EasySep™ Complete Kit for Human Whole Blood CD34+ Cells

Positive Selection

Catalog #15086

For labeling 120 mL of whole blood

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Description

Isolate highly purified CD34 cells from human whole 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, hematopoietic progenitors are pre-enriched using the RosetteSep™ Human Hematopoietic Progenitor Cell Enrichment Cocktail (15186C) with antibodies recognizing CD2, CD3, CD14, CD16, CD19, CD24, CD56, CD66b, and CD61 surface markers. CD34+ cells are then selected using the EasySep™ Human CD34 Positive Selection Cocktail (18066C.1), 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 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, culture, DNA/RNA extraction, or generation of induced pluripotent stem (iPS) cells.

- If isolating CD34+ cells from fresh cord blood the EasySep™ Human Cord Blood CD34 Positive Selection Kit II (Catalog #17896) is recommended.
- 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, the EasySep™ Human CD34 Positive Selection Kit (Catalog #18056) is recommended.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
RosetteSep™ Human Hematopoietic Progenitor Cell Enrichment Cocktail	15186C	3 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.
EasySep™ Human CD34 Positive Selection Cocktail	18066C.1	1 x 0.4 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A combination of monoclonal antibodies in PBS.
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

Use of whole peripheral blood less than 24 hours old that has been stored at room temperature (15 - 25°C) is recommended. Although RosetteSep™ has been optimized for use with whole blood, cells can be enriched from other sources (i.e. buffy coat). The concentration of nucleated cells in the sample should not exceed 5 x 10e7 cells/mL, and RBCs should be present at a ratio of at least 30 - 50, and preferably 100, RBCs per nucleated cell.

Recommended Medium

EasySep™ Buffer (Catalog #20144), RoboSep™ Buffer (#20104), RoboSep™ Buffer 2 (Catalog #20164) or PBS containing 2% fetal bovine serum (FBS) with 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.

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

Table 1. RosetteSep™ Human Hematopoietic Progenitor Cell Enrichment Protocol

		ROSETTESEP™	
STEP	**INSTRUCTIONS**	**Standard 50 mL Tube**	**SepMate™-50**
1	**Collect sample.**	15 mL per tube	15 - 17 mL per tube
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 20 minutes
3	**Dilute sample and mix gently.**	Equal volume of recommended medium to sample	Equal volume of recommended medium to sample
4	**Add density gradient medium to required tube.**	15 mL	15 mL
	Required tube.	50 mL conical tube (e.g. Corning® Catalog #352070)	SepMate™-50 tube (Catalog #15450, see Document #29251 for specific instructions)
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 pipet 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 pipet 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.**	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 as indicated in recommended medium.**	For an original sample volume of: • < 50 mL resuspend in 0.5 mL • ≥ 50 - 100 mL resuspend in 0.75 mL • > 100 - 150 mL resuspend in 1.0 mL	For an original sample volume of: • < 50 mL resuspend in 0.5 mL • ≥ 50 - 100 mL resuspend in 0.75 mL • > 100 - 150 mL resuspend in 1.0 mL
11	**The pre-enriched cells are now ready for use.**	Continue on to the EasySep™ or RoboSep™ Human CD34 Positive Selection protocol	Continue on to the EasySep™ or RoboSep™ Human CD34 Positive Selection 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. 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.

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 manual EasySep™ procedure for each magnet.

Table 2. EasySep™ Human CD34 Positive Selection 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 - 1 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 Selection Cocktail to sample.**	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 10 minutes	RT for 10 minutes
3	**Vortex RapidSpheres™.**	30 seconds	30 seconds
4	**Add RapidSpheres™ to sample.**	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 3 minutes	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
	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 now ready for use	Isolated cells are now 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.

