EasySep™ Human Cord Blood CD 34 Positive Selection Kit III

For processing 1000 mL of cord blood

Catalog #17897
Catalog #17897RF RoboSep™

Negative Selection

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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) cell isolation tube. For more information on SepMate™, see the associated Product Information Sheet.

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 #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

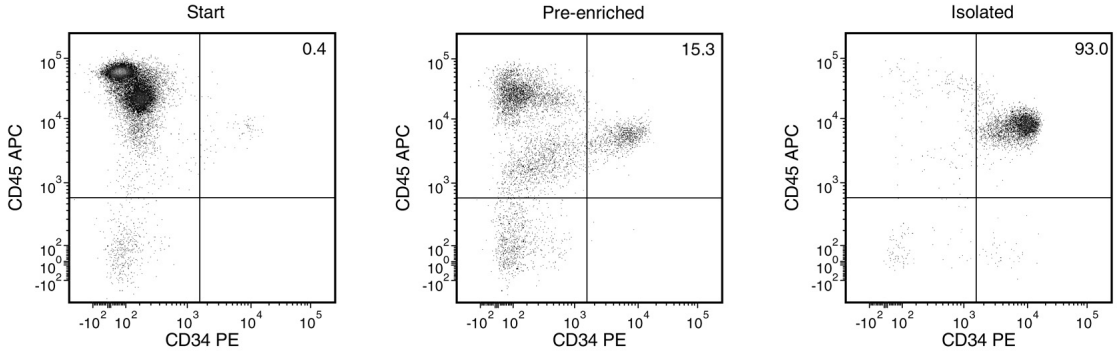
The EasySep™ Human Cord Blood CD34 Positive Selection Cocktail uses a class II anti-CD34 antibody clone that may block some class I and II anti-CD34 antibody clones used to assess purity by flow cytometry. For purity assessment by flow cytometry, use one of the following class III fluorochrome-conjugated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