PRODUCT INFORMATION SHEET

SepMate™

STEMCELL™ TECHNOLOGIES

SepMate™-50

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

SepMate™-15

CATALOG #15415 20 TUBES/BAG
CATALOG #15420 5X20 TUBES/BAG

INTENDED USE

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

PRODUCT DESCRIPTION

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 density gradient medium to the SepMate™ tube by carefully pipetting it through the central hole of the SepMate™ insert. Refer to Table 1 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. 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. 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.
4. 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.
5. 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. This will not affect performance.
6. Wash enriched MNCs with PBS + 2% FBS. Repeat wash.

   Note: Centrifuging at 300 x g for 8 minutes at room temperature, with the brake on, is recommended.

SEPMATE™ PROCEDURE

Numbers in brackets refer to steps under Directions for Use.

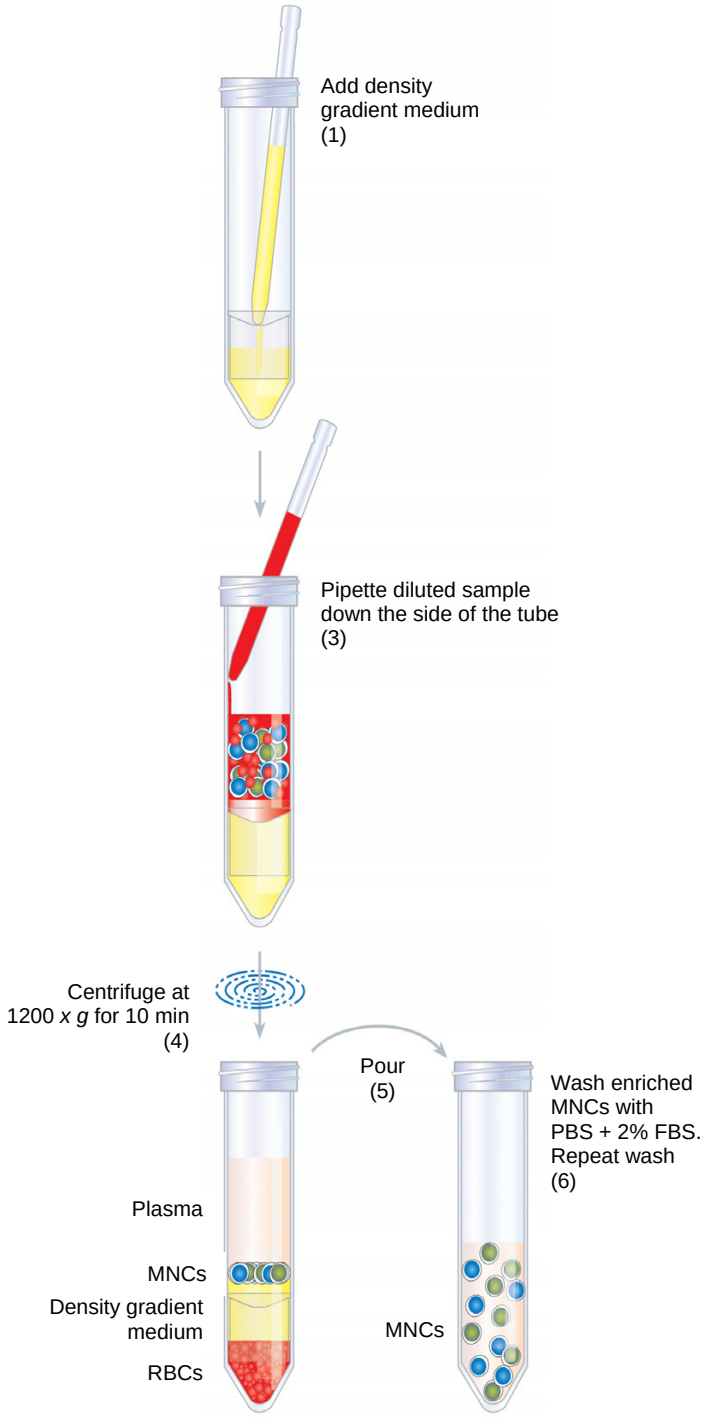

Table 1: Density Gradient Medium Volumes

SEPMATE™ TUBE	INITIAL BLOOD SAMPLE (mL)	DENSITY GRADIENT MEDIUM (mL)
15	0.5 - 4.0	4.5
15	> 4 - 5	3.5
50	4 - 17	15

NOTES

Samples

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 human whole peripheral blood or cord blood please contact STEMCELL Technologies' Technical Support at techsupport@stemcell.com.

SepMate™-15

SepMate™-15 is designed to process 0.5 - 5 mL of initial sample.

A minimum packed RBC volume of 0.25 mL is required. For patient samples with low hematocrits, the minimum sample volume may therefore be greater than 0.5 mL.

There is a maximum packed RBC volume of 3 mL. For patient samples with very high hematocrits, the maximum sample volume may therefore be less than 5 mL.

SepMate™-50

SepMate™-50 is designed to process 4 - 17 mL of initial sample.

A minimum packed RBC volume of 2 mL is required. For patient samples with low hematocrits, the minimum sample volume may therefore be greater than 4 mL.

There is a maximum packed RBC volume of 12 mL. For patient samples with very high hematocrits, the maximum sample volume may therefore be less than 17 mL.

Density Gradient Medium

Density gradient medium refers to Lymphoprep™ (Catalog #07801), Ficoll-Paque™ PLUS or other similar density gradient media.

Recommended Medium

The recommended medium is 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)

Troubleshooting

If the density gradient medium above the SepMate™ insert appears red after centrifugation (i.e. some RBCs have not pelleted), the SepMate™ tube can be spun at 1200 x *g* for another 10 minutes with the brake on. This situation may occur with samples that are older than 24 hours.

Platelet Removal (optional)

Platelets present in the plasma layer may be removed from the enriched MNCs in one of the following ways:

- In step 5, pipette off the supernatant above the MNC layer before pouring
- In step 6, perform one of the washes at 120 x *g* for 10 minutes at room temperature, with the brake off

SUPPLEMENTARY PROCEDURE

Use of SepMate™ with RosetteSep™ Cocktails

SepMate™ 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™ 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 to this procedure. It will have minimal effect on performance.
3. Follow the steps under SepMate™ Directions for Use, on reverse page.

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