RosetteSep™ HLA Total Lymphocyte Enrichment Cocktail

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Catalog #15263HLA	5 x 2 mL	For processing 250 mL whole blood
Catalog #15283HLA	20 x 2 mL	For processing 1000 mL whole blood

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Product Description

Enrich untouched and highly purified lymphocytes directly from human whole blood by negative selection.

- Fast and easy-to-use
- Requires no special equipment or training
- Untouched, viable cells
- Can be combined with SepMate™ for consistent, high-throughput sample processing

This kit targets non-lymphocytes for removal with antibodies recognizing specific surface markers. The RosetteSep™ antibody cocktail crosslinks unwanted cells in human whole blood to multiple red blood cells (RBCs), forming immunorosettes. 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. Isolated cells are immediately available for downstream applications such as flow cytometry, cell culture, or DNA/RNA extraction.

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.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
RosetteSep™ HLA Total Lymphocyte Enrichment Cocktail	15223HC.1	2 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A combination of monoclonal antibodies in PBS.

PBS - phosphate-buffered saline

Components may be shipped at room temperature (15 - 25°C) but should be stored as indicated above.

Precipitate may be observed in the cocktail vial but will not affect performance.

Materials Required But Not Provided

RosetteSep™ DM-L Density Medium (Catalog #15705), Lymphoprep™ (Catalog #07801), or other density gradient media with a density of 1.077 g/mL.

Recommended Medium

Dulbecco's Phosphate Buffered Saline with 2% Fetal Bovine Serum (Catalog #07905).

Directions for Use

Ensure that blood sample, recommended medium, density gradient medium, and centrifuge are all at room temperature (15 - 25°C).

1. 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 and Tips.
   NOTE: Do not vortex cocktail.
2. Incubate at room temperature (15 - 25°C) for 20 minutes.
3. Dilute sample with an equal volume of recommended medium 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. Be careful to minimize mixing of the density gradient medium and sample.
   NOTE: See Table 1 for volume recommendations. With 50 mL conical tubes (e.g. Catalog #38010), we suggest using a minimum of 15 mL of density gradient medium to make it easier to remove the enriched layer.
5. Centrifuge at 1200 x g (see Notes and Tips) for 20 minutes at room temperature 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. Remove some of the density gradient medium along with the enriched cells in order to ensure optimal recovery.
7. Wash enriched cells with recommended medium. Repeat.
8. Use enriched cells as desired. If you wish to evaluate the cell purity by flow cytometry, we recommend lysing enriched samples with Ammonium Chloride Solution (Catalog #07800) to remove residual RBCs (this can be done as the wash step).

Table 1. Recommended Volumes and Tube Sizes

WHOLE BLOOD VOLUME	RECOMMENDED MEDIUM VOLUME	TUBE SIZE	DENSITY GRADIENT MEDIUM VOLUME
1 mL	1 mL	5 mL	1.5 mL
2 mL	2 mL	14 mL	3 mL
3 mL	3 mL	14 mL	3 mL
4 mL	4 mL	14 mL	4 mL
5 mL	5 mL	50 mL	15 mL
10 mL	10 mL	50 mL	15 mL
15 mL	15 mL	50 mL	15 mL

Notes and Tips

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 x 10^7 cells/mL, and RBCs should be present at a ratio of at least 100 RBCs per nucleated cell.

CONVERSION OF g TO RPM

To convert g to RPM, use the following formula:

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

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

ASSESSING PURITY

For purity assessment of total lymphocytes (CD3+ and CD19+) by flow cytometry, use the following fluorochrome-conjugated antibody clones:

- Anti-Human CD3 Antibody, Clone UCHT1 (Catalog #60011), and
- Anti-Human CD19 Antibody, Clone HIB19 (Catalog #60005)

NOTE: 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+] and B [CD19+] cells)	> 80%

Technical Assistance

For technical support, 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, visit www.stemcell.com.

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