

Note: If using the kit #15276 (i.e. HetaSep™ with RosetteSep™ Complete Human Cord Blood Progenitor Enrichment Kit), please disregard this document and refer to **Document #28365** provided with the kit.

DIRECTIONS FOR USE

Ensure that blood sample, phosphate-buffered saline with 2% fetal bovine serum (PBS + 2% FBS; Catalog #07905), density gradient medium (see Notes and Tips, reverse page), and centrifuge are all at room temperature (15 - 25°C).

1. Add RosetteSep™ Enrichment Cocktail at **50 µL/mL** of cord blood (e.g. for 2 mL of cord blood, add 100 µL of cocktail). Mix well.
2. Incubate **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 gradient medium

OR

Layer the density gradient medium underneath the diluted sample.

Note: Be careful to minimize mixing of the density gradient medium and sample.

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

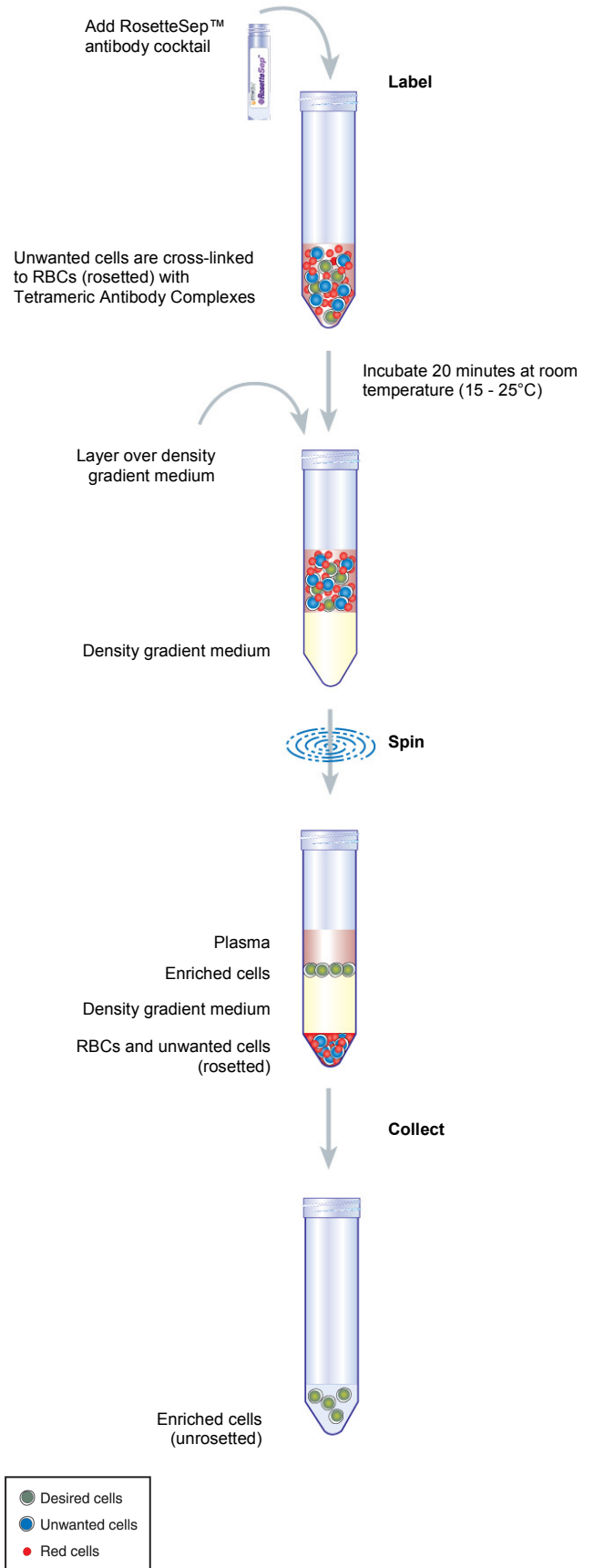
CORD BLOOD (mL)	PBS + 2% FBS (mL)	DENSITY GRADIENT MEDIUM (mL)	TUBE SIZE (mL)
1	1	1.5	5
2	2	3	14
3	3	3	14
4	4	4	14
5	5	15	50
10	10	15	50
15	15	15	50

5. Centrifuge for **20 minutes** at 1200 x g (see Notes and Tips) at room temperature (15 - 25°C), with the brake off.
6. Remove the enriched cells from the density gradient medium:plasma interface.

