

Apr 20, 2024 Version 2

# Flow Cytometry ECS Surface Antigens V.2

DOI

[dx.doi.org/10.17504/protocols.io.dm6gpwq11lzp/v2](https://dx.doi.org/10.17504/protocols.io.dm6gpwq11lzp/v2)



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Human Islet Research Ne...



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**Protocol Citation:** Michael Betts, Gregory Golden 2024. Flow Cytometry ECS Surface Antigens. [protocols.io](https://dx.doi.org/10.17504/protocols.io.dm6gpwq11lzp/v2)  
<https://dx.doi.org/10.17504/protocols.io.dm6gpwq11lzp/v2> Version 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

**Funders Acknowledgement:**

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 Bioproducts 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 Bioproducts 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  $2 \times 10^6$  cells/mL in R10 medium + 1  $\mu$ L/mL DNase I at  37 °C , 5% CO<sub>2</sub>.

**Note:** during resting, prepare antibody cocktail master mix. Adjust volume of ECS for  50  $\mu$ L 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  $1 \times 10^7$  cells/mL and count. If cells are too dilute, re-spin cells and resuspend at  $1 \times 10^7$  cells/mL. Transfer  200 mL of cells ( $2 \times 10^6$  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. Using a multichannel pipette, add  45  $\mu$ L of PBS to well.

d. Add  5  $\mu$ L 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  $\mu\text{L}$  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  $\mu\text{L}$  of FACS buffer to each well and spin plate at 500 xg for 5 minutes.
- h.** Discard supernatant and fix cells with  200  $\mu\text{L}$  of 1% PFA.
- i.** Run samples on flow cytometer.