EasySep™ Human Cord Blood CD 34 Positive Selection Kit III

For processing 1000 mL of cord blood

Catalog #17897

Negative Selection

Document #10000005307 | Version 01

STEMCELL™ TECHNOLOGIES

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Description

Isolate highly purified CD34+ cells from fresh whole umbilical cord blood using a simple, two-step procedure.

- Fast and easy-to-use
- Up to 96% purity
- No columns required
- Can be combined with SepMate™ for consistent, high-throughput sample processing

First, hematopoietic progenitor cells are pre-enriched using RosetteSep™ Human Progenitor Cell Basic Pre-Enrichment Cocktail (15226C) with antibodies recognizing T cell, B cell, and myeloid cell surface markers. CD34+ cells are then selected using EasySep™ Human CD34 Positive Selection Cocktail (18096C), which contains an antibody recognizing CD34. RosetteSep™ binds unwanted cells to red blood cells (RBCs), forming immunorosettes, which sediment during density gradient centrifugation. The pre-enriched fraction containing 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.

- If isolating CD34+ cells from fresh cord blood samples where platelet removal is desired, use EasySep™ Human Cord Blood CD34 Positive Selection Kit II (Catalog #17896).
- If isolating CD34+ cells from fresh adult peripheral blood or buffy coat, use Complete Kit for Human Whole Blood CD34+ Cells (Catalog #15086).
- If isolating CD34+ cells from other samples, including fresh or previously frozen mobilized peripheral blood or bone marrow mononuclear cells, or from previously frozen cord blood mononuclear cells, use EasySep™ Human CD34 Positive Selection Kit II (Catalog #17856).

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
RosetteSep™ Human Progenitor Cell Basic Pre-Enrichment Cocktail	15226C	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™ Dextran RapidSpheres™ 50100	50100	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.

PBS - phosphate-buffered saline

Components may be shipped at room temperature (15 - 25°C) but should be stored as indicated above.

Sample Preparation

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

CORD BLOOD

Collect cord blood in a blood collection container with anticoagulant.

Recommended Medium

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

Density Gradient Medium

Lymphoprep™ (Catalog #07801).

Directions for Use – RosetteSep™ Protocol

See page 1 for Sample Preparation and Recommended Medium. Refer to Table 1 for detailed instructions regarding the RosetteSep™ procedure.
For more rapid RosetteSep™ processing, this product can be combined with the SepMate™ RUO (Catalog #86450) or SepMate™ IVD* (Catalog #85450) cell isolation tube. For more information on SepMate™, see the associated Product Information Sheets.
* 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).

Ensure that cord blood sample, recommended medium, density gradient medium, and centrifuge are all at room temperature (15 - 25°C).

Table 1. RosetteSep™ Human Cord Blood CD34 Pre-Enrichment Protocol

		ROSETTESEP™	
STEP	**INSTRUCTIONS**	**Standard 50 mL Tube**	**SepMate™-50**
1	**Collect cord blood sample within the volume range.**	5 - 15 mL	4 - 17 mL
2	**Add RosetteSep™ Cocktail to sample.** **NOTE: Do not vortex cocktail.**	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.**	15 mL	15 mL
5	**Add diluted sample to the tube containing the density gradient medium.**	Layer diluted sample on density gradient medium, being careful to minimize 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
7	**Collect pre-enriched cells.** **§ For platelet removal see footnote below.**	Harvest enriched cell layer with a pipette and transfer to new 50 mL tube**	Pour supernatant into a new standard 50 mL tube
8	**Wash pre-enriched cells.**	Top up with recommended medium	Top up with recommended medium
9	**Centrifuge.** ***** For platelet reduction see footnote below.**	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 pre-enriched cells as indicated, in recommended medium.‡** **NOTE: If working with a sample which contains a large volume of RBCs, the RosetteSep™ pre-enriched cell pellet may be slightly larger than the recommended resuspension volume. Do not add any additional recommended medium to the sample.**	For an original cord blood volume of: • < 100 mL resuspend in 0.5 mL • ≥ 100 - 150 mL resuspend in 0.75 mL • > 150 mL resuspend in 1 mL	For an original cord blood volume of: • < 100 mL resuspend in 0.5 mL • ≥ 100 - 150 mL resuspend in 0.75 mL • > 150 mL resuspend in 1 mL
11	**The pre-enriched cells are ready for use.**	Continue with the EasySep™ or RoboSep™ Human Cord Blood CD34 Positive Selection Kit III protocol	Continue with the EasySep™ or RoboSep™ Human Cord Blood CD34 Positive Selection Kit III protocol

