RosetteSep™ HLA Myeloid Cell Enrichment Kit

REF 15272HLA **For 20 tests (10 mL of whole blood per test)**

Components:

RosetteSep™ HLA Myeloid Cell Enrichment Cocktail	**5 x 2 mL**
RosetteSep™ DM-M Density Medium	**2 x 100 mL**

ENGLISH

INTENDED USE

RosetteSep™ cell enrichment cocktails are designed for the in vitro enrichment of specific cell subsets from human whole blood.

PRODUCT DESCRIPTION

The RosetteSep™ antibody cocktail crosslinks unwanted cells in human whole blood to multiple red blood cells (RBCs), forming immunorosettes. These dense immunorosettes pellet along with the free RBCs when centrifuged over a density gradient medium such as RosetteSep™ DM-M (Catalog #15725). 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.

RosetteSep™ HLA Myeloid Cell Enrichment Cocktail **REF #15272HC.1**

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, CD8, CD19, CD56) and glycophorin A on RBCs. The mouse monoclonal antibody subclass is IgG_1.

RosetteSep™ DM-M Density Medium **REF #15725**

RosetteSep™ DM-M Density Medium is a density separation medium designed specifically for use with the RosetteSep™ cocktail for the enrichment of human myeloid ($CD33^+$) cells.

Density: 1.085 g/mL

QUALITY CONTROL

RosetteSep™ HLA Myeloid Cell Enrichment Cocktail **REF #15272HC.1**

RosetteSep™ cell enrichment cocktails are manufactured using aseptic technique and tightly controlled processes.

Each lot of RosetteSep™ cell enrichment cocktail is sterility tested according to USP methods and Quality Control performance tested in cell separation assays using human whole blood.

RosetteSep™ DM-M Density Medium **REF #15725**

RosetteSep™ DM-M Density Medium is manufactured using aseptic technique and tightly controlled processes.

Each lot of RosetteSep™ DM-M Density Medium is sterility tested according to USP methods.

STORAGE AND STABILITY

RosetteSep™ HLA Myeloid Cell Enrichment Cocktail **REF #15272HC.1**

Store at 2 - 8°C. This product may be shipped at 15 - 25°C, but should be refrigerated upon receipt. Do not freeze. Product stable at 2 - 8°C until expiry date (EXP) on label.

RosetteSep™ DM-M Density Medium **REF #15725**

Store at 15 - 25°C. Storage at 2 - 8°C is acceptable, but ensure that the medium equilibrates to 15 - 25°C and invert bottle to mix contents before use. Keep protected from direct light. Product stable at 15 - 25°C until expiry date (EXP) on label.

WARNINGS AND PRECAUTIONS

1. For professional users only.
2. This product is for in vitro diagnostic use.
3. Do not use cocktail or density medium if vial or bottle contents have leaked. Unused cocktail or density medium may be disposed of according to standard laboratory procedures for non-hazardous liquids.
4. This product should be handled by trained personnel observing good laboratory practices. Once this product is added to human cells, treat the suspension as potentially biohazardous. Handling of reagents and disposal of wastes should observe all local, state, or national regulations.
5. These products are a potential irritant to eyes, respiratory system, and skin. They may also be harmful if ingested. Avoid exposure through skin, eye contact, inhalation, and ingestion.

SPECIAL MATERIALS REQUIRED BUT NOT PROVIDED

Ethylenediaminetetraacetic acid (EDTA) solution

0.5 M EDTA solution (e.g. Ethylenediaminetetraacetic acid disodium salt solution, 0.5M, Catalog #E7889 from Sigma)

Recommended Medium

Phosphate buffered saline with 2% fetal bovine serum (PBS + 2% FBS, Catalog #07905), both with and without 1 mM EDTA.

To make PBS + 2% FBS + 1 mM EDTA, add 1 mL of 0.5 M EDTA to 499 mL of PBS + 2% FBS.

HANDLING AND DIRECTIONS FOR USE

Ensure that blood sample, recommended medium both with and without EDTA (see Special Materials Required but Not Provided), RosetteSep™ DM-M Density Medium, and centrifuge are all at room temperature (15 - 25°C).

1. Aliquot 10 mL of whole blood into a 50 mL tube. If desired, retain a small aliquot of blood (500 µL) for flow cytometric analysis of the start sample.
2. Add RosetteSep™ HLA Cocktail at **50 µL/mL** of whole blood (e.g. for 10 mL of whole blood, add 500 µL of cocktail). Mix well.
3. Incubate **20 minutes** at room temperature (15 - 25°C).
4. Dilute sample with an equal volume of PBS + 2% FBS with EDTA and mix gently.
5. Layer the diluted sample on top of 10 mL of RosetteSep™ DM-M Density Medium (Catalog #15725).

