PRODUCT INFORMATION SHEET

SepMate™

SepMate™-50

STEMCELL™ TECHNOLOGIES

CATALOG #15450 20 TUBES/BAG
CATALOG #15460 5X20 TUBES/BAG

INTENDED USE

SepMate™-50 tubes are designed for the *in vitro* isolation of mononuclear cells (MNC) from human whole peripheral blood and cord blood samples by density gradient centrifugation.

PRODUCT DESCRIPTION

50 mL polypropylene tube with insert. Gamma-irradiated.

STORAGE

Store at room temperature (15 - 25°C).

DIRECTIONS FOR USE

Ensure that sample, phosphate buffered saline with 2% fetal bovine serum (PBS + 2% FBS; Catalog #07905), density gradient medium (*see* "Notes" on reverse page), and centrifuge are all at room temperature (15 - 25°C).

1. Add **15 mL** of density gradient medium to the SepMate™-50 tube by carefully, yet quickly, pipetting it through the central hole of the SepMate™-50 insert.

   Note: 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.

   For example, dilute 5 mL of sample with 5 mL of PBS + 2% FBS.

3. Add diluted sample by pipetting it down the side of the SepMate™-50 tube.

4. Centrifuge at **1200 x *g*** (*see* "Notes") for **10 minutes** at room temperature (15 - 25°C), with the **brake on**.

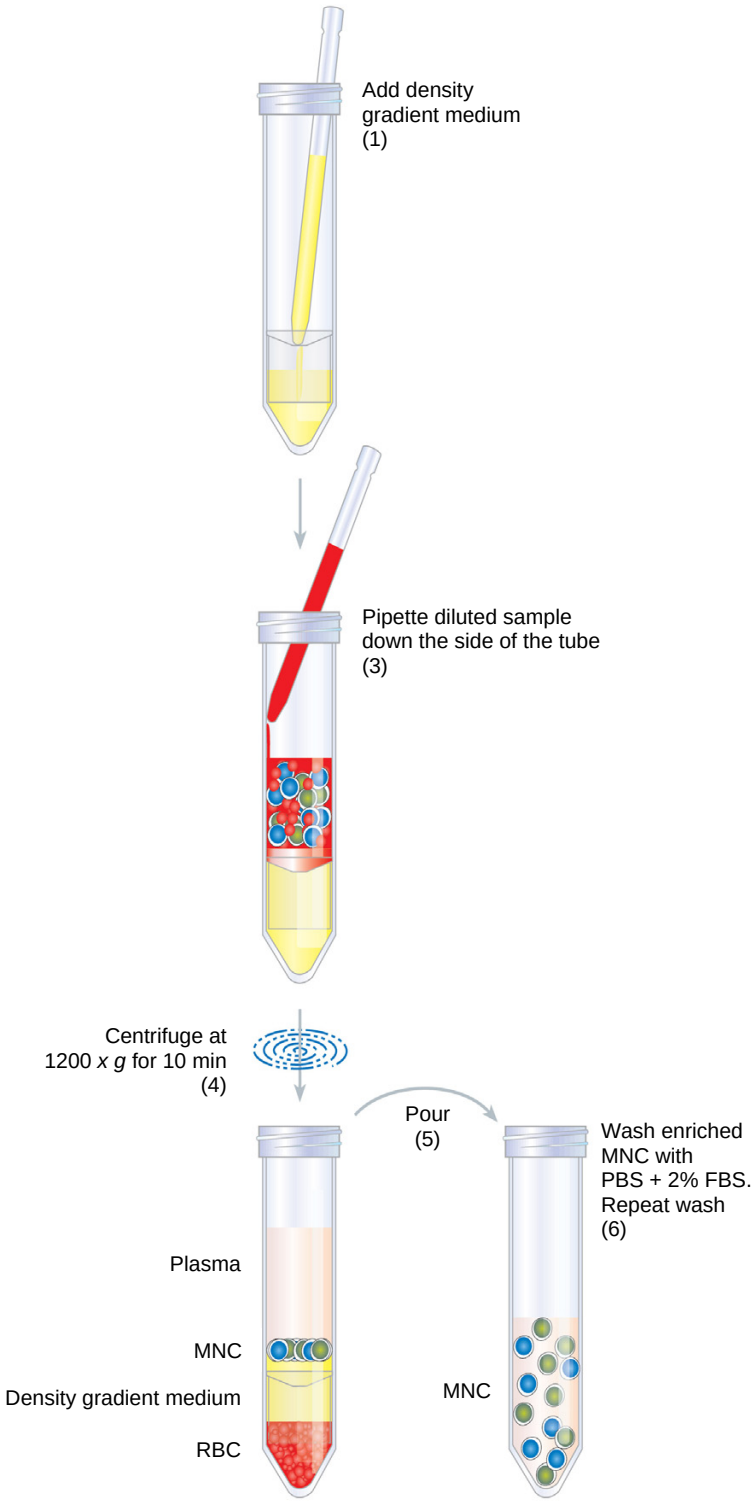
5. Pour off top layer, which contains the enriched MNC, into a new tube. Do not hold the SepMate™-50 tube in the inverted position for longer than 2 seconds.

   Note: Some red blood cells (RBC) may be present on the surface of the SepMate™-50 insert after centrifugation. This will not affect performance.

6. Wash enriched MNC with PBS + 2% FBS. Repeat wash.

SEPMATE™-50 PROCEDURE

Numbers in brackets refer to steps under "Directions for Use".



STEMCELL™ TECHNOLOGIES

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NOTES

Samples

SepMate™-50 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™-50 with samples other than human whole peripheral blood or cord blood please contact Technical Support at techsupport@stemcell.com.

SepMate™-50 is designed to process 5 - 17 mL of initial sample.

A minimum red blood cell volume of 2 mL is required. For patient samples with low hematocrit, the minimum sample volume may therefore be greater than 5 mL.

There is a maximum red blood cell volume of 12 mL. For patient samples with very high hematocrit, the maximum sample volume may therefore be less than 17 mL.

Density Gradient Medium

Density gradient medium refers to Ficoll-Paque™ PLUS or other similar density gradient media.

Recommended Medium

The recommended medium is Dulbecco's phosphate buffered saline with 2% fetal bovine serum (PBS + 2% FBS, Catalog #07905).

Conversion of *g* to RPM

To convert *g* to rpm, use the following formula:

$$RPM = \sqrt{\frac{RCF}{(1.118 \times 10^{-5}) \times (Radius)}}$$

Where: RPM = centrifuge speed in revolutions per minute
RCF = relative centrifugal force (*g*)
Radius = radius of centrifuge rotor in centimeters (cm)

SUPPLEMENTARY PROCEDURE

Use of SepMate™-50 with RosetteSep™ Cocktails

SepMate™-50 tubes can be used with RosetteSep™ cell enrichment cocktails to isolate specific cell types from human whole blood. For available RosetteSep™ cocktails please refer to www.rosettesep.com.

To use SepMate™-50 with RosetteSep™ cocktails:

1. Add RosetteSep™ 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 performance.
3. Follow the steps under SepMate™-50 "Directions for Use", on reverse page.

   Note: Use density gradient medium recommended in the RosetteSep™ cocktail Product Information Sheet.