RosetteSep® Procedure:

Ensure that blood sample, PBS + 2% FBS (Catalog #07905), density medium (See Notes & Tips, opposite page) and centrifuge are all at room temperature.

1. Add RosetteSep® Human Total Lymphocyte Enrichment Cocktail at **50 µL/mL** of whole blood* (e.g. for 2 mL of whole blood, add 100 µL of cocktail). Mix well.

   *If using samples other than fresh whole blood, please see Notes and Tips

2. Incubate 20 minutes at room temperature.

3. Dilute sample with an equal volume of PBS + 2% FBS and mix gently.

4. Layer the diluted sample on top of the density medium
   OR
   Layer the density medium underneath the diluted sample.

   Be careful to minimize mixing of density medium and sample.

   See table below for volume recommendations. With 50 mL centrifuge tubes, we suggest using a minimum of 15 mL density medium to make it easier to remove the enriched cell layer.

Recommended Volumes and Tube Sizes			
Whole Blood	PBS + 2% FBS	Density Medium	Tube Size
1 mL	1 mL	1.5 mL	5 mL
2 mL	2 mL	3 mL	14 mL
3 mL	3 mL	3 mL	14 mL
4 mL	4 mL	4 mL	14 mL
5 mL	5 mL	15 mL	50 mL
10 mL	10 mL	15 mL	50 mL
15 mL	15 mL	15 mL	50 mL

5. Centrifuge for 20 minutes at 1200 x *g* (See Notes and Tips) at room temperature, with the brake off.

6. Remove the enriched cells from the density medium : plasma interface.

   Sometimes it is difficult to see the cells at the interface, especially when very rare cells are enriched. It is advisable to remove some of the density medium along with the enriched cells in order to ensure their complete recovery.

7. Wash enriched cells with PBS + 2% FBS. Repeat.

8. Use enriched cells as desired. We recommend that enriched samples are lysed with ammonium chloride to remove residual red blood cells prior to flow cytometric analysis (this can be done as one of the wash steps) or if residual red blood cells will interfere with subsequent assays.

STEMCELL Technologies

In North America
Tel: 1.604.877.0713
Fax: 1.604.877.0704
Toll Free Tel: 1.800.667.0322
Toll Free Fax: 1.800.567.2899
e-mail: info@stemcell.com
www.stemcell.com

In Europe
Tel: +33.(0).4.76.04.75.30
Fax: +33.(0).4.76.18.99.63
Toll Free Tel: 00.800.7836.2355
Toll Free Fax: 00.800.7836.2300
e-mail: info.eu@stemcell.com

In Australia
Fax: +61.(03).9338.4320
Toll Free Tel: 1.800.060.350
e-mail: info.aus@stemcell.com

In Singapore
Tel: (65) 6776 7754
Fax: (65) 6776 7114
e-mail: info.sg@stemcell.com

October 2009

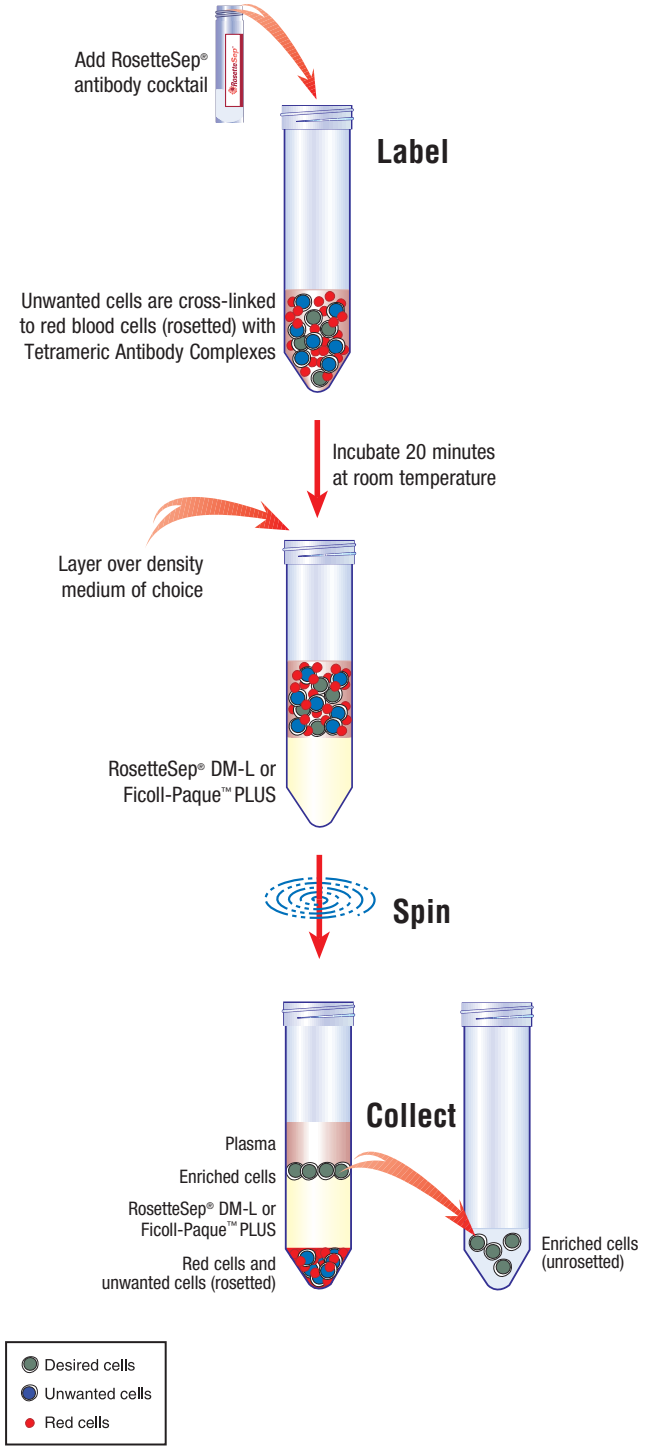
#29947

PROCEDURE

RosetteSep®

Human Total Lymphocyte Enrichment Cocktail

CATALOG #15563



Catalog #15563 5 mL

RosetteSep® Product Information Sheet

PRODUCT DESCRIPTION & APPLICATIONS:

The RosetteSep® Human Total Lymphocyte Enrichment Cocktail is designed to enrich lymphocytes from whole blood.

