use this protocol and it's working

Created: April 20, 2024

Last Modified: April 20, 2024

Protocol Integer ID: 98530

Keywords: extracellular epitope staining (ECS), intra-cellular epitope staining (ICS), HIRN, flow cytometry ics nuclear antigen, standardized protocol for cryopreserved sample recovery, cryopreserved sample recovery, flow cytometry, combined analysis of extracellular epitope, parameter flow cytometry, ics procedures for cell, extracellular epitope, preserved cell suspension, proper cryopreservation, cell suspension, cryopreservation, cell population, labeled antibody, discrete cell population, biological sample, sample recovery, cell, ics procedure

Funders Acknowledgements:
NIH
Grant ID: U01 DK112217

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.

Materials

Materials Required

1. 1x phosphate saline buffer (PBS) **Corning Catalog #**21-031-CM
2. Bovine serum albumin (BSA) **Gemini Bio-Products Catalog #**700-101P
3. Sodium Azide **Fisher Scientific Catalog #**S2271-500
4. FACS buffer (1xPBS, 10g/L BSA, 1 g/L sodium azide)
5. RPMI **Corning Catalog #**10-040-CM
6. Fetal calf serum (FCS) **Gemini Bio-Products Catalog #**900-108
7. Penicillin/streptomycin 10000 U/mL penicillin 10000 µg/mL streptomycin **Lonza Catalog #**17-602E
   Lonza (**RRID:SCR_000377**)
8. "R10" medium (RPMI, 10% FCS, 1% penicillin/streptomycin)
9. DNAse I **Roche Catalog #**04716728001
10. Live/Dead fixable Aqua Dead Cell Stain kit **Invitrogen - Thermo 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
12. Paraformaldehyde (PFA) **Electron Microscopy 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.

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 2×10^6 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 1×10^7 cells/mL and count. If cells are too dilute, re-spin cells and resuspend at 1×10^7 cells/mL. Transfer 200 mL of cells (2×10^6 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.

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.