RosetteSep™ Human Cord Blood Progenitor Enrichment Kit

Catalog #15276

For processing 500 mL cord blood

STEMCELL™ TECHNOLOGIES
Scientists Helping Scientists™ | WWW.STEMCELL.COM
TOLL FREE PHONE 1 800 667 0322 • PHONE +1 604 877 0713
INFO@STEMCELL.COM • TECHSUPPORT@STEMCELL.COM
FOR GLOBAL CONTACT DETAILS VISIT OUR WEBSITE

Document #28365 | Version 2_4_0

Description

Enrich untouched human cord blood progenitor cells directly from human cord blood by negative selection.

- Fast and easy-to-use
- Requires no special equipment or training
- Untouched, viable cells

This kit targets non-progenitor cells for removal with antibodies recognizing specific cell surface markers. The RosetteSep™ antibody cocktail crosslinks unwanted cells in human whole blood to multiple 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.

NOTE: Disregard Document #28581 provided in the cocktail box for #15026. These instructions will replace those provided in the cocktail box when using kit #15276.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
RosetteSep™ Human Hematopoietic Progenitor Cell Enrichment Cocktail	15186C	2 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.
HetaSep™	07906	100 mL	Store at 15 - 25°C.	Stable until expiry date (EXP) on label.	6% Hetastarch Solution.

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 cord blood products see www.stemcell.com/primarycells.

CORD BLOOD

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

Recommended Medium

Dulbecco's PBS containing 2% fetal bovine serum (FBS; 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 cord blood sample, HetaSep™, recommended medium, density gradient medium, and centrifuge are all at room temperature (15 - 25°C).

NOTE: This protocol was developed for separations in 50 mL centrifuge tubes but can be adapted for use with blood bags.

Table 1. Concentrate Leukocytes

STEP	INSTRUCTIONS	Standard Tube
1	Prepare sample.	Add 2 mL of HetaSep™ per 10 mL cord blood
		Mix well
2	Centrifuge.	50 x *g* for 5 minutes, **brake off**
		Retain supernatant plus top 10% of pellet volume and place into a new tube. Discard remainder of pellet.
3	Wash cells.	Top up with recommended medium
4	Centrifuge. Remove and discard supernatant using a pipette.	300 x *g* for 10 minutes brake low **Do not pour off**, as pellet is not firmly packed.
5	Resuspend cells in recommended medium.	Add 0.5 mL per 10 mL of **original** cord blood volume. Final volume should be approximately 10 - 15% of original blood volume.

Table 2. RosetteSep™ Human Cord Blood Progenitor Enrichment Kit Protocol

		ROSETTESEP™
STEP	INSTRUCTIONS	Standard Tube
1	Add RosetteSep™ Cocktail to sample.	75 µL/10 mL of **original** cord blood volume
	Mix and incubate.	RT for 10 minutes
2	Dilute sample with recommended medium and mix gently.	Use twice as much volume of recommended medium
3	Add density gradient medium to required tube.	See Table 3 for volumes and tubes
4	Add diluted sample to the tube containing the density gradient medium. Use at least half as much density gradient medium as the total volume of the diluted sample	Layer diluted sample on density gradient medium, being careful to minimize their mixing
5	Centrifuge.	1200 x *g* for 20 minutes, **brake off**
6	Collect enriched cells. * For platelet removal see footnote below.	Harvest enriched cell layer with a pipette and transfer to new tube**
7	Wash enriched cells.	Top up with recommended medium
8	Centrifuge.	300 x *g* for 10 minutes brake low***
		Discard supernatant
9	Resuspend cells in recommended medium.	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 8.

** 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.

*** The wash step can be done with Ammonium Chloride Solution (Catalog #07800) prior to flow cytometry analysis or if residual RBCs will interfere with subsequent assays.

Table 3. Recommended Volumes and Tube Sizes

WHOLE BLOOD VOLUME	PBS + 2% FBS VOLUME	STANDARD TUBE	
		TUBE SIZE	DENSITY MEDIUM VOLUME
0.5 mL	1 mL	5 mL	0.75 mL
1 mL	2 mL	5 mL	1.5 mL
2 mL	4 mL	14 mL	3 mL
3 mL	6 mL	14 mL	4.5 mL
4 mL	8 mL	50 mL	6 mL
5 mL	10 mL	50 mL	7.5 mL
10 mL	20 mL	50 mL	15 mL

* Small bubbles may be present in the density gradient medium after pipetting. This will not affect performance.

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 human cord blood progenitor cells by flow cytometry use one of the following fluorochrome-conjugated antibodies:

- Anti-Human CD34 Antibody, Clone 581 (Catalog #60013), or
- Anti-Human CD34 Antibody, Clone 8G12 (Catalog #60121)