RosetteSep™ Human CD8 Depletion Cocktail

Catalog #15623 For processing 40 mL whole blood
Catalog #15663 For processing 200 mL whole blood

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Description

Deplete CD8+ cells directly from human whole blood.

- 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 CD8+ cells for removal with antibodies recognizing specific cell 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.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
RosetteSep™ Human CD8 Depletion Cocktail	15623C	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.

Sample Preparation

For available whole blood products, see www.stemcell.com/primarycells.

For optimal performance, use whole peripheral blood collected within the last 24 hours and stored at room temperature (15 - 25°C).

Although RosetteSep™ has been optimized for use with whole blood, cells can be enriched from other sources (i.e. buffy coat) provided that RBCs are present at a ratio of at least 100 RBCs per nucleated cell. The concentration of nucleated cells in the sample should not exceed 5 x 10^7 cells/mL.

For more rapid RosetteSep™ processing, this product can be combined with the SepMate™ RUO (Catalog #86450/86415) or SepMate™ IVD* (Catalog #85450/85415) cell isolation tube. For more information on SepMate™, see the associated Product Information Sheets.

If using SepMate™ with samples with hematocrits outside the normal range, note that a minimum packed RBC volume is required. See table below for details.

	SEPMATE™-15	SEPMATE™-50
Sample volume range	0.5 - 5 mL	4 - 17 mL
Minimum packed RBC volume	0.25 mL	2 mL
Maximum packed RBC volume	3 mL	12 mL

- For samples with low hematocrits, the required sample volume may be greater than the minimum volume stated above.
- For samples with very high hematocrits, the maximum sample volume may be less than the maximum volume stated above.

* SepMate™ IVD is only available in select regions where it is registered as an In Vitro Diagnostic (IVD) device for the isolation of mononuclear cells (MNCs) from whole blood or bone marrow by density gradient centrifugation. In all other regions SepMate™ is available for research use only (RUO).

Recommended Medium

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

Density Gradient Medium

Lymphoprep™ (Catalog #07801) or other density gradient medium with a density of 1.077 g/mL.

Directions for Use – RosetteSep™ Protocol

See page 1 for Sample Preparation and Recommended Medium.

Ensure that whole blood sample, recommended medium, density gradient medium, and centrifuge are all at room temperature (15 - 25°C). For more information on using the SepMate™-15 or SepMate™-50 tube, refer to the applicable Product Information Sheet.

Table 1. RosetteSep™ Human CD8 Depletion Cocktail Protocol

		ROSETTESEP™	
STEP	INSTRUCTIONS	Standard Tube	SepMate™ Tube
1	Collect sample.	Up to 15 mL per tube (see Table 2)	0.5 - 17 mL per tube (see Table 2)
2	Add RosetteSep™ Cocktail to sample.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 20 minutes	RT for 10 minutes
3	Dilute sample with recommended medium and mix gently.	Equal volume to sample	Equal volume to sample
4	Add density gradient medium to required tube.	See Table 2 for volumes and tubes	See Table 2 for volumes and tubes
5	Add diluted sample to the tube containing the density gradient medium.	Layer diluted sample on density gradient medium, being careful to minimize their mixing	Pour or pipette diluted sample into tube
6	Centrifuge.	1200 x g for 20 minutes, **brake off**	1200 x g for 10 minutes, **brake on** NOTE: For samples > 24 hours old it may be necessary to centrifuge for an additional 10 minutes.
7	Collect enriched cells. * For platelet removal see footnote below.	Harvest enriched cell layer with a pipette and transfer to new tube**	Pour supernatant into a new standard tube NOTE: Some RBCs may be present on the surface of the SepMate™ insert after centrifugation. This will not affect performance.
8	Wash enriched cells.	Top up with recommended medium	Top up with recommended medium
9	Centrifuge.	300 x g for 10 minutes brake low	300 x g for 10 minutes brake low
		Discard supernatant	Discard supernatant
10	Repeat steps as indicated.	Steps 8 and 9***	Steps 8 and 9***
11	Resuspend cells in recommended medium.	The enriched cells are ready for use	The enriched cells are ready for use

RT - room temperature (15 - 25°C)
* To minimize platelet contamination, remove and discard the top third of the plasma layer before collecting the cells at the density gradient medium:plasma interface. Platelets may also be removed by including an extra wash with centrifugation at 120 x g for 10 minutes at room temperature with no brake after step 9.
** Sometimes it is difficult to see the cells at the interface. It is recommended to remove some of the density gradient medium along with the pre-enriched cells in order to ensure complete recovery.
*** One of the wash steps can be done with Ammonium Chloride Solution (Catalog #07800) prior to flow cytometry analysis or if residual RBCs will interfere with subsequent assays.

Table 2. Recommended Volumes and Tube Sizes

WHOLE BLOOD VOLUME	RECOMMENDED MEDIUM VOLUME	STANDARD TUBE		SEPMATE™ TUBE	
		TUBE SIZE	DENSITY GRADIENT MEDIUM VOLUME	TUBE SIZE	DENSITY GRADIENT MEDIUM VOLUME*
0.5 mL	0.5 mL	5 mL	1.5 mL	15 mL	4.5 mL
1 mL	1 mL	5 mL	1.5 mL	15 mL	4.5 mL
2 mL	2 mL	14 mL	3 mL	15 mL	4.5 mL
3 mL	3 mL	14 mL	3 mL	15 mL	4.5 mL
4 mL	4 mL	14 mL	4 mL	15 mL / 50 mL	4.5 mL** / 15 mL
5 mL	5 mL	50 mL	15 mL	15 mL / 50 mL	3.5 mL / 15 mL
10 mL	10 mL	50 mL	15 mL	50 mL	15 mL
15 mL	15 mL	50 mL	15 mL	50 mL	15 mL
17 mL	17 mL	--	--	50 mL	15 mL

* Small bubbles may be present in the density gradient medium after pipetting. This will not affect performance.
** If using a sample size of > 4 - 5 mL in the SepMate™-15 tube, use 3.5 mL of density gradient medium.

Notes and Tips

CONVERSION OF g TO RPM

To convert g to RPM, use the following formula:

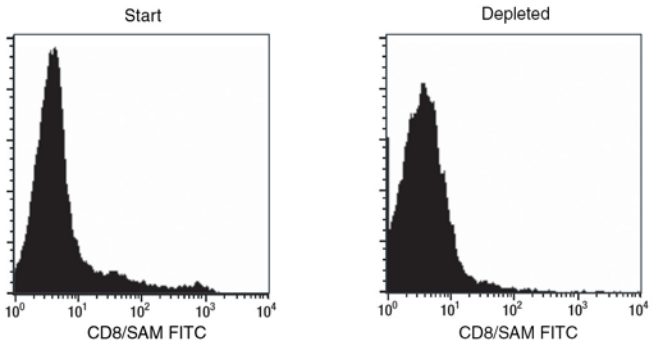
$$RPM = \sqrt{\frac{RCF}{(1.118 \times 10^{-5}) \times (Radius)}}$$

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 CD8 depletion by flow cytometry, use a sheep anti-mouse FITC, which will label any residual CD8+ cells.

Data



In the above example, the purities of the start and final depleted fractions are 13% and 0.4%, respectively (2.0 Log depletion).