PROCEDURE

RosetteSep™

NEGATIVE SELECTION

STEMCELL™ TECHNOLOGIES

CTC ENRICHMENT COCKTAIL CONTAINING ANTI-CD56

CATALOG #15137 / 15177

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™ CTC Enrichment Cocktail Containing Anti-CD56 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 fresh whole blood, please see Notes and Tips.
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.

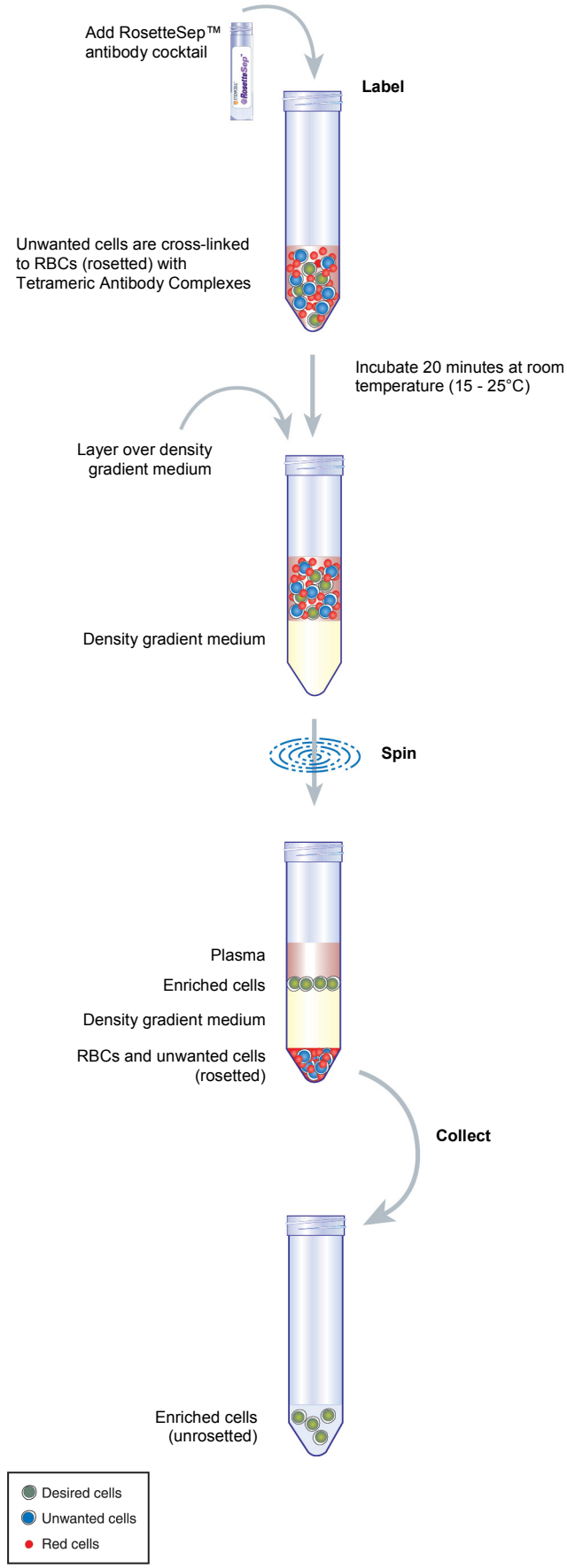
   See table below for volume recommendations. With 50 mL centrifuge tubes, we suggest using a minimum of 15 mL density gradient medium to make it easier to remove the enriched cell layer.

WHOLE 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 #15137	2 mL	For labeling 40 mL of whole blood
CATALOG #15177	10 mL	For labeling 200 mL of whole blood

RosetteSep™
PRODUCT INFORMATION SHEET

PRODUCT DESCRIPTION AND APPLICATIONS:

The RosetteSep™ CTC Enrichment Cocktail Containing Anti-CD56 is designed to enrich epithelial tumor cells from whole blood.

ROSETTESEP™ LABELING OF HUMAN CELLS

The RosetteSep™ antibody cocktail crosslinks unwanted cells in human whole 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.

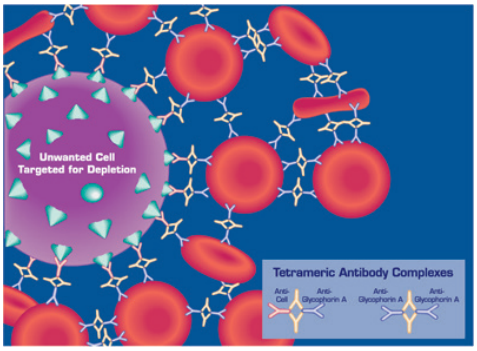


Figure 1 Rosette of unwanted cell and RBCs formed by RosetteSep™ Tetrameric Antibody Complexes (TACs)

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)

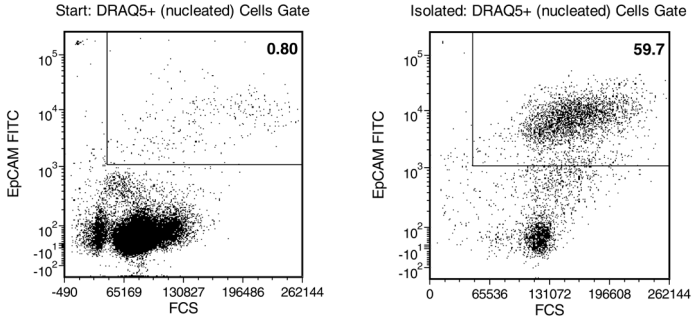
SAMPLES OTHER THAN WHOLE BLOOD

Although RosetteSep™ has been optimized for use with whole blood, cells can be enriched from other sources (i.e. buffy coat, leukapheresis). The concentration of nucleated cells in the sample should not exceed 5×10^7 cells/mL, and RBCs should be present at a ratio of at least 50 - 100 RBCs per nucleated cell.

ASSESSING PURITY

Purity of epithelial tumor cells can be measured by flow cytometry after staining with a fluorochrome-conjugated tumor-specific antibody.

TYPICAL ROSETTESEP™ CTC ENRICHMENT PROFILE:



In the example above, CAMA (epithelial tumor cell line) cells were seeded into whole blood at a starting frequency of 0.8%. The CAMA cell content of the enriched fraction is 60%. Typically 3.2 to 4.4 log depletion of targeted CD45+ cells is attained.

COMPONENT DESCRIPTION:

ROSETTESEP™ CTC ENRICHMENT COCKTAIL CONTAINING ANTI-CD56

CODE #15137C

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 (CD3, CD14, CD16, CD19, CD38, CD45, CD56, CD61, 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™ CTC ENRICHMENT COCKTAIL CONTAINING ANTI-CD56

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.

STEMCELL™ TECHNOLOGIES

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