Isolating PBMCs or Specific Cell Subsets from Whole Blood Using SepMate™

The following procedure provides guidelines for isolating peripheral blood mononuclear cells (PBMCs) from whole blood using SepMate™, a tube that enables consistent and hassle-free PBMC isolation in just 15 minutes. The unique SepMate™ insert allows blood to be rapidly pipetted over the density gradient medium, and prevents the layers from mixing. After density gradient centrifugation with the brake on, PBMCs are simply poured into a fresh tube. SepMate™ can also be used with RosetteSep™ cell enrichment cocktails to isolate specific cell subsets from whole blood in less than 30 minutes.

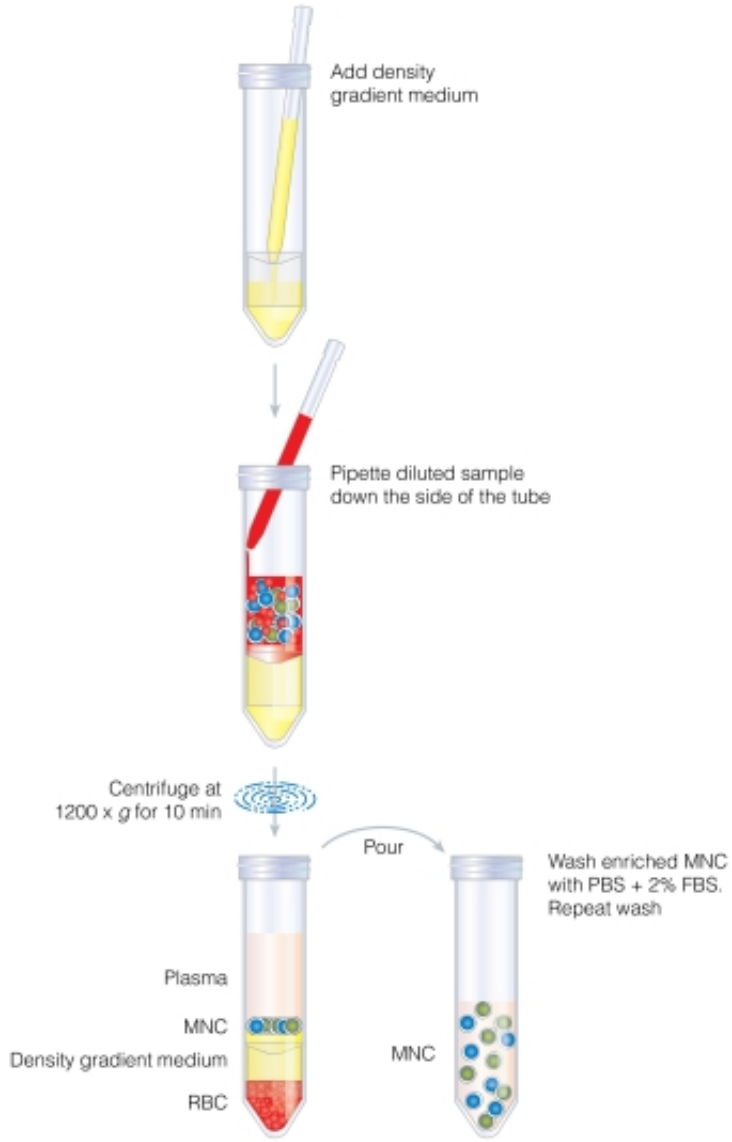
SepMate™ can be used with human whole peripheral blood and cord blood samples. It has not been tested with samples older than 48 hours. For use of SepMate™ with samples other than those listed here, please contact STEMCELL Technologies' Technical Support at techsupport@stemcell.com.

PBMC Isolation Protocol

Ensure that the sample, phosphate-buffered saline with 2% fetal bovine serum (PBS + 2% FBS), density gradient medium (e.g. Ficoll™ or Lymphoprep™) and centrifuge tubes are all at room temperature (15 - 25 ° C).

1. Add 15 mL of density gradient medium to the SepMate™-50 tube by carefully pipetting it through the central hole of the SepMate™-50 insert. 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. This will not affect performance.
2. Dilute sample with an equal volume of PBS + 2% FBS. Mix gently.
   Example: Dilute 5 mL of sample with 5 mL of PBS + 2% FBS.
   Note: SepMate™-50 is designed to process 5 - 17 mL of initial sample.
3. Keeping the SepMate™-50 tube vertical, add the diluted sample by pipetting it down the side of the SepMate™-50 tube. The sample will mix with the density gradient medium above the insert.
4. Centrifuge at 1200 x g 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.
5. Pour off the top layer, which contains the enriched mononuclear cells (MNCs), into a new tube. Do not hold the SepMate™-50 tube in the inverted position for longer than 2 seconds.
   Note: Some red blood cells may be present on the surface of the SepMate™-50 insert after centrifugation. This will not affect performance.
6. Wash enriched MNCs with PBS + 2% FBS. Repeat wash.

Figure 1. Schematic of the PBMC isolation protocol using SepMate™



Ficoll is a trademark of GE Healthcare Ltd. Lymphoprep is a trademark of Axis-Shield.

Specific Cell Subset Isolation Protocol

SepMate™ can be used with RosetteSep™ cell enrichment cocktails to isolate specific cell subsets from human whole blood. This is a general procedure; specific conditions may vary according to the cell type being enriched. Find RosetteSep™ protocols for a specific cell type by clicking here.

1. Add RosetteSep™ enrichment cocktail to the whole blood sample using volumes recommended in the RosetteSep™ cocktail Product Information Sheet.
2. Incubate for 10 minutes at room temperature (15 - 25 ° C).
   Note: The 10-minute incubation time is specific for this procedure. It will have minimal effect on the performance of most RosetteSep™ cell enrichment cocktails. For enriching very rare cells such as hematopoietic progenitor cells or circulating tumor cells, an incubation time of 20 minutes is still recommended.
3. Follow the steps under the PBMC Isolation Protocol above, using the density gradient medium recommended in the RosetteSep™ cocktail Product Information Sheet.