   Be careful to minimize mixing of density medium and sample.
6. Centrifuge for **25 minutes** at 330 x *g* (see Notes) at room temperature (15 - 25°C) with the brake off.
7. Remove the enriched cells from the RosetteSep™ DM-M Density Medium : plasma interface.

   Note: It is sometimes 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 optimal recovery.
8. Wash enriched cells with PBS + 2% FBS.
9. Use enriched cells as desired. If you wish to evaluate the cell purity by flow cytometry, we recommend lysing both the start and enriched samples with ammonium chloride to remove residual RBCs (this can be done as the wash step).

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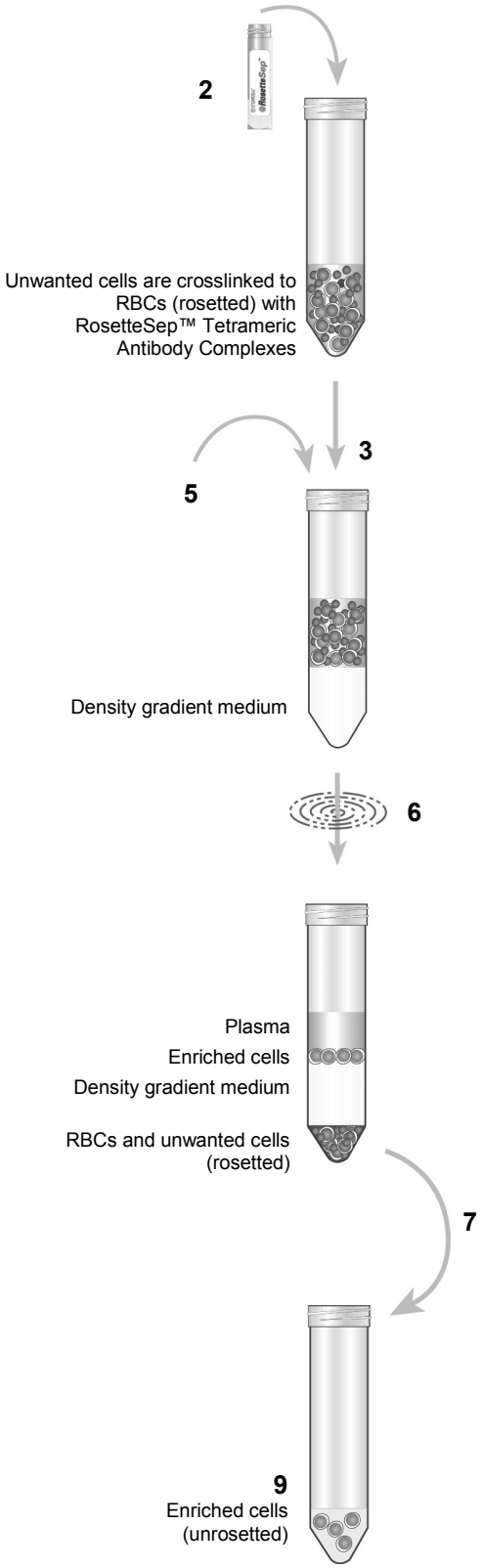
ROSETTESEP™ PROCEDURE

Numbers refer to steps in Handling and Directions for Use.



NOTES

Density Medium

RosetteSep™ DM-M Density Medium has been formulated to optimize myeloid cell recovery. Using a different density medium may cause cell loss.

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 rotor (cm)

Assessing Purity

Purity of myeloid ($CD33^+$) cells can be measured by flow cytometry after staining with a fluorochrome-conjugated anti-CD33 antibody (e.g. PE anti-CD33, Catalog #10536) or a combination of other myeloid cell-specific antibodies.

To reduce non-specific antibody binding, add normal human serum to all flow cytometry samples (start and enriched) prior to the addition of the antibody stain, at a concentration of 2 µL human serum/100 µL cells.

Typical Results

These results are for illustrative purposes only. They were obtained using samples from normal, healthy adults. Results from individual patient samples may vary.

CATALOG #	CELL TYPE ENRICHED	PURITY
15272HLA	Myeloid Cells ($CD33^+$)	> 85%

TECHNICAL ASSISTANCE

For technical support please contact us by email at techsupport@stemcell.com or call either **+1.604.877.0713** or the European Toll-Free number 00800 7836 2355. For more information please visit www.stemcell.com.

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