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Isolation of mononuclear cells using Septmate

Ajit N Shah¹

¹UCSF

Ajit N Shah

ATTACHMENTS

[Sepmate_PBMC.pdf](#)

OPEN ACCESS

DOI:

dx.doi.org/10.17504/protocols.io.88chzsw

External link:

https://cdn.stemcell.com/media/files/pis/10000000612-PIS_01.pdf?_ga=2.182160867.53291116.1573512387-262712883.1573512387

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Protocol status: Working
We use this protocol and it's working

Created: Nov 11, 2019

Last Modified: Nov 12, 2019

MATERIALS

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⊗ SepMate™-50 (IVD) 500 Tubes **Stemcell Technologies Catalog #85460**

⊗ Dulbecco's Phosphate Buffered Saline with 2% Fetal Bovine Serum 500 mL **Stemcell Technologies Catalog #7905**

⊗ Lymphoprep™ **Stemcell Technologies Catalog #07801**

⊗ ACK Lysing Buffer Thermo Fisher Catalog #A1049201

- 1 Add density gradient medium to the SepMate™ tube by carefully pipetting it through the central hole of the SepMate™ insert. Refer to step 2 for required volumes. The top of the density gradient medium will be above the insert.

NOTE: Small bubbles may be present in the density gradient medium after pipetting. These bubbles will not affect performance.

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SEPM ATET M TUBE	INITI AL SAMP LE (mL)	DENS ITY GRAD IENT MEDI UM (mL)
15	0.5 - 4.0	4.5
15	>4-5	3.5
50	4 - 17	15

Sample with Density Gradient Medium

2.1

Overview of Procedure

-
- 3 Dilute sample with an equal volume of PBS + 2% FBS. Mix gently.
For example, dilute 5 mL of sample with 5 mL of PBS + 2% FBS.
 - 4 Keeping the SepMate™ tube vertical, add the diluted sample by pipetting it down the side of the tube. The sample will mix with the density gradient medium above the insert.
NOTE: The sample can be poured down the side of the tube. Take care not to pour the diluted sample directly through the central hole.
 - 5 Centrifuge at 1200 x g (see Notes) for 10 minutes at room temperature, with the brake on.
NOTE: For samples older than 24 hours, a centrifugation time of 20 minutes is recommended.
 - 6 Pour off the top layer, which contains the enriched MNCs, into a new tube. Do not hold the SepMate™ tube in the inverted position for longer than 2 seconds.
NOTE: Some red blood cells (RBCs) may be present on the surface of the SepMate™ insert after centrifugation. These RBCs will not affect performance.

NOTE: To reduce platelet contamination in the enriched MNCs, pipette off some of the supernatant above the MNC layer before pouring.
 - 7 Wash enriched MNCs with PBS + 2% FBS. Centrifuging at 300 xg for 8 minutes at RT.
 - 8 Add ACK Lysis buffer and incubate for 3 minutes. Add 3-4 volumes of PBS + 2% FBS. Centrifuging at 400 xg for 5 minutes at RT.
 - 9 Wash enriched MNCs with PBS + 2% FBS. Centrifuging at 400 xg for 5 minutes at RT.
 - 10 Re-suspend in desired volume of media/buffer and perform cell count.

11 PMBC are ready for downstream process.