Table 3. EasySep™ Human CD34 Positive Selection Protocol

		EASYSEP™ MAGNETS	
		EasyEights™ (Catalog #18103)	
STEP	INSTRUCTIONS	5 mL tube	14 mL tube
1	Prepare RosetteSep™ pre-enriched sample according to Table 1.	0.5 mL	0.5 - 1 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 Selection Cocktail to sample.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 10 minutes	RT for 10 minutes
3	Vortex RapidSpheres™.	30 seconds	30 seconds
4	Add RapidSpheres™ to sample.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 3 minutes	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 2.5 mL	Top up to 3 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 10 minutes	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	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 2.5 mL	Top up to 3 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 5 minutes	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	Discard supernatant
9	Repeat steps as indicated.	Steps 7 and 8 (total of 1 x 10-minute and 2 x 5-minute separations)	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	300 x *g* for 10 minutes brake low
		Carefully aspirate and discard supernatant	Carefully aspirate and discard supernatant
11	Resuspend cells in desired medium.	Isolated cells are now ready for use	Isolated cells are now ready for use

RT - room temperature (15 - 25°C)
** Collect the entire supernatant, all at once, into a single pipette (e.g. for the EasyEights™ 5 mL tube use a 2 mL serological pipette and for the EasyEights™ 14 mL tube use a 10 mL serological pipette).

Directions for Use – Fully Automated RoboSep™ Protocol

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

Table 4. RoboSep™ Human CD34 Positive Selection Protocol

STEP	INSTRUCTIONS	RoboSep™ (Catalog #20000 and #21000) 
1	Prepare RosetteSep™ pre-enriched sample according to Table 1.	0.5 - 1 mL
	Add sample to required tube.	14 mL (17 x 100 mm) polystyrene round-bottom tube (e.g. Corning® Catalog #352057)
2	Select protocol.	Human CD34 Whole Blood Positive Selection 15086 v2
3	Vortex RapidSpheres™.	30 seconds
4	Load the carousel.	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 containing the isolated cells 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 now ready for use

Notes and Tips

ASSESSING PURITY:

The EasySep™ Human Whole Blood CD34 Positive Selection Cocktail uses the anti-CD34 antibody clone QBend10. QBend10 is a class II antibody and 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 a fluorochrome-conjugated anti-CD45 antibody and one of the following class III fluorochrome-conjugated anti-CD34 antibody clones:

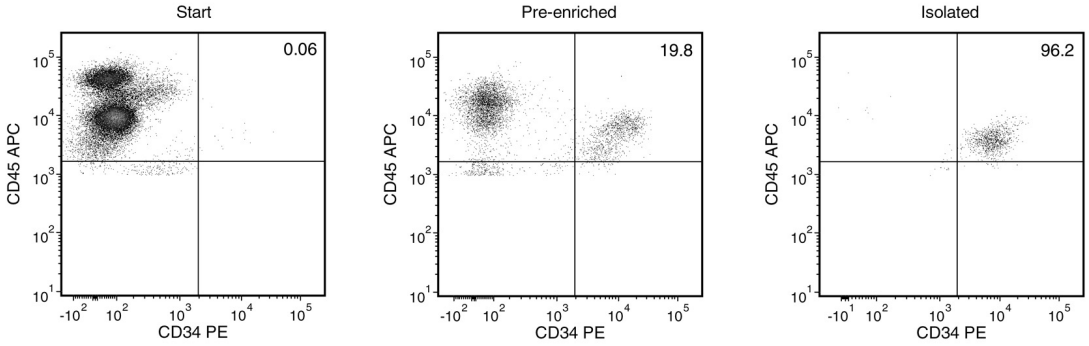
- Anti-Human CD45 Antibody, Clone HI30 (Catalog #60018), and
- Anti-Human CD34 Antibody, Clone 581 (Catalog #60013)
- anti-human CD34 antibody, clone 8G12
- anti-human CD34 antibody, clone AC136
- anti-human CD34 antibody, clone BirmaK3

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).

ReproTeSR™ (Catalog #05920) can be used to reprogram isolated cells to human iPS cells.

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

Data



Starting with whole peripheral blood, the CD34+ cell content of the isolated fraction is typically 95.1 ± 4.5% (gated on viable CD45+ cells; mean ± SD for the silver “The Big Easy” EasySep™ Magnet). In the above example, the purities of the start and final isolated fractions are 0.06% and 96.2%, respectively.