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EPCAM Sorting Protocol March

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

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ABSTRACT

Epcam 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 EpCAM microbeads, human (cat#130-061-101)

Other:

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. EPCAM Sorting Protocol March. **protocols.io**
https://protocols.io/view/epcam-sorting-protocol-march-bs5mng46

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

2 After completing HT2-280 sorting protocol centrifuge HT2-280 – cells for 5 min @ 300g(rcf).

3 Resuspend pellet in 100uls αEpcam microbeads and 900uls FACS.

4 Incubate in 4C for 30 min in darkness.

5 Wash pellet with 20mL FACS and spin for 5 min @ 300g(rcf).

6 Resuspend pellet in FACS (the # of ml should = 2x the number of columns being used).

7 Set up columns and wash with 3mL FACS 3x.

8 Add 2mL of cells per column.

9 Wash each column 3x with 3mL FACS.

10 10. Discard flow through-- negative cells.

11 11. Plunge all columns into 1 tube using 5mL FACS per column.

12 12. Spin +cells for 5 min @300g(rcf) and resuspend in 0.04%BSA+PBS.

13 13. Count cells and record viability on cellometer (ViaStain) or EVOS using trypan blue (1:1).

14 14. Proceed to cytospin protocol, organoid protocol, or bank cells for RNA and/or protein.