Isolation of mononuclear cells using Septmate

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1 Works for me

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

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EXTERNAL LINK

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

Sepmate_PBMC.pdf

MATERIALS

NAME	CATALOG #	VENDOR
SepMate™-50 (IVD) 500 Tubes	85460	Stemcell Technologies
Dulbecco's Phosphate Buffered Saline with 2% Fetal Bovine Serum 500 mL	7905	Stemcell Technologies
Lymphoprep™	07801	Stemcell Technologies
ACK Lysing Buffer	A1049201	Thermo Fisher

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

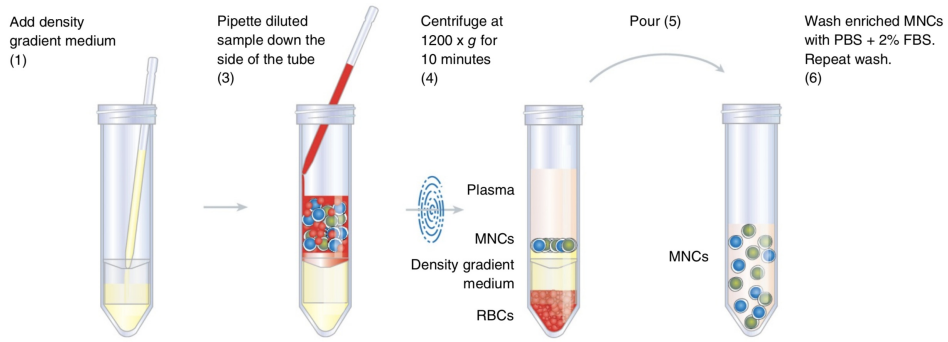
NOTE: Small bubbles may be present in the density gradient medium after pipetting. These bubbles will not affect performance.

2

SEPMATETM TUBE	INITIAL SAMPLE (mL)	DENSITY GRADIENT MEDIUM (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 SepMateTM 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 SepMateTM tube in the inverted position for longer than 2 seconds.
NOTE: Some red blood cells (RBCs) may be present on the surface of the SepMateTM 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 xgfor 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.