SepMate™

SepMate™-50
Catalog #86450 100 tubes
Catalog #86460 500 tubes

SepMate™-15
Catalog #86415 100 tubes
Catalog #86420 500 tubes

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Intended Use

SepMate™ is used to isolate mononuclear cells (MNCs, comprising lymphocytes and monocytes) from whole blood or bone marrow by density centrifugation.

For research use only.

Product Description

MNCs are commonly isolated by density centrifugation. With this method, defibrinated or anticoagulant-treated blood is carefully layered on a density gradient medium and centrifuged for a short period of time. Differential migration during centrifugation results in the formation of layers containing different cell types. The bottom layer contains erythrocytes which have been aggregated by the density gradient medium and therefore sediment completely through the density gradient medium. The layer immediately above the erythrocyte layer contains mostly granulocytes, which at the osmotic pressure of the density gradient medium solution attain a density great enough to migrate through the density gradient medium layer. Because of their lower density, the MNCs are found at the interface between the plasma and the density gradient medium with other slowly sedimenting particles (platelets). The MNCs are carefully recovered from the interface and washed.

The specialized insert in SepMate™ minimizes mixing of the sample and the density gradient medium, thereby avoiding the need for careful layering and careful cell removal from the interface. Density gradient medium is pipetted through a central hole in the insert, partially filling the tube. Whole blood is then rapidly pipetted down the side of the tube to rest upon the density gradient medium. After centrifugation for 10 minutes with the brake on, the enriched cell layer is simply poured off into a new tube, while the density gradient medium, erythrocytes, and granulocytes are retained below the insert. The MNCs are washed and are then ready for use.

Storage and Stability

Store at ambient temperature. Product stable at ambient temperature until expiry date on label. Do not use if tubes are damaged.

Warnings and Precautions

1. For professional users only.
2. Do not re-use SepMate™ tubes.
3. Do not use SepMate™ tubes after the expiry date indicated on the label.
4. This product should be handled by trained personnel observing good laboratory practices. Dispose of tubes and biologic waste in accordance with appropriate local, state, or national biohazard safety regulations.
5. SepMate™ can be used with human whole peripheral blood, bone marrow, and cord blood samples. It is not intended for use with leukapheresis samples, buffy coat samples, or samples older than 48 hours.
6. Centrifuge tubes at recommended settings.
7. Following centrifugation, cells may aggregate on the SepMate™ tube wall above the MNC layer. This aggregation is normal and is influenced by sample quality and age, and type of anticoagulant used. This aggregation is not related to the use of SepMate™. The cells can be dislodged by using a pipette tip to scrape the side of the tube.

Microbial State

SepMate™ tubes were irradiated by an electron beam process that conforms to the applicable requirements of ISO 11137-1. Do not use if the integrity of the packaging is compromised. Do not re-use.

Materials Required but Not Provided

Density Gradient Medium

Lymphoprep™ (Catalog #07801), Ficoll-Paque™ PLUS, or any similar medium with a density of 1.077 g/mL designed for the separation of mononuclear cells.

Recommended Medium

Dulbecco's Phosphate Buffered Saline with 2% Fetal Bovine Serum (PBS + 2% FBS; Catalog #07905).

Directions for Use

Ensure that sample, recommended medium (PBS + 2% FBS), density gradient medium (see Materials Required but Not Provided), 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. These bubbles 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.

   NOTE: Different makes and models of centrifuges may provide different rates of deceleration when braking. If a layer of MNCs is not visible following centrifugation or the recovery of MNCs is low, reduce the rate of deceleration (i.e. braking) to medium or low.
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. 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.
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.

   NOTE: To remove platelets from the enriched MNCs, perform one of the washes at 120 x *g* for 10 minutes at room temperature, with the brake off.

   NOTE: 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 step may be necessary when processing samples that are older than 24 hours.

SepMate™ Procedure

Numbers in brackets refer to steps under Directions for Use.

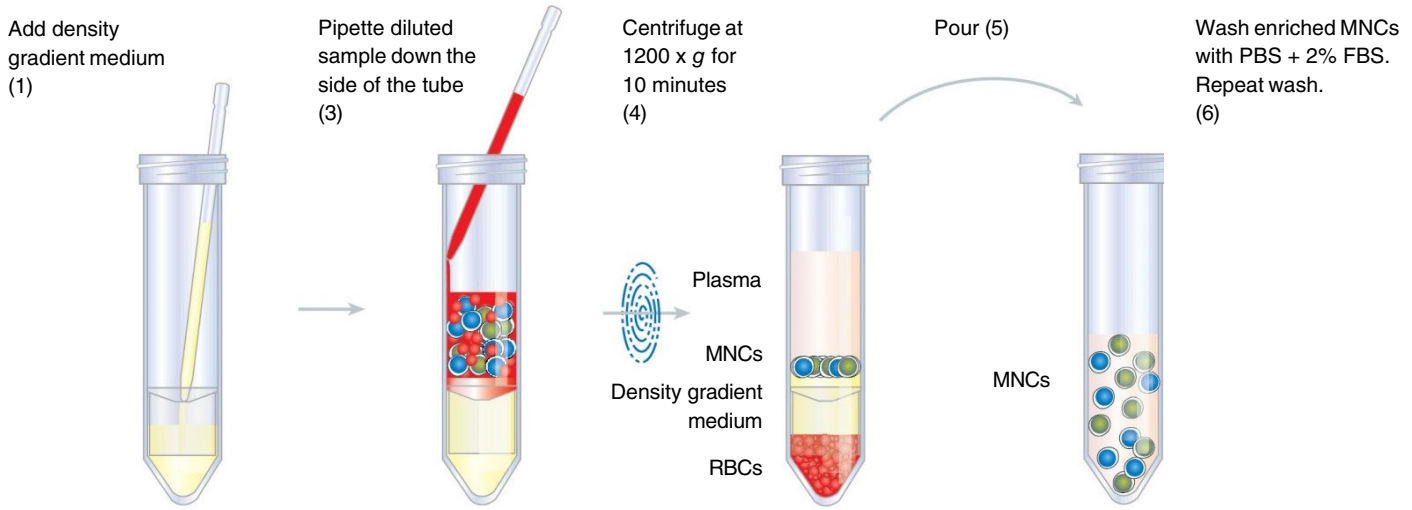

Table 1. Sample and Density Gradient Medium Volumes

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

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, 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 appropriate RosetteSep™ Product Information Sheet.
2. Incubate at room temperature (15 - 25°C) for 10 minutes.
   NOTE: The 10-minute incubation time is specific to this procedure. It will have minimal effect on performance.
3. Follow the steps for SepMate™ under Directions for Use.
   NOTE: Use density gradient medium recommended in the RosetteSep™ Product Information Sheet.

Notes

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 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 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 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 samples with very high hematocrits, the maximum sample volume may therefore be less than 17 mL.

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)

Technical Assistance

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