Mar 05, 2021

CD45 Depletion Protocol

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

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ABSTRACT

CD45 Depletion: Sorting for CD45 Negative Cells from a single cell suspension

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 CD45 Microbeads, human, 2mL (cat# 130-045-801)

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)

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PROTOCOL CITATION

Morrisey Lab 2021. CD45 Depletion Protocol. **protocols.io**
https://protocols.io/view/cd45-depletion-protocol-bs2pngdn

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CREATED

Mar 05, 2021

LAST MODIFIED

Mar 05, 2021

PROTOCOL INTEGER ID

47919

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1 After completing Human lung digestion protocol count cells and record viability on EVOS using trypan blue (this is a 1:1 dil-10uls/10uls) --You might have to dilute cells if too concentrated to count.

2 Can also use cellometer to count cells (20uls/20uls ; using ViaStain™ AOPI Staining Solution).

3 Decide number of total cells you want to deplete (we aim for 12 million cells at 2 million per column; for a total of 6 columns).

4 Place desired cell amount in 50mL conical and centrifuge at 4C --300x g(rcf) for 5 minutes.

5 Note **All centrifugation for entire protocol should be done in 4C centrifuge**

6 Remove supernatant and resuspend pellet in FACS buffer/°CD45 magnetic beads antibody (1:50). ie- 980uls FACS buffer/20uls CD45 MACS microbeads

Incubate for 30 min @4C in darkness.

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8 Can set up columns on the magnetic strip labelling flow through tubes as CD45 negative.

9 After incubation wash pellet 2x with 10ml FACS buffer and spin at 300xg (rcf) for 5 minutes. (It is important to break up the pellet before centrifuging)

10 On the final spin wash columns previously set up with 3mL FACS at a time for a total of 6mL to each column and do not let column dry out. Discard flow through. (If you do not discard flow through will not have enough room for depletion)

11 10. Resuspend pellet in FACS buffer (the amt of FACS = 6 columns = 12mL of FACS).

12 11. Add 2mL of resuspended cells to each column and allow to flow through (Each column works best if you use 1-2mL cells per LS column (do not use LD with the lungs). The max vol per column is <5mL)

13 12. Wash columns 3x with 3mLs of FACS buffer. At the end, should have approx. 11mL of collected flow through.

14 13. Combine flow through in a new 50mL conical tube and keep on ice. This is the CD45 negative bulk lung population. This is the population we want to keep.

15 14. Centrifuge the collected CD45- cells at 300x g(rcf) for 5 minutes and resuspend in 0.04% PBS+BSA.

16 15. Count CD45- cells on EVOS or using cellometer to determine cell viability.

17 16. Continue with HT2-280 sort protocol or scRNA 10x genomics protocol.

18 17. For CD45 positive cells (the cells on the column) collect these cells by plunging with 5mL FACS per column. Can use this as QC to compare them to the CD45 neg population for a control.

19 a. Remove columns from magnet and place it in a 50mL tube.

20 b. Add 5mLs of FACs buffer and immediately flush out the magnetically labeled cells by firmly and fairly quickly pushing the plunger into the column.

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18. Centrifuge the collected cells at 300xg for 5 min and resuspend in 0.04% BSA+PBS.

19. Count cells on EVOS or cellometer.

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