ROSETTESEP® LABELING OF HUMAN CELLS:

The RosetteSep® antibody cocktail crosslinks unwanted cells in human whole blood to multiple red blood cells (RBCs), forming immunorosettes (Figure 1). This increases the density of the unwanted (rosetted) cells, such that they pellet along with the free RBCs when centrifuged over a buoyant density medium such as RosetteSep® DM-L or Ficoll-Paque™ PLUS. Desired cells are never labeled with antibody and are easily collected as a highly enriched population at the interface between the plasma and the buoyant density medium.

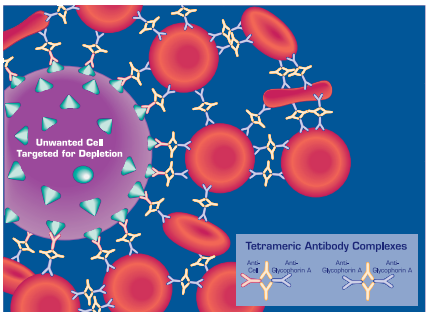


Figure 1.
Rosette of Unwanted Cell and RBCs Formed by RosetteSep® Tetrameric Antibody Complexes (TAC)

NOTES AND TIPS:

Recommended Medium. Phosphate Buffered Saline (PBS) + 2% Fetal Bovine Serum (FBS) (Catalog #07905).

Density Medium. Density medium refers to RosetteSep® DM-L (Catalog #15705) or Ficoll-Paque® (Catalog #07957). Recovery of lymphocytes may be improved with the use of STEMCELL's unique density medium, RosetteSep® DML.

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

Samples Other than Whole Blood. Although RosetteSep® has been optimized for use with whole blood, cells can be enriched from other sources (i.e. buffy coat, leukaphereses). The concentration of nucleated cells in the sample should not exceed 5 x 10^7 cells/mL, and red blood cells (RBCs) should be present at a ratio of at least 30-50 RBCs per nucleated cell.

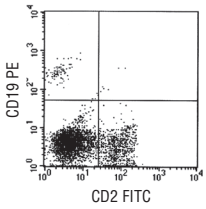
ASSESSING PURITY:

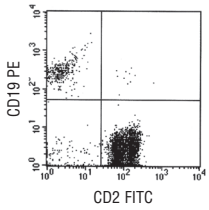
Purity of lymphocytes can be measured by flow cytometry after staining with fluorochrome conjugated antibodies against CD2 (e.g. FITC anti-CD2, Catalog #10401) and CD19 (e.g. FITC anti-CD19, Catalog #10409). Lymphocytes are either $CD2^+$ or $CD19^+$ (Note that $CD2^+CD16^+$ NK cells are removed during the RosetteSep® procedure).

TYPICAL ROSETTESEP® LYMPHOCYTE ENRICHMENT PROFILE:

Start: 20% $CD2^+$ or $CD19^+$ Cells

Enriched: 95% $CD2^+$ or $CD19^+$ Cells
34% Recovery of $CD2^+$ or $CD19^+$ Cells*





Results (mean ±1 S.D.): n=5
Purity: 94 ± 2% $CD2^+$ or $CD19^+$ Cells Recovery: 34 ± 6% $CD2^+$ or $CD19^+$ Cells
*Recovery reflects depletion of $CD2^+CD16^+$ NK cells.

COMPONENT DESCRIPTION:

RosetteSep® Human Total Lymphocyte Enrichment Cocktail.
This cocktail contains a combination of mouse and rat monoclonal antibodies purified from mouse ascites fluid or hybridoma culture supernatant. Purified by affinity chromatography using Protein A or Protein G Sepharose. These antibodies are bound in bispecific TAC which are directed against cell surface antigens in human hematopoietic cells (CD16, CD36, CD66b) and glycophorin A on red blood cells. The mouse monoclonal antibody subclass is IgG_1. It should be kept in mind that this product is a biological reagent, and as such cannot be completely characterized or quantified. Some variability is unavoidable.

STABILITY AND STORAGE:

RosetteSep® Human Total Lymphocyte Enrichment Cocktail.
Stable at 4°C for two years. Do not freeze this product. Contents sterile in unopened tube. This product may be shipped at room temperature, and should be refrigerated upon receipt.

STEMCELL Technologies

In North America
Tel: 1.604.877.0713
Fax: 1.604.877.0704
Toll Free Tel: 1.800.667.0322
Toll Free Fax: 1.800.567.2899
e-mail: info@stemcell.com
www.stemcell.com

In Europe
Tel: +33.(0).4.76.04.75.30
Fax: +33.(0).4.76.18.99.63
Toll Free Tel: 00.800.7836.2355
Toll Free Fax: 00.800.7836.2300
e-mail: info.eu@stemcell.com

In Australia
Fax: +61.(03).9338.4320
Toll Free Tel: 1.800.060.350
e-mail: info.aus@stemcell.com

In Singapore
Tel: (65) 6776 7754
Fax: (65) 6776 7114
e-mail: info.sg@stemcell.com

October 2009

#29947