PROCEDURE

RosetteSep™

NEGATIVE SELECTION

STEMCELL™ TECHNOLOGIES

HUMAN CORD BLOOD PROGENITOR CELL ENRICHMENT COCKTAIL

CATALOG #15026 / 15066

*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 cord 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™ Human Progenitor 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.

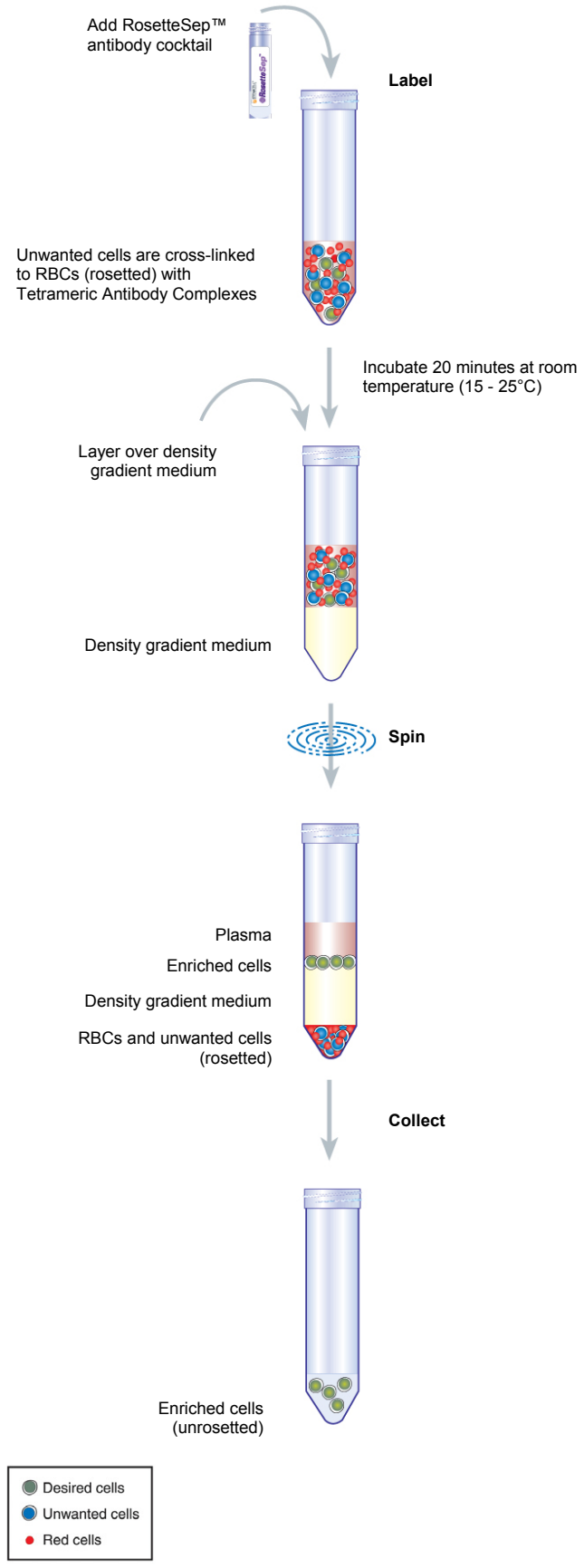
   See table below for volume recommendations. With 50 mL centrifuge tubes, we suggest using a minimum of 15 mL density gradient medium to make it easier to remove the enriched cell layer.

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 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



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

RosetteSep™

PRODUCT INFORMATION SHEET

PRODUCT DESCRIPTION AND APPLICATIONS:

The RosetteSep™ Human Cord Blood Progenitor Cell Enrichment Cocktail is designed to enrich epithelial tumor cells from cord 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.

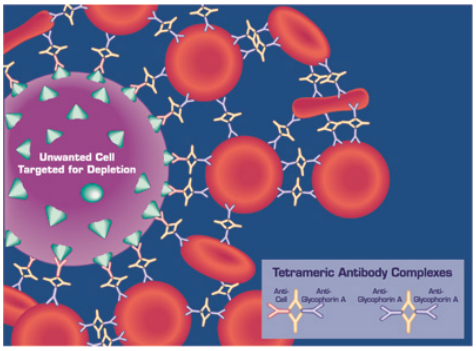


Figure 1 Rosette of unwanted cell and RBCs formed by RosetteSep™ Tetrameric Antibody Complexes (TACs)

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:

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

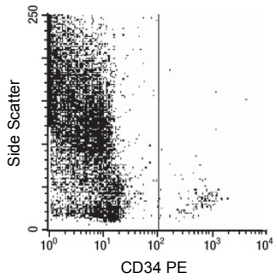
Where: RPM = centrifuge speed in revolutions per minute
RCF = relative centrifugal force (*g*)
Radius = radius of centrifuge rotor in centimeters (cm)

ASSESSING PURITY

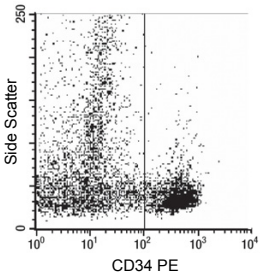
Purity of cord blood progenitor cells can be measured by flow cytometry after staining with a fluorochrome-conjugated anti-CD34 antibody (e.g. CD34 Antibody, Clone 8G12, FITC-Conjugated, Catalog #10413).

TYPICAL ROSETTESEP™ CORD BLOOD 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 PROGENITOR ENRICHMENT COCKTAIL

CODE #15026C

This cocktail contains a combination of mouse and rat monoclonal antibodies. These antibodies are bound in bispecific Tetrameric Antibody Complexes (TACs) which are directed against cell surface antigens on human hematopoietic cells (CD2, CD3, CD14, CD16, CD19, CD24, CD56, and CD66b) and glycophorin A on RBCs. 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 PROGENITOR ENRICHMENT COCKTAIL

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