RosetteSep™ HLA Granulocyte Depletion Cocktail

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Catalog #15664HLA	5 x 2 mL	For processing 250 mL whole blood
Catalog #15684HLA	20 x 2 mL	For processing 1000 mL whole blood

Document #10000003342 | Version 02

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. This increases the density of 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.

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 as indicated on label.

Warnings and Precautions

1. For professional users only.
2. This product is for in vitro diagnostic use.
3. Do not use cocktail if vial contents have leaked. Unused cocktail 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. 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.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	FORMAT
RosetteSep™ HLA Granulocyte Depletion Cocktail	15624HC.1	2 mL	A combination of monoclonal antibodies in PBS.

PBS - phosphate-buffered saline

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

Materials Required But Not Provided

Lymphoprep™ (Catalog #07801) or other density gradient medium 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 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 (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. 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

Granulocyte depletion can be monitored by flow cytometry to evaluate depletion of high side scatter 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
15664HLA/15684HLA	Mononuclear Cells (Granulocyte Depletion)	> 90%

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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