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HT2-280 Sorting Protocol

Morrisey Lab[1]

[1]University of Pennsylvania

1 *Works for me* This protocol is published without a DOI.

Chelseahortman

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ABSTRACT

HT2-280 Sorting Protocol

FACS Buffer:

- 1L dPBS (Cell Center, cat# 14190136, without Ca++ & Mg++)

- 2mL EDTA (Invitrogen, cat# 15575020, UltraPure, 0.5M, ph 8.0, 100mL)

- 10mL of FBS (Invitrogen, cat# 10437-028, 500mL)

0.04% BSA+PBS:

- 10mg BSA (Jackson, cat# 001-000-162, IgG free, protease free)

- 25mL PBS (Cell Center, cat# 14190136, without Ca++ & Mg++)

MACS Accessories: (Miltenyi Biotec):

QuadroMACS Separator (cat# 130-090-976)

MACS multistand (cat# 130-042-303)

MACS LS Cloumns (25pk) (cat# 130-042-401)

MACS Rat Anti-mouse IgM Microbeads, 2mL (cat# 130-047-301)

Other:

HT2-280 antibody (-20C benchtop freezer)(Terrace, cat#TB-27AHT2-280)

Cellometer K2 (Nexcelome)

Countess™ Cell Counting Chamber Slides (Thermo Fisher cat#C10228)

ViaStain™ AOPI Staining Solution (Nexcelom/ Fisher cat #CS2-0106)

Evos (Lifetech)

Trypan Blue (Fisher cat #ICN1691049)

PROTOCOL CITATION

Morrisey Lab 2021. HT2-280 Sorting Protocol. **protocols.io**
https://protocols.io/view/ht2-280-sorting-protocol-bs5nng5e

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1 Make sure 4C centrifuge is on and at correct temperature.

2 After completing human lung digestion protocol and/or CD45 depletion, count total number of cells and place desired amount of cells into 50mL conical (We typically want 24 million cells).

3 Spin at 300xg(rcf) for 5 minutes, remove supernatant, and wash with 20mL FACS buffer.

4 Spin at 300xg(rcf) for 5 minutes, remove supernatant, resuspend pellet in 20uls of 1o antibody (HT2-280) and 980uls FACS buffer *HT2-280 ab is a 1:50 dilution*

5 Incubate at 4C for 1 hour in darkness.

6 Wash 2x with 10ml FACS buffer and spin at 300x g(rcf) for 5 min.

7 Resuspend pellet with 200uls rat anti-mouse IgM secondary ab (2o antibody for HT2-280) and 800uls FACS buffer.

8 Incubate for 30 minutes at 4C in the dark.

9 Wash 2x with 10ml FACS buffer and spin at 300xg (rcf) for 5 min.

10 10. Resuspend pellet in 24mLs of FACS buffer (the # of ml should = 2x the number of columns being used).

11 11. Set up MACS columns and label the flow through tubes as HT2-280 Negative.

12 12. Note**When adding buffer/cells to columns it is important to not have bubbles. You can use P1000 to get rid of them.

13 13. Add 3mL of FACs buffer to each column, allow flow through, wash columns 2 more times. We use multiple columns to improve yield of AT2 cells; using less columns depending on quality of single cell suspension can reduce yield.

14 14. Add 2mL of resuspended cells to each column and allow to flow through.

15 15. Wash columns 3x with 3mLs of FACs buffer. You will have approx. 11mL of collected flow through.

16 16. Combine all negative cells in one tube and centrifuge 300xg (rcf) for 5 min.

17 17. Combine all positive cells (AT2 cells) by taking columns off the magnetic rack and adding 5mLs of FACs to each one, pushing the plunger into the column. Centrifuge 300xg (rcf) for 5 min.

18 18. Resuspend both positive and negative cells in 0.04% BSA+PBS and place on ice.

19 19. Note** Volume of 0.04%BSA+PBS depends on pellet size.

20 20. Count both positive and negative cells on cellometer (ViaStain) or EVOS using trypan blue (1:1) recording viability and place on ice.

21 21. Follow cytospin protocol and make 4-6 slides per cell type.

22 22. If there are enough cells can collect RNA and Protein to bank. Make sure to record lung Id, date, disease (if applicable), cell type.

23 a. If doing RNA, resuspend cells in 500uL of Trizol for any amount of cells less than 1 million and put in -80C

24 b. If Protein Lysis buffer, resuspend at 100 uL of lysis buffer per million cells and put in -80C

25 23. Can proceed to Epcam staining to do a further sort in which +cells can be used for cytospins and/or organoids.