Note: 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 gradient 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 Solution (Catalog #07800) to remove residual red blood cells (RBCs) prior to flow cytometric analysis (this can be done as one of the wash steps) or if residual RBCs will interfere with subsequent assays.

ROSETTESEP™ PROTOCOL DIAGRAM



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TOLL FREE PHONE 1 800 667 0322 • PHONE +1 604 877 0713

INFO@STEMCELL.COM • TECHSUPPORT@STEMCELL.COM • FOR GLOBAL CONTACT DETAILS VISIT WWW.STEMCELL.COM

VERSION 1.3.0
DOCUMENT #28581

CATALOG #15026 2 mL For labeling up to 40 mL of blood
 CATALOG #15066 10 mL For labeling up to 200 mL of blood

PRODUCT DESCRIPTION AND APPLICATIONS:

The RosetteSep™ Human Hematopoietic Progenitor Cell Enrichment Cocktail is designed to enrich hematopoietic progenitor cells from blood.

ROSETTESEP™ LABELING OF HUMAN CELLS

The RosetteSep™ antibody cocktail crosslinks unwanted cells in human cord blood to multiple 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 density gradient medium. Desired cells are never labeled with antibody and are easily collected as a highly enriched population at the interface between the plasma and the density gradient medium.

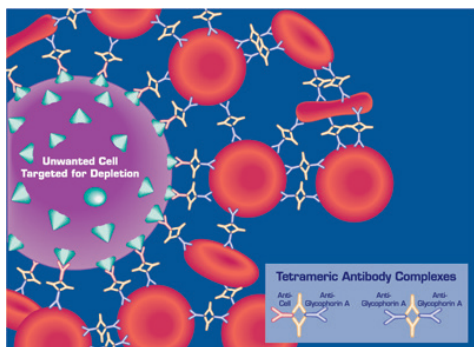


Figure 1 Rosette of unwanted cell and RBCs formed by RosetteSep™ Tetrameric Antibody Complexes (TACs)

NOTES AND TIPS

RECOMMENDED MEDIUM

The recommended medium is PBS + 2% FBS (Catalog #07905).

DENSITY GRADIENT MEDIUM

Density gradient medium refers to Lymphoprep™ (Catalog #07801), Ficoll-Paque™ PLUS, or other similar density gradient media.

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

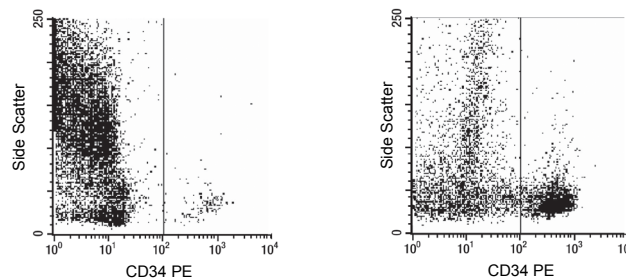
ASSESSING PURITY

Purity of hematopoietic progenitor cells can be measured by flow cytometry after staining with a fluorochrome-conjugated anti-CD34 antibody such as Anti-Human CD34 Antibody, Clone 581 (Catalog #60013).

TYPICAL ROSETTESEP™ HUMAN HEMATOPOIETIC PROGENITOR CELL ENRICHMENT PROFILE:

Start: 0.3% CD34+ Cells

Enriched: 30% CD34+ Cells



Starting with fresh human cord blood, the CD34+ cell content of the enriched fraction is typically 29 ± 9%.

COMPONENT DESCRIPTION:

ROSETTESEP™ HUMAN HEMATOPOIETIC PROGENITOR CELL ENRICHMENT COCKTAIL CODE #15186C

This cocktail contains a combination of monoclonal antibodies. These antibodies are bound in bispecific Tetrameric Antibody Complexes (TACs) which are directed against cell surface antigens on non-hematopoietic progenitor cells. 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.

Precipitate may be observed in the cocktail vial but will not affect performance.

STABILITY AND STORAGE:

ROSETTESEP™ HUMAN HEMATOPOIETIC PROGENITOR CELL ENRICHMENT COCKTAIL

Product stable at 2 - 8°C until expiry date as indicated on label. 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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