Apr 20, 2024 Version 2

Flow Cytometry ECS Surface Antigens V.2

DOI
https://dx.doi.org/10.17504/protocols.io.dm6gpwq11lzp/v2

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DOI: https://dx.doi.org/10.17504/protocols.io.dm6gpwq11lzp/v2

Protocol Citation: Michael Betts, Gregory Golden 2024. Flow Cytometry ECS Surface Antigens. **protocols.io** https://dx.doi.org/10.17504/protocols.io.dm6gpwq11lzp/v2Version created by **Sandy Beshir**

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

Created: April 20, 2024

Last Modified: April 20, 2024

Protocol Integer ID: 98531

Keywords: extracellular epitope staining (ECS), intra-cellular epitope staining (ICS), HIRN, flow cytometry ecs surface antigen, standardized protocol for cryopreserved sample recovery, flow cytometry, cryopreserved sample recovery, parameter flow cytometry, preserved cell suspension, ecs procedures for cell, surface epitope labeling, proper cryopreservation, cell suspension, analysis of cell, cryopreservation, extracellular stain, surface epitope, labeled antibody, cell population, biological sample, cell, tissue culture media, sample recovery, antibody

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. 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.

Materials

Materials Required

1. 1x phosphate saline buffer (PBS) Corning 21-031-CM **Corning Catalog #**21-031-CM
2. Bovine serum albumin (BSA) Gemini 700-101P **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. Paraformaldehyde (PFA) **Electron Microscopy 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.

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 (2×10^6cells) 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.