EasySep™ Human Cord Blood CD34 Positive Selection Kit

Positive Selection

Catalog #18096

For processing 2 x 10^9 cells

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Description

Isolate untouched and highly purified CD34+ cells directly from human cord blood using a simple, two-step procedure.

- Fast and easy-to-use
- Up to 98% purity
- No columns required
- Can be combined with SepMate™ for consistent, high-throughput sample processing

First, CD34+ cells are pre-enriched by negative selection using RosetteSep™ Cord Blood CD34 Pre-Enrichment Cockatil (15631C), with antibodies recognizing specific cell surface markers. CD34+ cells are then selected using EasySep™ Human CD34 Positive Selection Kit (Catalog #18096), which contains an antibody recognizing CD34.

RosetteSep™ binds unwanted cells to RBCs, forming immunorosettes, which sediment during density gradient centrifugation. The pre-enriched fraction containing the CD34+ cells is harvested from the interface between the plasma and density gradient medium. The pre-enriched CD34+ cells are then labeled with antibodies and magnetic particles, and separated without columns using an EasySep™ magnet. Unwanted cells are simply poured off, while desired cells remain in the tube. Isolated CD34+ cells are immediately available for downstream applications such as flow cytometry, cell culture, DNA/RNA extraction, or generation of induced pluripotent stem (iPS) cells.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
RosetteSep™ Cord Blood CD34 Pre-Enrichment Cocktail	15631C	2 x 2.5 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A combination of monoclonal antibodies in PBS.
EasySep™ Human CD34 Positive Selection Cocktail	18096C	2 x 1 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A combination of monoclonal antibodies in PBS. Includes an Fc receptor blocking antibody.
EasySep™ Magnetic Nanoparticles Positive Selection	18150	1 x 1 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A suspension of magnetic particles in water.
EasySep™ Red Blood Cell Lysis Buffer, 10X Concentrate	20110	1 x 10 mL	Store at 15 - 25°C.	Stable until expiry date (EXP) on label.	A 10X concentrated red blood cell lysis reagent.

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.

Additional Reagent Stability Information

REAGENT NAME	STORAGE	SHELF LIFE
EasySep™ Red Blood Cell Lysis Buffer (1X dilution)	Store at 2 - 8°C. Do not freeze.	Stable for up to 3 months. Do not exceed the expiry date (EXP) of the original component.

Sample Preparation

For available fresh and frozen samples, see www.stemcell.com/primarycells.

CORD BLOOD

For optimal performance use cord blood collected within the last 24 hours and stored at room temperature (15 - 25°C). Remove aggregates by passing cord blood through a 70 µm cell strainer. 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, please 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

EasySep™ Buffer (Catalog #20144), RoboSep™ Buffer (Catalog #20104), or PBS containing 2% fetal bovine serum and 1 mM EDTA. Medium should be free of Ca++ and Mg++.

Density Gradient Medium

Lymphoprep™ (Catalog #07801) or other density gradient medium with a density of 1.077 g/mL. For use with HetaSep™, contact Technical Support at techsupport@stemcell.com.

Directions for Use – RosetteSep™ Protocol

See page 1 for Sample Preparation and Recommended Medium.

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

Table 1. RosetteSep™ Human Cord Blood CD34 Positive Selection Kit 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.**	5 µL/mL of sample	5 µL/mL of sample
	Mix and incubate.	RT for 10 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.**	400 x *g* for 30 minutes, **brake off**	400 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*** NOTE: For the second wash, centrifuge at 120 x g for 10 minutes	Steps 8 and 9*** NOTE: For the second wash, centrifuge at 120 x g for 10 minutes
11	**Resuspend cells in recommended medium.**	Resuspend at 2 x 10^8 cells/mL The pre-enriched cells are ready for EasySep™ procedure (see Table 3)	Resuspend at 2 x 10^8 cells/mL The pre-enriched cells are ready for EasySep™ procedure (see Table 3)

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 cytometric analysis or if residual RBCs will interfere with subsequent assays.

Table 2. Recommended Volumes and Tube Sizes

WHOLE BLOOD VOLUME	PBS + 2% FBS VOLUME	STANDARD TUBE		SEPMATE™ TUBE	
		TUBE SIZE	DENSITY MEDIUM VOLUME	TUBE SIZE	DENSITY 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

Directions for Use – Manual EasySep™ Protocols

See page 1 for Sample Preparation and Recommended Medium. Refer to Table 3 for detailed instructions regarding the EasySep™ procedure for each magnet.

