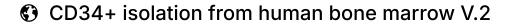


Apr 20, 2024

Version 2



DOI

dx.doi.org/10.17504/protocols.io.36wgq4p53vk5/v2



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Human Islet Research N...



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DOI: https://dx.doi.org/10.17504/protocols.io.36wgq4p53vk5/v2

Protocol Citation: Mohsen Khosravi-Maharlooei, Markus Holzl, Austin Chen, Megan Sykes 2024. CD34+ isolation from human bone marrow. **protocols.io** https://dx.doi.org/10.17504/protocols.io.36wgq4p53vk5/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: 98527

Keywords: isolating cd34, isolation from human bone marrow, cells from human bone marrow, cd34, immune cells via iv injection, human bone marrow, immune cell, bone marrow ablation, mixed lymphocyte reaction experiment, bone marrow, humanized mice, cell, mice through reconstitution, iv injection

Funders Acknowledgements:

NIH

Grant ID: U01 DK123559

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

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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 (<u>130-046-702</u>)
- Human serum
- MACS supply
- antibodies

Required Buffers

- BM Medium (♣ 500 mL Media 199, ♣ 5 mL Hepes, ♣ 5 mL DNAse, ♣ 40 μL Gentamycin)
- MACS buffer (\$\overline{\Lambda}\$ 500 mL PBS, \$\overline{\Lambda}\$ 5 g BSA, \$\overline{\Lambda}\$ 2 mL EDTA, sterile filtrated and degassed)
- Cryomedium (A 90 mL PBS, A 10 mL FBS, A 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 4 250 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 4 500 g without brake at RT
- 6 Collect the leukocyte ring in 4 50 mL Falcons and fill up with BM medium
- 7
- 8 Resuspend the cells in MACS buffer and count (take 4 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 4 300 µL of MACS buffer per 10^8 cells
- 11 Add \perp 100 μ L of FcR-B reagent per 10^8 cells, mix it and incubate in fridge for 15 minutes
- 12 Add 4 100 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 450 mL MACS buffer and strain through a blue strainer (40 uM)



- 14 Wash cells (Δ 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 4 3 mL prerinsed) MACS column.
- 15 Wash with buffer \perp 3 mL 3 times and keep negative fraction (*Take* \perp 50 μ L *for* FCM = POST neg)
- 16 Put the column out of the magnet and push out positive fraction with 45 mL Buffer and the plunger
- 17 Collect the positive fractions. (Take \perp 50 μ L for FCM = Post pos)
- 18 Process the cells as desired (injection in mouse or cryopreservation)
- 19 Check the puritiy with FACS

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FACS pane	<u>el</u>	
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