

RosetteSep™ PROCEDURE:

Before commencing, ensure that blood sample, PBS + 2% FBS (Catalog #07905), density medium (See Notes & Tips, reverse side) and centrifuge are all at room temperature (15 - 25°C).

1. Add RosetteSep™ Human NK Cell Enrichment Cocktail at **50 µL/mL** of whole blood* (e.g. for 2 mL of whole blood, add 100 µL of cocktail). Mix well.
**Note: If using samples other than whole blood, please see Notes and Tips (reverse side).*
2. Incubate for **20 minutes** at room temperature (15 - 25°C).
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 (See Notes & Tips, reverse side).

OR

Layer the density medium underneath the diluted sample.

Be careful to minimize mixing of density medium and sample.

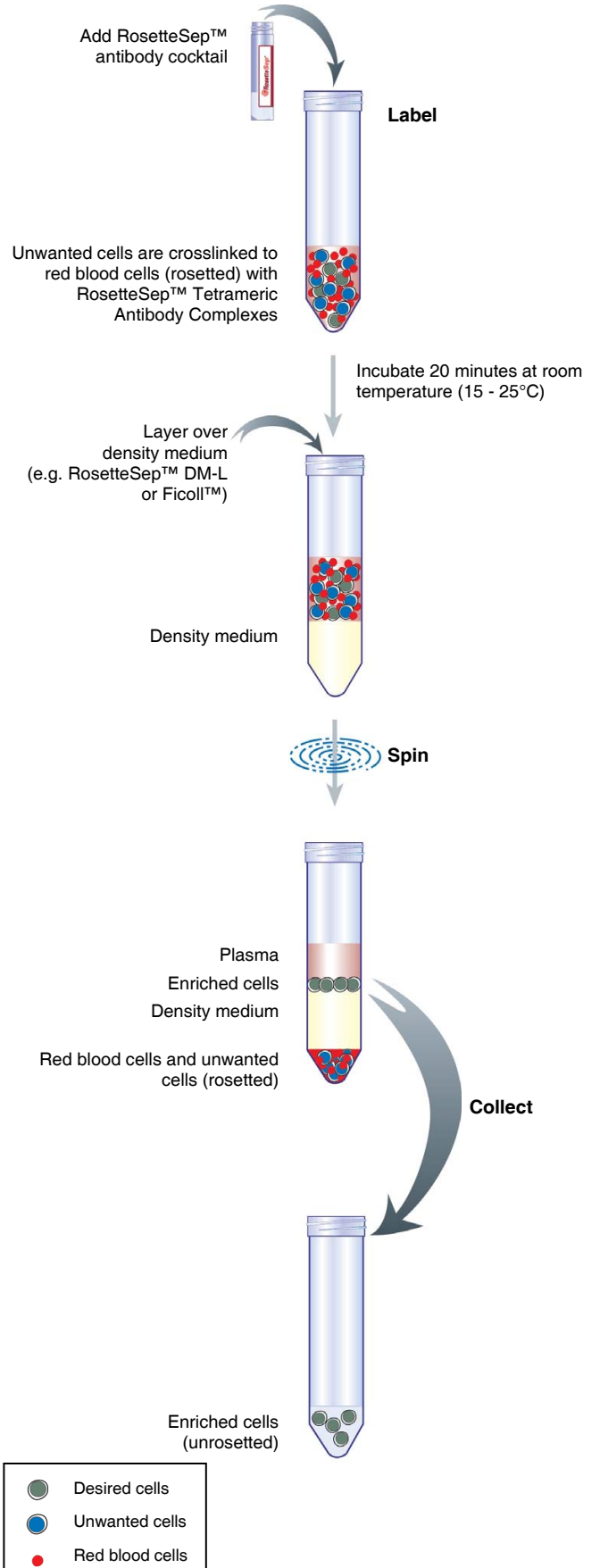
See Table 1 below for volume recommendations. With 50 mL centrifuge tubes, we suggest using a minimum of 15 mL density medium to make it easier to remove the enriched cell layer.

