

RosetteSep[®] HLA Procedure:

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

1. Add RosetteSep[®] HLA Human B Cell Enrichment Cocktail at **40 µL/mL** of whole blood* (e.g. for 2 mL of whole blood, add 80 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

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


to remove the enriched cell layer.

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.

PROCEDURE



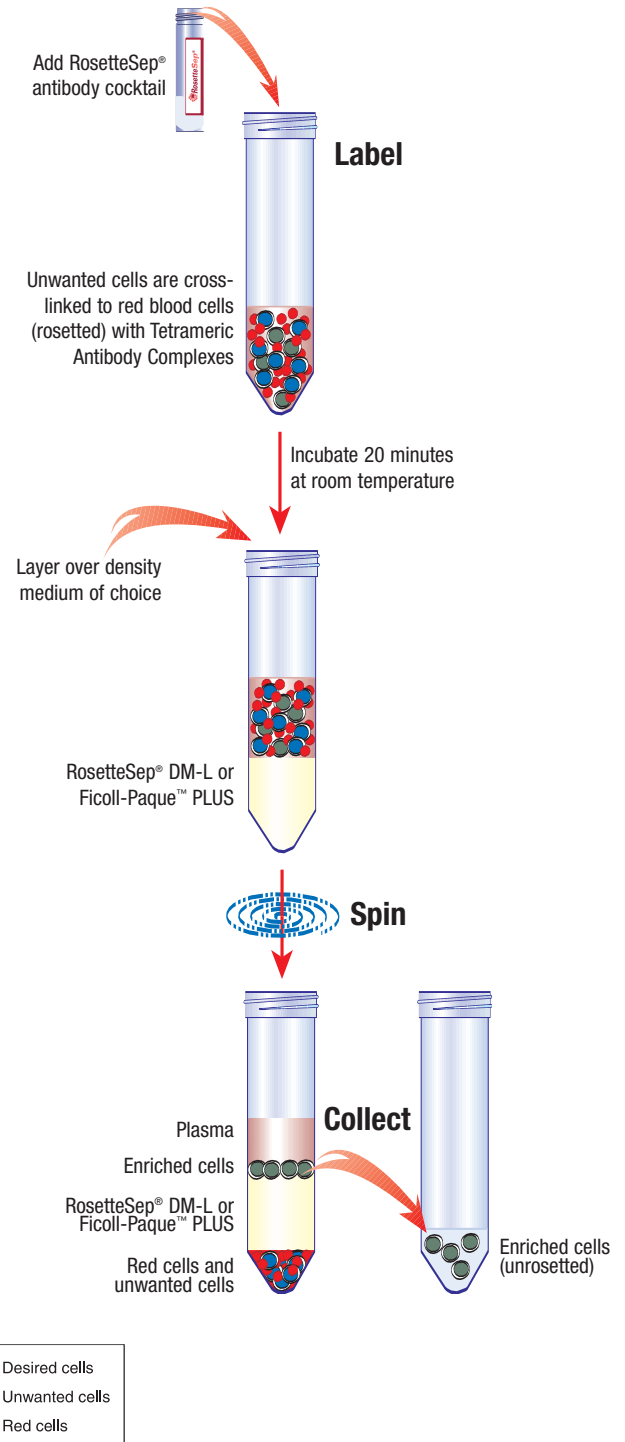
RosetteSep[®] HLA



Version 1.0.0

Human B Cell Enrichment Cocktail

CATALOG #15064HLA
#15084HLA



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Catalog #15064HLA	10 mL	For labeling 250 mL of whole blood
Catalog #15084HLA	40 mL	For labeling 1000 mL of whole blood

PRODUCT DESCRIPTION & APPLICATIONS:

The RosetteSep® HLA Human B cell enrichment cocktail is designed to enrich B cells 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, so 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.

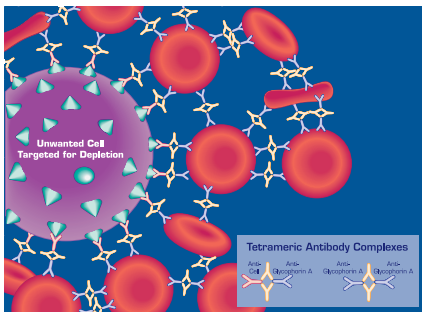


Figure 1.
Rosette of Unwanted Cell and RBCs Formed by RosetteSep® Tetrameric Antibody Complexes (TAC)

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™ PLUS (Catalog #07957). Recovery of lymphocytes may be improved with the use of StemCell's unique density medium, RosetteSep® DM-L.

Conversion of g to rpm. To convert g to rpm, use the following formula:

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

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 5×10^7 cells/mL, and red blood cells (RBCs) should be present at a ratio of at least 30 - 50 RBCs per nucleated cell.

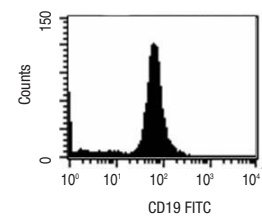
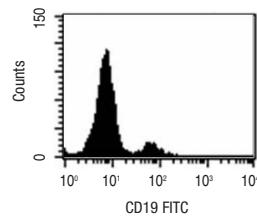
ASSESSING PURITY:

Purity of B cells can be measured by flow cytometry after staining with a fluorochrome conjugated antibody against CD19 (e.g. FITC anti-CD19, Catalog #10409), CD20 (e.g. FITC anti-CD20, Catalog #10410) or other B cell markers.

TYPICAL ROSETTESEP® HLA B CELL ENRICHMENT PROFILE:

Start: 3.8% CD19⁺ Cells

Enriched: 94.6% CD19⁺ Cells



Starting with whole blood, the CD19⁺ cell content of the enriched fraction typically ranges from 59.3 - 97.2%.

COMPONENT DESCRIPTION:

RosetteSep® HLA Human B Cell Enrichment Cocktail code #15024HC

This cocktail contains a combination of mouse and rat monoclonal antibodies purified from mouse ascites fluid or hybridoma culture supernatant, by affinity chromatography using Protein A or Protein G Sepharose. These antibodies are bound in bispecific TAC which are directed against cell surface antigens on human hematopoietic cells (CD2, CD3, CD16, CD36, CD56, CD66b) and glycoprotein A on red blood cells. The mouse monoclonal antibody subclass is IgG₁. 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® HLA Human B Cell 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.

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