

RosetteSep[®] Procedure:

Ensure that blood sample, PBS + 2% FBS (Catalog #07905), Ficoll-Paque[®] (Catalog #07907) and centrifuge are all at room temperature.

1. Add EDTA to whole blood to a final concentration of 1 mM (i.e. add 10 µL of a 100 mM solution of EDTA per 1 mL of whole blood). Add RosetteSep[®] Human Monocyte 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 1 mM EDTA. Mix gently.

4. Layer the diluted sample on top of the Ficoll-Paque[®]
OR
Layer the Ficoll-Paque[®] underneath the diluted sample.

Be careful to minimize mixing of Ficoll-Paque[®] and sample.

See table below for volume recommendations. With 50 mL centrifuge tubes, we suggest using a minimum of 15 mL Ficoll-Paque[®] to make it easier to remove the enriched cell layer.

Recommended Volumes and Tube Sizes			
Whole Blood	PBS + 2% FBS + 1 mM EDTA	Ficoll-Paque [®]	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 </td <td>15 mL</td> <td>50 mL</td>	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 Ficoll-Paque[®] : 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 Ficoll-Paque[®] along with the enriched cells in order to ensure their complete recovery.

7. Wash enriched cells with PBS + 2% FBS and 1 mM EDTA. 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.

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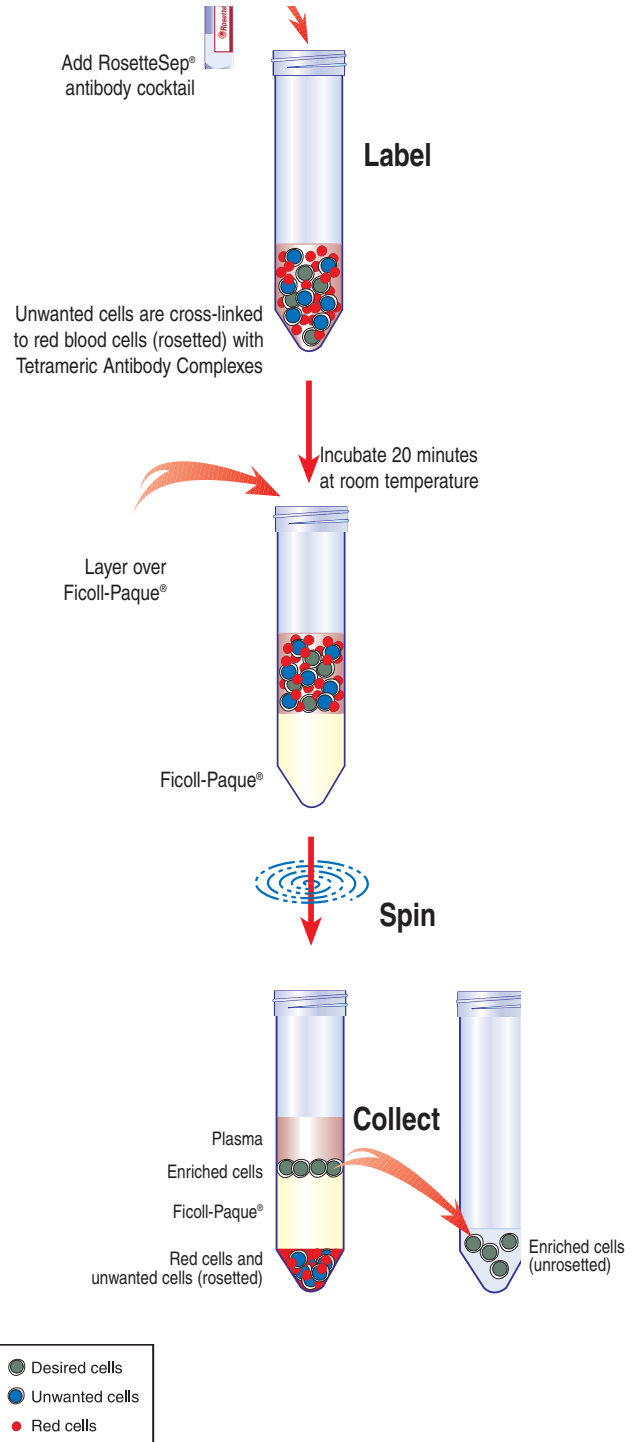
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PROCEDURE

Human Monocyte Enrichment Cocktail

CATALOG #15028/15068



Catalog #15028	2 mL	For labeling 40 mL of whole blood
Catalog #15068	10 mL	For labeling 200 mL of whole blood

PRODUCT DESCRIPTION & APPLICATIONS:

The RosetteSep® Human Monocyte enrichment cocktail is designed to enrich monocytes 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 Ficoll-Paque®. 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.

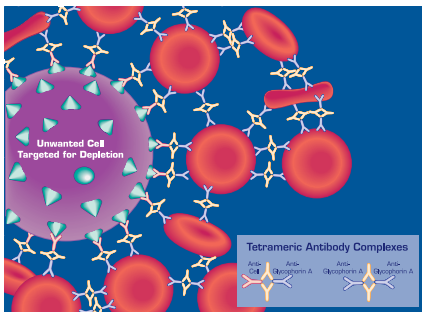


Figure 1.
Rosette of Unwanted Cell and RBCs Formed by RosetteSep® Tetrameric Antibody Complexes (TAC)

NOTES AND TIPS:

Recommended Medium. Phosphate Buffered Saline (PBS) + 2% Fetal Bovine Serum (FBS) (Catalog #07905) + 1 mM EDTA.

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.

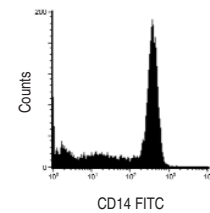
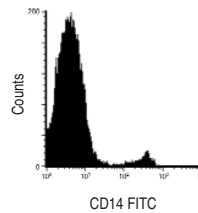
ASSESSING PURITY:

Purity of Monocytes can be measured by flow cytometry after staining with a fluorochrome conjugated anti-CD14 antibody (e.g. FITC anti-CD14, Catalog #10406).

TYPICAL ROSETTESEP® MONOCYTE ENRICHMENT PROFILE:

Start: 7% CD14⁺ Cells

Enriched: 78% CD14⁺ Cells
72% Recovery of CD14⁺ Cells



Results (mean \pm 1 S.D.):
Purity: $71 \pm 9\%$ CD14⁺ Cells

n=19
Recovery: $57 \pm 23\%$ CD14⁺ Cells

COMPONENT DESCRIPTION:

RosetteSep® Human Monocyte Enrichment Cocktail. 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, CD8, CD19, CD56, CD66b) and glycophorin 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® Human Monocyte 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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