TABLE 1. RECOMMENDED VOLUMES AND TUBE SIZES

Whole Blood (mL)	PBS + 2% FBS (mL)	Density Medium (mL)	Tube Size (mL)
1	1	1.5	5
2	2	3	14
3	3	3	14
4	4	4	14
5	5	15	50
10	10	15	50
15	15	15	50

5. Centrifuge for **20 minutes** at 1200 x g (See Notes and Tips, reverse side) at room temperature (15 - 25°C), 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.

ROSETTESEP™ PROTOCOL DIAGRAM



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PRODUCT DESCRIPTION AND APPLICATIONS:

The RosetteSep™ Human NK Cell Enrichment Cocktail is designed to enrich NK 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, such that they pellet along with the free RBCs when centrifuged over a buoyant density medium such as RosetteSep™ DM-L (Catalog #15705) or Ficoll-Paque™ PLUS (Catalog #07957). 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.

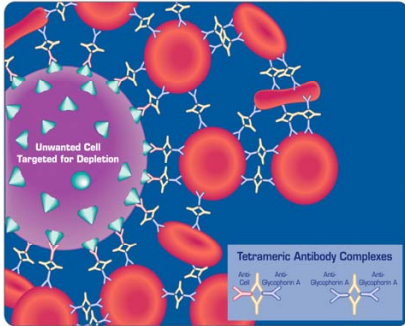


Figure 1.
 Rosette of Unwanted Cell and RBCs Formed by RosetteSep™ Tetrameric Antibody Complexes (TAC)

NOTES AND TIPS:

RECOMMENDED MEDIUM. The recommended medium is 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

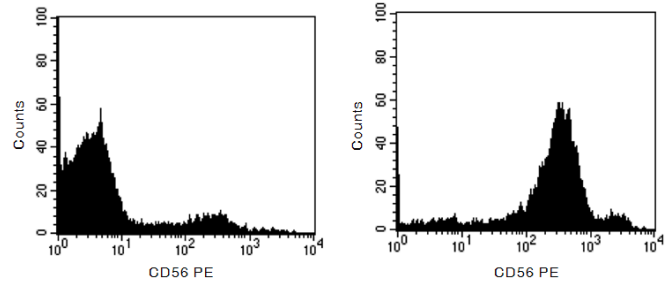
SAMPLES OTHER THAN WHOLE BLOOD. Although RosetteSep™ has been optimized for use with whole blood, cells can be enriched from other sources (e.g. buffy coat or leukapheresis samples). The concentration of nucleated cells in the sample should not exceed 5×10^7 cells/mL, and red blood cells (RBCs) should be present at a ratio of at least 30 - 50 RBCs per nucleated cell.

ASSESSING PURITY. The purity of NK cells can be measured by flow cytometry after staining with a fluorochrome-conjugated anti-CD56 antibody (e.g. PE anti-CD56, Catalog #10526).

TYPICAL ROSETTESEP™ HUMAN NK CELL ENRICHMENT PROFILE:

Start*: 7% CD56⁺ Cells

Enriched*: 94% CD56⁺ Cells



Starting with fresh peripheral blood the CD56⁺ cell content of the enriched fraction typically ranges from 80 - 98%.

*Note: Red blood cells were removed by lysis prior to flow cytometry.

COMPONENT DESCRIPTIONS:

ROSETTESEP™ HUMAN NK CELL ENRICHMENT COCKTAIL

CODE #15025C.1

This cocktail contains a combination of monoclonal antibodies bound in bispecific TAC which are directed against cell surface antigens on human hematopoietic cells (CD3, CD4, CD19, CD36, CD66b, CD123) and glycophorin A on RBCs. The mouse monoclonal antibody subclass is IgG₁. It should be noted 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 NK CELL ENRICHMENT COCKTAIL

Product stable at 2 - 8°C until expiry date as indicated on label. Contents have been sterility tested. Do not freeze this product. This product may be shipped at room temperature (15 - 25°C), and should be refrigerated upon receipt.

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