RosetteSep™ HLA Myeloid Cell Enrichment Kit

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Catalog #15272HLA For 20 tests (10 mL of whole blood per test)

Document #10000003559 | Version 02

Product Description

RosetteSep™ cell enrichment cocktails are designed for the in vitro enrichment of specific cell subsets from human whole blood. 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™ HLA Myeloid Cell Enrichment Cocktail (Catalog #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 (Catalog #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.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
RosetteSep™ HLA Myeloid Cell Enrichment Cocktail	15272HC.1	5 x 2 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A combination of monoclonal antibodies in PBS.
RosetteSep™ DM-M Density Medium	15725	2 x 100 mL	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.	Stable until expiry date (EXP) on label.	A density separation medium with a density of 1.085g/mL.

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

0.5 M Ethylenediaminetetraacetic acid (EDTA) solution (e.g. Sigma Catalog #E7889).

Recommended Medium

Dulbecco's 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.

Directions for Use

Ensure that blood sample, recommended medium both with and without EDTA, 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 (e.g Catalog #38010). 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.
   NOTE: Do not vortex cocktail.
3. Incubate at room temperature (15 - 25°C) for 20 minutes.
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. Be careful to minimize mixing of density medium and sample.
6. Centrifuge at 330 x g (see Notes) for 25 minutes at room temperature with the brake off.
7. Remove the enriched cells from the RosetteSep™ DM-M Density 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.
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 Solution (Catalog #07800) to remove residual RBCs (this can be done as the wash step).

Notes and Tips

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

ASSESSING PURITY

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.

For purity assessment of myeloid cells (CD33+) by flow cytometry, use the following fluorochrome-conjugated antibody clone:

- Anti-Human CD33 Antibody, Clone P67.6 (Catalog #60126)

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, 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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