


PROCEDURE



NEGATIVE SELECTION




**HUMAN BONE
MARROW
PROGENITOR CELL
PRE-ENRICHMENT
COCKTAIL**

CATALOG #15027 / 15067

DIRECTIONS FOR USE

Ensure that bone marrow 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™ Pre-Enrichment Cocktail at **50 µL/mL** of bone marrow (e.g. for 2 mL of bone marrow, 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.

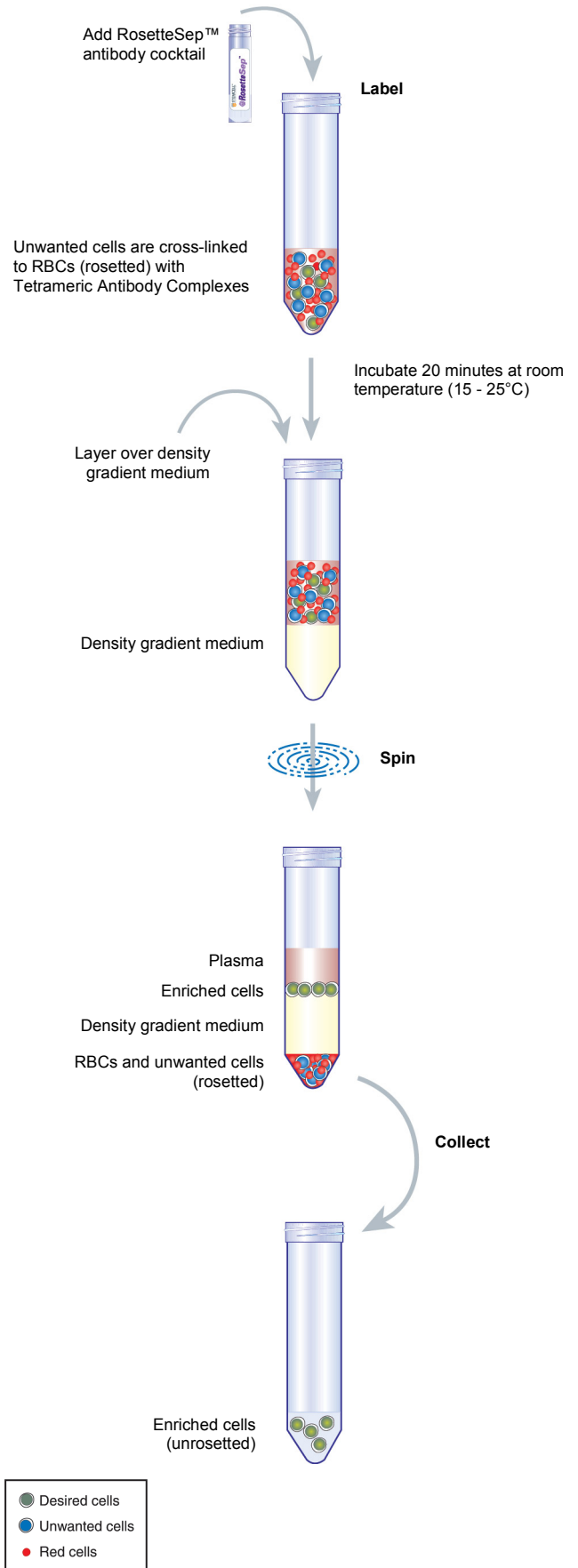
BONE MARROW (mL)	PBS + 2% FBS (mL)	DENSITY GRADIENT MEDIUM (mL)	TUBE SIZE (mL)
1	1	1.5	5
2	2	3	14
3	3	3	14
4	4	4	14
5	5	15	50
10	10	15	50
15	15	15	50

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



STEMCELL TECHNOLOGIES INC.'S QUALITY MANAGEMENT SYSTEM IS CERTIFIED TO ISO 13485 MEDICAL DEVICE STANDARDS. FOR RESEARCH USE ONLY. NOT INTENDED FOR HUMAN OR ANIMAL DIAGNOSTIC OR THERAPEUTIC USES.

CATALOG #15027 2 mL For labeling 40 mL of whole blood
 CATALOG #15067 10 mL For labeling 200 mL of whole blood

PRODUCT DESCRIPTION AND APPLICATIONS:

The RosetteSep™ Human Bone Marrow Progenitor Cell Pre-Enrichment Cocktail is designed to remove mature lineage-committed cells from whole bone marrow.

ROSETTESEP™ LABELING OF HUMAN CELLS

The RosetteSep™ antibody cocktail crosslinks unwanted cells in human whole blood or bone marrow 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.

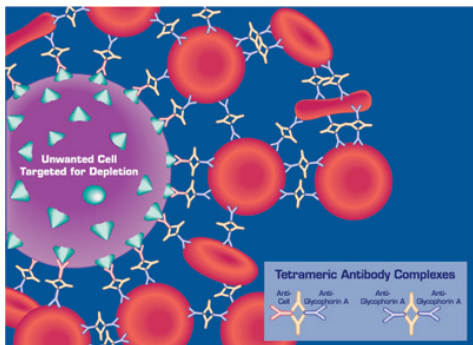


Figure 1 Rosette of unwanted cell and RBCs formed by RosetteSep™ Tetrameric Antibody Complexes (TACs)

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)

SAMPLES OTHER THAN BONE MARROW

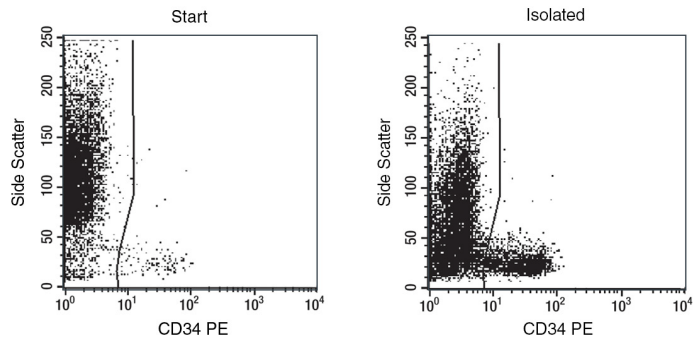
Although RosetteSep™ has been optimized for use with whole blood or bone marrow, cells can be enriched from other sources (i.e. buffy coat, leukaphereses). The concentration of nucleated cells in the sample should not exceed 5 x 10⁷ cells/mL, and RBCs should be present at a ratio of at least 50 - 100 RBCs per nucleated cell.

ASSESSING PURITY

Purity of bone marrow 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 BONE MARROW PROGENITOR CELL PRE-ENRICHMENT PROFILE:

Start: 0.6% CD34+ Cells
 De-bulked: 17% CD34+ Cells
 29-Fold Enrichment of CD34+ Cells
 51% Recovery of CD34+ Cells



Results (mean ± 1 SD): n=4
 Enrichment of CD34+ Cells: 25 ± 10 Fold
 Recovery: 29 ± 16% CD34+ Cells

Enrichment of CFC: 10 - 53 Fold n=3
 Recovery: 24 - 51% CFC

COMPONENT DESCRIPTION:

ROSETTESEP™ HUMAN BONE MARROW PROGENITOR CELL PRE-ENRICHMENT COCKTAIL CODE #15027C

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-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 BONE MARROW PROGENITOR CELL PRE-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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