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.
** Sometimes it is difficult to see the cells at the interface. For maximum recovery, remove some of the density gradient medium along with the pre-enriched cells.
*** For additional platelet removal, centrifuge cells at 120 x *g* for 10 minutes with the brake low. Carefully aspirate and discard the supernatant. Repeat if desired. Continue with step 10.
‡ Cell pellets from separate cord bloods, resuspended according to Table 1, step 10, may be combined to obtain a maximum sample volume of 4 mL when using "The Big Easy" or the EasyEights™ EasySep™ magnet.

Directions for Use – Manual EasySep™ Protocols

See page 1 for Sample Preparation and Recommended Medium. Refer to Tables 2 and 3 for detailed instructions regarding the EasySep™ procedure for each magnet.

Table 2. Easy Sep™ Human Cord Blood CD34 Positive Selection Kit III Protocol

		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS	 **EasySep™ (Catalog #18000)**	**"The Big Easy" (Catalog #18001)** 
1	Prepare RosetteSep™ pre-enriched sample according to Table 1.‡	0.5 mL	0.5 - 4 mL
	Add sample to required tube.	5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Catalog #38007)	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	Add Selection Cocktail to sample. NOTE: Do not vortex cocktail.	100 µL/mL of sample	100 µL/mL of sample
	Mix and incubate.	RT for 10 minutes	RT for 10 minutes
3	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
4	Add RapidSpheres™ to sample.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 1 minute	RT for 3 minutes
5	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 3 mL for samples ≤ 1 mL • Top up to 10 mL for samples > 1 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 3 minutes	RT for 3 minutes
6	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
7	Repeat steps as indicated.	Steps 5 and 6, three more times (total of 4 x 3-minute separations)	Steps 5 and 6, three more times (total of 4 x 3-minute separations)
8	Remove the tube from the magnet and top up the sample 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
9	Resuspend cells in desired medium.	Isolated cells are ready for use	Isolated cells are ready for use

RT - room temperature (15 - 25°C)
‡ Cell pellets from separate cord bloods, resuspended according to Table 1, step 10, may be combined to obtain a maximum sample volume of 4 mL when using "The Big Easy" EasySep™ magnet.
* 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.

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Table 3. Easy Sep™ Human Cord Blood CD34 Positive Selection Kit III Protocol

		EASYSEP™ MAGNET
STEP	INSTRUCTIONS	 EasyEights™ (Catalog #18103) — 14 mL tube
1	Prepare RosetteSep™ pre-enriched sample according to Table 1.‡	0.5 - 4 mL
	Add sample to required tube.	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	Add Selection Cocktail to sample. NOTE: Do not vortex cocktail.	100 µL/mL of sample
	Mix and incubate.	RT for 10 minutes
3	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds
4	Add RapidSpheres™ to sample.	50 µL/mL of sample
	Mix and incubate.	RT for 3 minutes
5	Add recommended medium to top up sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.	• Top up to 3 mL for samples ≤ 1 mL • Top up to 10 mL for samples > 1 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 10 minutes
6	Carefully pipette** (do not pour) off the supernatant. Remove the tube from the magnet; this tube contains the isolated cells.	Discard supernatant
7	Add recommended medium to top up sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.	• Top up to 3 mL for samples ≤ 1 mL • Top up to 10 mL for samples > 1 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 5 minutes
8	Carefully pipette** (do not pour) off the supernatant. Remove the tube from the magnet; this tube contains the isolated cells.	Discard supernatant
9	Repeat steps as indicated.	Steps 7 and 8 (total of 1 x 10-minute and 2 x 5-minute separations)
10	Remove the tube from the magnet and top up the sample with recommended medium. Centrifuge.	300 x g for 10 minutes brake low
		Carefully aspirate and discard supernatant
11	Resuspend cells in desired medium.	Isolated cells are ready for use

RT - room temperature (15 - 25°C)
‡ Cell pellets from separate cord bloods, resuspended according to Table 1, step 10, may be combined to obtain a maximum sample volume of 4 mL.
** Collect the entire supernatant, all at once, into a single pipette (e.g. for EasyEights™ 14 mL tube use a 10 mL serological pipette [Catalog #38004]).

