



RosetteSep™ HLA Total Lymphocyte Enrichment Cocktail

REF 15263HLA	10 mL	For labeling 250 mL of whole blood
REF 15283HLA	40 mL	For labeling 1000 mL of whole blood

ENGLISH

INTENDED USE

RosetteSep™ cell enrichment cocktails are designed for the in vitro enrichment of specific cell subsets from human cell sources, including 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-L (Catalog #15705), Lymphoprep™ (Catalog #07801) or Ficoll-Paque™ PLUS. 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 Total Lymphocyte Enrichment Cocktail REF #15223HC.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 (CD16, CD36, CD66b), and glycophorin A on RBCs. The mouse monoclonal antibody subclass is IgG₁.

QUALITY CONTROL

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.

STORAGE AND STABILITY

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.

WARNINGS AND PRECAUTIONS

- For professional users only.
- This product is for in vitro diagnostic use.
- Do not use cocktail if vial contents have leaked. Unused cocktail may be disposed of according to standard laboratory procedures for non-hazardous liquids.
- 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.
- This product is a potential irritant to eyes, respiratory system, and skin. This product may also be harmful if ingested. Avoid exposure through skin, eye contact, inhalation, and ingestion.

SPECIAL MATERIALS REQUIRED BUT NOT PROVIDED

Density Gradient Medium

Density gradient medium refers to RosetteSep™ DM-L (Catalog #15705), Lymphoprep™ (Catalog #07801), Ficoll-Paque™ PLUS, or other similar density gradient media.

Recommended Medium

Phosphate buffered saline with 2% fetal bovine serum (PBS + 2% FBS, Catalog #07905).

Lymphoprep is a trademark of AXIS-SHIELD. Ficoll-Paque™ PLUS is a trademark of GE Healthcare Limited.

HANDLING AND DIRECTIONS FOR USE

Ensure that blood sample, recommended medium, density gradient medium (see Special Materials Required but Not Provided), and centrifuge are all at room temperature (15 - 25°C).

- Add RosetteSep™ HLA Cocktail at **40 µL/mL** of whole blood* (e.g. for 2 mL of whole blood, add 80 µL of cocktail). Mix well.
**If using samples other than fresh whole blood, see Notes.*
- Incubate **20 minutes** at room temperature (15 - 25°C).
- Dilute sample with an equal volume of PBS + 2% FBS and mix gently.
- Layer the diluted sample on top of the density gradient medium

OR

Layer the density gradient medium underneath the diluted sample.

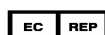
Be careful to minimize mixing of the density gradient medium and sample.

See Table 1 for volume recommendations. With 50 mL conical tubes, we suggest using a minimum of 15 mL density gradient medium to make it easier to remove the enriched layer.

Table 1: Recommended Volumes and Tube Sizes

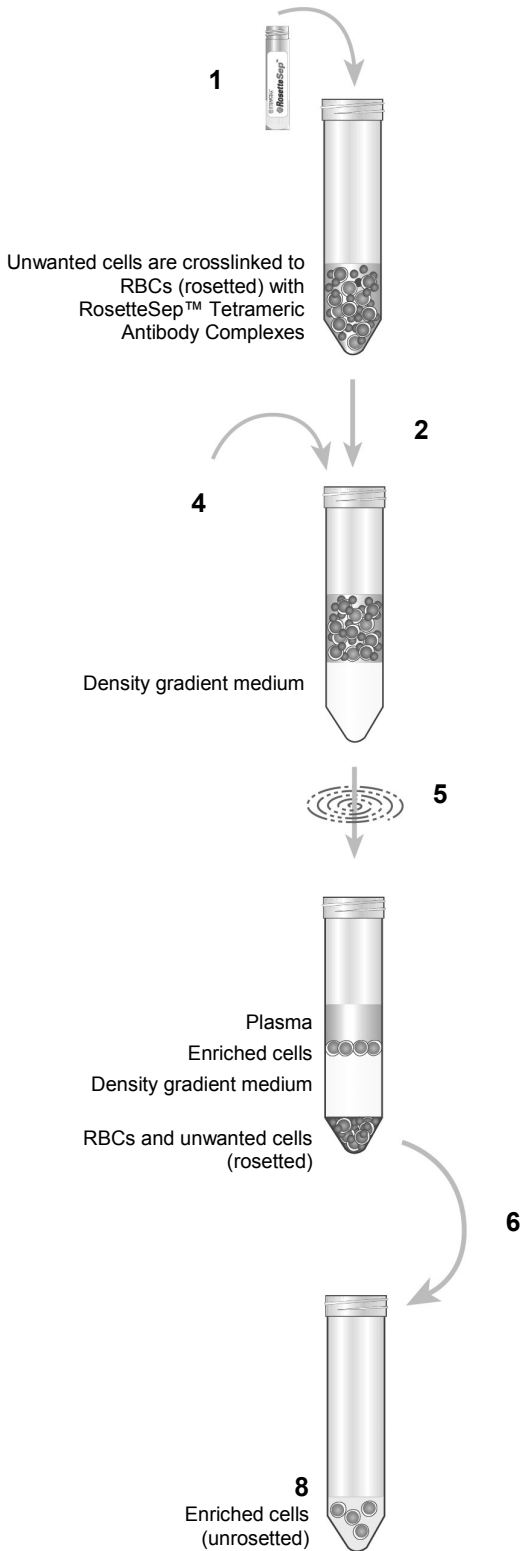
WHOLE BLOOD (mL)	PBS + 2% FBS (mL)	DENSITY GRADIENT MEDIUM (mL)	TUBE SIZE (mL)
1	1	1.5	5
2	2	3	14
3	3	3	14
4	4	4	14
5	5	15	50
10	10	15	50
15	15	15	50

- Centrifuge for **20 minutes** at 1200 x g (see Notes) at room temperature (15 - 25°C), with the brake off.
- 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 optimal recovery.
- Wash enriched cells with PBS + 2% FBS. Repeat.
- Use enriched cells as desired. If you wish to evaluate the cell purity by flow cytometry, we recommend lysing enriched samples with ammonium chloride to remove residual RBCs (this can be done as the wash step).



ROSETTESEP™ PROCEDURE

Numbers refer to steps in Handling and Directions for Use.



NOTES

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, leukapheresis samples). 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 30 - 100 RBCs per nucleated cell.

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 rotor (cm)

Assessing Purity

The purity of lymphocytes can be measured by flow cytometry after staining with fluorochrome-conjugated antibodies against CD3 (e.g. Anti-Human CD3, Clone UCHT1, Catalog #60011) and CD19 (e.g. Anti-Human CD19, Clone HIB19, Catalog #60005). Lymphocytes are either CD3⁺ or CD19⁺ (Note that CD3⁺CD16⁺ NK cells are removed during the RosetteSep™ procedure).

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
15263HLA/15283HLA	Total Lymphocytes (T (CD3 ⁺) & B (CD19 ⁺) Cells)	> 80%

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.

If you require a printed copy or a translated version of this document in a certain language please contact technical support.

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REF Catalog or reference number	LOT Batch code	 Use by: YYYY-MM
 Caution, consult accompanying documents	IVD In Vitro Diagnostic Medical Device	 For storage within temperature limits
 Manufacturers identification (name & address)	EC REP Authorized EC representative in the European Community	 CE Mark

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