Table 3. EasySep™ Human Cord Blood CD34 Positive Selection Kit Protocol

		EASYSEP™ MAGNETS	
STEP	**INSTRUCTIONS**	 **EasySep™ (Catalog #18000)**	**"The Big Easy" (Catalog #18001)** 
1	Prepare RosetteSep™ pre-enriched sample according to Table 1.	2 x 10^8 cells/mL 0.1 - 0.5 mL	2 x 10^8 cells/mL 0.1 - 0.5 mL
	Add sample to required tube.	5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352058)	14 mL (17 x 100 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352057)
2	Add 1X RBC Lysis Buffer. Mix well.	Equal volume to sample	Equal volume to sample
3	Add Selection Cocktail to sample.	100 µL/mL of sample	100 µL/mL of sample
	Mix and incubate.	RT for 15 minutes	RT for 15 minutes
4	Mix Magnetic Particles. NOTE: Particles should appear evenly dispersed.	Pipette up and down more than 5 times	Pipette up and down more than 5 times
5	Add Magnetic Particles to sample.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 10 minutes	RT for 10 minutes
6	Add recommended medium to top up the sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.	Top up to 2.5 mL	• Top up to 5 mL for samples < 0.5 mL • Top up to 10 mL for samples ≥ 0.5 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 5 minutes
7	Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring off the supernatant. Remove the tube from the magnet; this tube contains the isolated cells.	Discard supernatant	Discard supernatant
8	Repeat steps as indicated.	Steps 6 and 7, four more times (total of 5 x 5-minute separations)	Steps 6 and 7, four more times (total of 5 x 5-minute separations)
9	Remove the tube from the magnet and top up with recommended medium. Centrifuge.	300 x g for 10 minutes brake low	300 x g for 10 minutes brake low
		Carefully aspirate and discard supernatant	Carefully aspirate and discard supernatant
10	Resuspend cells in desired medium.	Isolated cells are ready for use	Isolated cells are ready for use

RT - room temperature (15 - 25°C)

* Leave the magnet and tube inverted for 2 - 3 seconds, then return upright. Do not shake or blot off any drops that may remain hanging from the mouth of the tube.

Directions for Use – Fully Automated RoboSep™ Protocol

See page 1 for Sample Preparation and Recommended Medium. Refer to Table 4 for detailed instructions regarding the RoboSep™ procedure.

Table 4. RoboSep™ Human Cord Blood CD34 Positive Selection Kit Protocol

STEP	INSTRUCTIONS	RoboSep™ (Catalog #20000 and #21000) 
1	Prepare RosetteSep™ pre-enriched sample according to Table 1.	2 x 10^8 cells/mL 0.25 - 4.25 mL
	Add sample to required tube.	14 mL (17 x 100 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352057)
2	Add 1X RBC Lysis Buffer. Mix well.	Equal volume to sample
3	Select protocol.	Human CD34 Cord Blood Positive Selection 18096
4	Mix Magnetic Particles. NOTE: Particles should appear evenly dispersed.	Pipette up and down more than 5 times
5	Load the carousel.	Follow on-screen prompts
	Start the protocol.	Press the green “Run” button
6	Unload the carousel when the run is complete and remove the tube containing the isolated cells. Centrifuge.	300 x g for 10 minutes
		Carefully aspirate and discard supernatant
7	Resuspend cells in desired medium.	Isolated cells are ready for use

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

EASYSEP™ RED BLOOD CELL LYSIS BUFFER

EasySep™ Red Blood Cell Lysis Buffer is supplied as a 10X concentrate. Prepare 1X lysis buffer at least 1 hour before use by adding 1 part 10X lysis buffer to 9 parts distilled or Type 1 water. Mix gently and completely before use.

SAMPLE VARIABILITY

Some cord blood red cells may be less dense than in other whole blood samples, which can lead to incomplete removal of the RBCs after centrifugation. RBC aggregation may be observed at the interface following centrifugation. This does not adversely affect granulocyte depletion or subsequent EasySep™ positive selection.

ASSESSING DEPLETION

Granulocyte depletion can be monitored by flow cytometry to evaluate depletion of high side scatter cells.

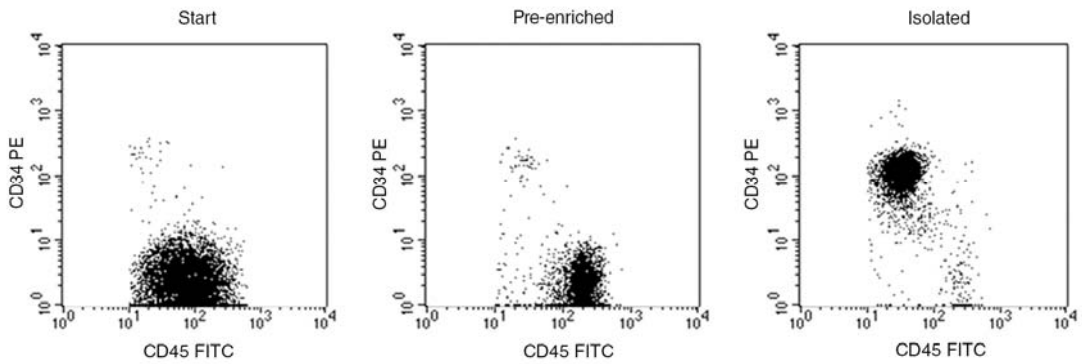
ASSESSING PURITY

For purity assessment of CD34+ cells by flow cytometry use the following fluorochrome-conjugated antibodies:

- Anti-Human CD34 Antibody, Clone 581 (Catalog #60013), or
- Anti-Human CD34 Antibody, Clone 8G12 (Catalog #60121), or
- Anti-human CD34 antibody, clone AC136, or
- Anti-human CD34 antibody, clone BirmaK3

NOTE: Flow cytometry analysis of the positively selected cells may show slightly increased side scatter relative to the start sample.

Data



Starting with fresh cord blood, the CD34+ cell content of the isolated fraction typically ranges from 81 - 98% (gated on CD45+ cells). In the above example, the purities of the start and final isolated fractions are 0.5% and 94.6%, respectively.

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