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 III Protocol

STEP	INSTRUCTIONS	RoboSep™ (Catalog #20000 and #21000) 
1	Prepare RosetteSep™ pre-enriched sample according to Table 1.‡	0.5 - 4 mL
	Add sample to required tube.	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	Select protocol.	• Human CD34 Positive Selection III from CB 17897 • Human CD34 Positive Selection III from CB 17897 - high purity
3	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds
4	Load the carousel. NOTE: Do not vortex cocktail.	Follow on-screen prompts
	Start the protocol.	Press the green “Run” button
5	Unload the carousel when the run is complete and remove the tube from the magnet. Centrifuge.	300 x *g* for 10 minutes brake low
		Carefully aspirate and discard supernatant
6	Resuspend cells in desired medium.	Isolated cells are ready for use

‡ Cell pellets from separate cord bloods, resuspended according to Table 1, step 10, may be combined to obtain a maximum sample volume of 4 mL.

Notes and Tips

ASSESSING PURITY

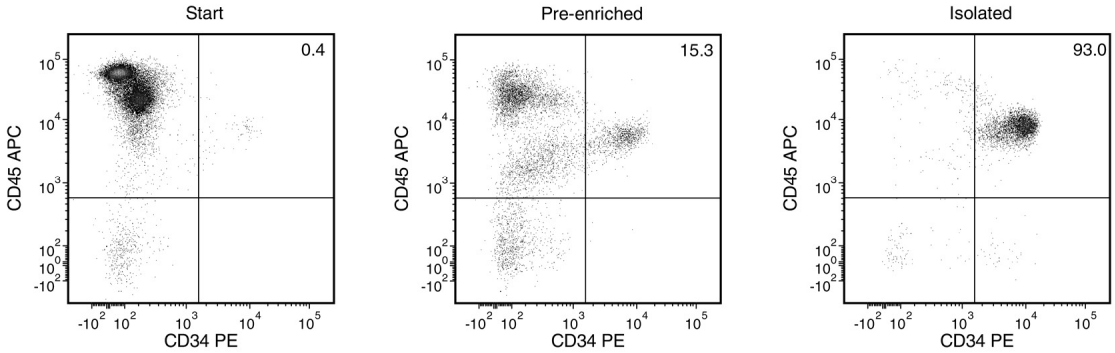
The EasySep™ Human Cord Blood CD34 Positive Selection Cocktail uses a class II anti-CD34 antibody clone that may block some class I and II anti-CD34 antibody clones used to assess purity by flow cytometry. For purity assessment by flow cytometry, use one of the following class III fluorochrome-conjugated anti-CD34 antibody clones and a fluorochrome-conjugated anti-CD45 antibody:

- Anti-Human CD34 Antibody, Clone 581 (Catalog #60013), Clone 8G12 (Catalog #60121), clone AC136, or clone BirmaK3, and
- Anti-Human CD45 Antibody, Clone HI30 (Catalog #60018)

Isolated CD34+ cells can be expanded and/or differentiated into mature hematopoietic cells of specific lineages using StemSpan™ Serum-Free Expansion Media and Supplements (for more information, visit www.stemcell.com).

The frequency of erythroid (BFU-E/CFU-E), myeloid (CFU-GM), and multilineage (CFU-GEMM) progenitor cells can be assessed in colony-forming unit (CFU) assays in semi-solid culture media using MethoCult™ H4034 Optimum (Catalog #04034) or MethoCult™ H4035 Optimum Without EPO (Catalog #04035).

Data



Starting with fresh cord blood, the CD34+ cell content of the isolated fraction is typically 87 ± 12% (mean ± SD, n = 10; data obtained using the purple EasySep™ Magnet). The CD34+ cell content of the starting sample is typically 0.5 ± 0.25% (mean ± SD).