PROCEDURE

RosetteSep™

NEGATIVE SELECTION

HUMAN MONOCYTE ENRICHMENT COCKTAIL

CATALOG #15028 / 15068

STEMCELL™ TECHNOLOGIES

V 2.1.0

RosetteSep™ PROCEDURE:

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

1. Add EDTA to whole blood to a final concentration of 1 mM (e.g. add 10 µL of a 100 mM solution of EDTA per 1 mL of whole blood). Add RosetteSep™ Human Monocyte 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 1 mM EDTA. Mix gently.
4. Layer the diluted sample on top of the density medium

   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

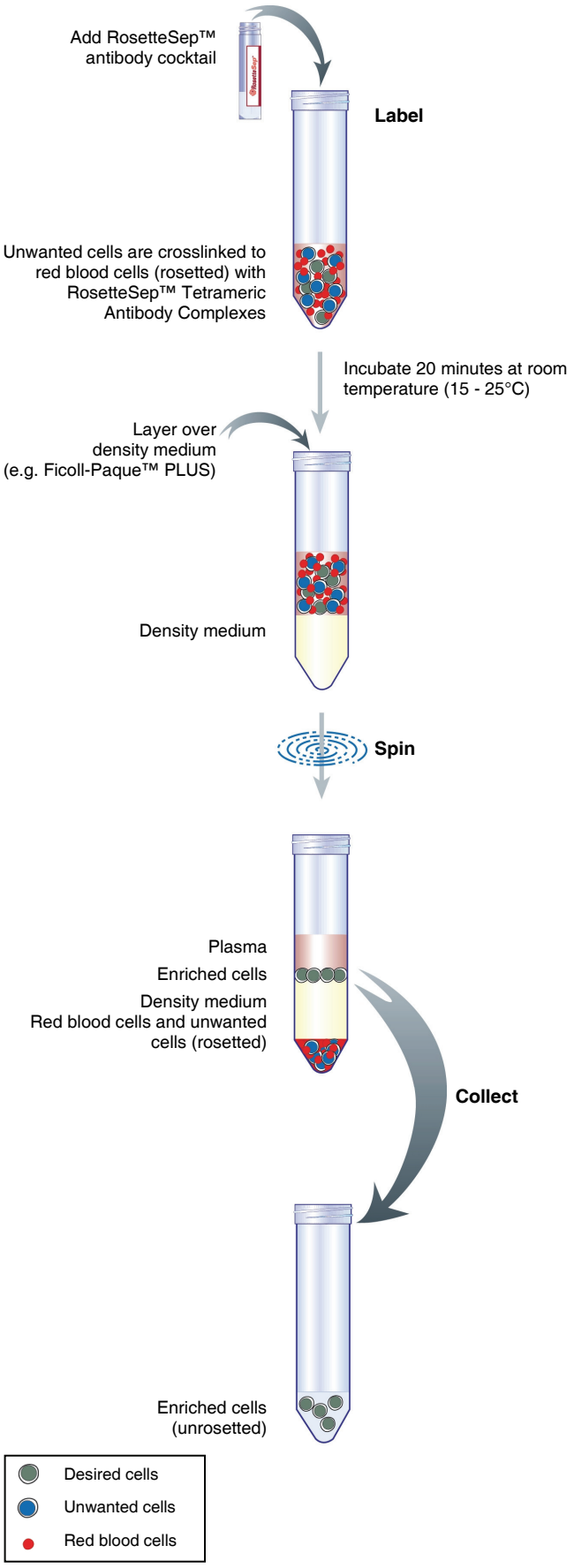
Whole Blood (mL)	PBS + 2 % FBS (mL)	Density Medium (mL)	Tube Size (mL)
1	1	1.5	5
2	2	3	14
3	3	3	14
4	4	4	14
5	5	15	50
10	10	15	50
15	15	15	50

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 and 1 mM EDTA. 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



CATALOG #15028	2 mL	For labeling 40 mL of whole blood
CATALOG #15068	10 mL	For labeling 200 mL of whole blood

RosetteSep™

PRODUCT INFORMATION SHEET

PRODUCT DESCRIPTION AND APPLICATIONS:

The RosetteSep™ Human Monocyte Enrichment Cocktail is designed to enrich monocytes 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 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.

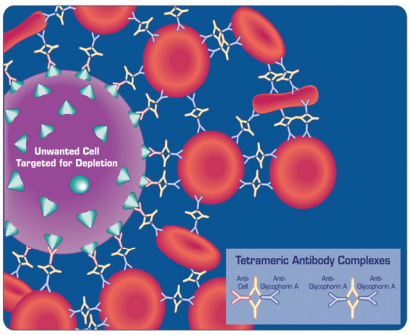


Figure 1.
Rosette of Unwanted Cell and RBCs Formed by RosetteSep™ Tetrameric Antibody Complexes (TAC)

NOTES AND TIPS:

RECOMMENDED MEDIUM. The recommended medium is Phosphate Buffered Saline (PBS) + 2% Fetal Bovine Serum (FBS) (Catalog #07905) with 1 mM EDTA added.

DENSITY MEDIUM. Density medium refers to Ficoll-Paque™ PLUS (Catalog #07957).

CONVERSION OF *g* TO RPM. To convert *g* to rpm, use the following formula:

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

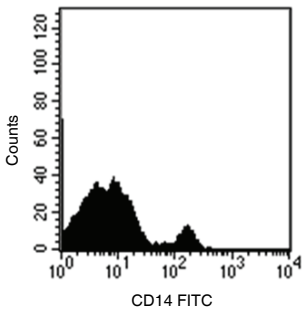
Where: RCF = relative centrifugal force (*g*)
RPM = centrifuge speed in revolutions per minute
Radius = radius of rotor in cm

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 x 10^7 cells/mL, and red blood cells (RBCs) should be present at a ratio of at least 30 - 50 RBCs per nucleated cell.

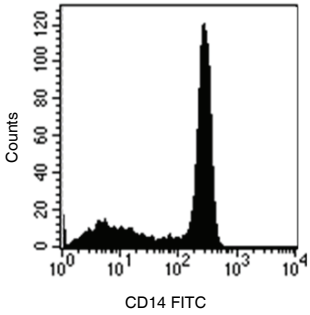
ASSESSING PURITY. The purity of monocytes can be measured by flow cytometry after staining with a fluorochrome-conjugated anti-CD14 antibody (e.g. PE anti-CD14, Catalog #10406).

TYPICAL ROSETTESEP™ HUMAN MONOCYTE ENRICHMENT PROFILE:

Start*: 9.6% $CD14^+$ Cells



Enriched*: 78% $CD14^+$ Cells



Starting with fresh peripheral blood the $CD14^+$ cell content of the enriched fraction typically ranges from 72% - 85%.

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

COMPONENT DESCRIPTIONS:

ROSETTESEP™ HUMAN MONOCYTE ENRICHMENT COCKTAIL **CODE #15028C.1**

This cocktail contains a combination of monoclonal antibodies bound in bispecific Tetrameric Antibody Complexes (TAC) which are directed against cell surface antigens on human hematopoietic cells (CD2, CD3, CD8, CD19, CD56, CD66b, CD123) and glycophorin A on RBCs. The mouse monoclonal antibody subclass is IgG_1. 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 MONOCYTE 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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