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CD34+ isolation from human bone marrow V.2

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Mohsen Khosravi-Maharlooei[1], Markus Holzl[1], Austin Chen[1], Megan Sykes[1]
[1]Columbia Center for Translational Immunology, Columbia University, New York

Human Islet Research N...

Sandy Beshir
City of Hope

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We use this protocol and it's working

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Abstract

This protocol details the steps for isolating CD34+ cells from human bone marrow. The CD34+ cells isolated from this protocol can be used for generating humanized mice through reconstitution of immune cells via IV injection after bone marrow ablation. These cells can also be used for mixed lymphocyte reaction experiments.

Note

Corresponding Authors

Mohsen Khosravi-Maharlooei
Email: mkm2182@cumc.columbia.edu

Austin Chen
Email: ac4274@cumc.columbia.edu
Tel: 425-283-6900

Materials

Required material

- Bone Marrow Medium
- MACS buffer(degassed)
- Sterile flask for BM rinse
- 15 and 50 mL Falcons
- Histopaque
- Sterile pipetts
- CD34+ MACS kit (130-046-702)
- Human serum
- MACS supply
- antibodies

Required Buffers

- BM Medium (500 mL Media 199, 5 mL Hepes, 5 mL DNAse, 40 µL Gentamycin)
- MACS buffer (500 mL PBS, 5 g BSA, 2 mL EDTA, sterile filtrated and degassed)
- Cryomedium (90 mL PBS, 10 mL FBS, 10 mL DMSO)

Troubleshooting

Before start

Human bone marrow is a rich source for CD34+ hematopoietic stem cells. CD34+ cells can be easily isolated and further processed.

1 Transfer the content of the collection bag into a sterile flask

2 Add 250 mL BM Medium to the bag and rinse it thoroughly

3 Transfer the content of the collection bag into the sterile flask

4 Layer 35 mL of the suspension over 15 mL of Histopaque

5 Centrifuge the tubes for 30 minutes 500 g without brake at RT

6 Collect the leukocyte ring in 50 mL Falcons and fill up with BM medium

7 Wash once by centrifuging 6 minutes 500 g

8 Resuspend the cells in MACS buffer and count (***take*** 50 µL ***for FACS confirmation = PRE***)

9 Wash down again and resuspend the pellet according to the protocol (**130-046-702** MACS Human CD34+ kit)

10 Add 300 µL of MACS buffer per 10^8 cells

11 Add 100 µL of FcR-B reagent per 10^8 cells, mix it and incubate in fridge for 15 minutes

12 Add 100 µL of CD34 beads per 10^8 cells to the suspension and mix and let it sit for 30 min (fridge)

13 Fill up with 50 mL MACS buffer and strain through a blue strainer (40 uM)

14 Wash cells (500 g 6 min) and resuspend in MACS buffer 500 uL/200,000,000 cells. If you have more cells, increase volume accordingly. E.g 3 *10^9 cells = 7,5mL. Aliquot this volume to more than one (with 3 mL prerinsed) MACS column.

15 Wash with buffer 3 mL 3 times and keep negative fraction (***Take*** 50 µL ***for FCM = POST neg***)

16 Put the column out of the magnet and push out positive fraction with 5 mL Buffer and the plunger

17 Collect the positive fractions. ***(Take*** 50 µL ***for FCM = Post pos)***

18 Process the cells as desired (injection in mouse or cryopreservation)

19 Check the puritiy with FACS

20

FACS panel		
CD45	FITC	5 ul
CD3	PercpC5.5	5 ul
CD14	PacBlue	5 ul
CD19	APC	5 ul
CD34	PE	5 ul
CD38	PeC7	5 ul
Human Serum		5 ul
FACSbuffer		15 ul
Total		50 ul/sample