EasySep™ Positive Selection

Catalog #17879

EasySep™ Human Whole Blood CD34 Positive Selection Kit II

For processing 75 mL whole blood (37 mL buffy coat)

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Description

Isolate highly purified CD34+ cells from whole blood or buffy coat samples by immunomagnetic positive selection.

- Fast and easy-to-use
- No columns required

This kit targets CD34+ cells for positive selection with an antibody recognizing the CD34 surface marker. Desired cells are 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 cells are immediately available for downstream applications such as flow cytometry, culture, or DNA/RNA extraction.

- For isolating CD34+ cells from whole blood without lysis buffer, use Complete Kit for Human Whole Blood CD34+ Cells (Catalog #15086).
- For isolating CD34+ cells from fresh cord blood, use EasySep™ Human Cord Blood CD34 Positive Selection Kit II (Catalog #17896).
- For isolating CD34+ cells from any other sample type, use EasySep™ Human CD34 Positive Selection Kit II (Catalog #17856).

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Human CD34 Positive Selection Cocktail	18096C	3 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	3 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.

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 expiry date (EXP) of original component.

Sample Preparation

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

WHOLE BLOOD

Collect whole blood in a blood collection tube containing anticoagulant.

BUFFY COAT

1. Add an equal volume of recommended medium to whole blood.
2. Centrifuge at 800 x g for 10 minutes at room temperature (15 - 25°C) with the brake off.
3. Remove the concentrated leukocyte band (this is the buffy coat), plus a small portion of the plasma and concentrated red blood cells (RBCs). The target is to concentrate the leukocytes approximately 5-fold while maintaining the same hematocrit (e.g. collect 2 mL of buffy coat when starting with 10 mL of whole blood).
4. Transfer buffy coat to the required tube (see Table 1).

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++.

Directions for Use – Manual EasySep™ Protocols

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

Table 1. EasySep™ Human Whole Blood CD34 Positive Selection Kit II Protocol

		"THE BIG EASY" EASYSEP™ MAGNET (CATALOG #18001)	
STEP	**INSTRUCTIONS**	 **Whole Blood**	**Buffy Coat** 
1	Prepare sample within the volume range.	Up to 4.5 mL	Up to 4.5 mL
	Add sample to required tube.	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	Add 1X EasySep™ RBC Lysis Buffer to sample.	Equal volume to sample	Equal volume to sample
3	Add Selection Cocktail to sample.	20 µL/mL of diluted sample	40 µL/mL of diluted sample
	Mix and incubate.	RT for 15 minutes	RT for 15 minutes
4	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
5	Add RapidSpheres™ to sample.	20 µL/mL of diluted sample	40 µL/mL of diluted 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 10 mL	Top up to 10 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 10 minutes	RT for 10 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	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 10 mL	Top up to 10 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 5 minutes
9	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
10	Repeat steps as indicated.	Steps 8 and 9 (total of 1 x 10-minute and 2 x 5-minute separations)	Steps 8 and 9 (total of 1 x 10-minute and 2 x 5-minute separations)
11	Resuspend cells in desired medium. Be sure to collect cells from the sides of the tube.	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 2 for detailed instructions regarding the RoboSep™ procedure.

Table 2. RoboSep™ Human Whole Blood CD34 Positive Selection Kit II Protocol

STEP	INSTRUCTIONS	RoboSep™ (Catalog #20000 and #21000) 
1	Prepare sample within the volume range.	0.25 - 4.5 mL
	Add sample to required tube.	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	Add 1X EasySep™ RBC Lysis Buffer to sample.	Equal volume to sample
3	Select protocol. NOTE: Enter volume.	Human CD34 Positive Selection 17879 NOTE: Enter diluted sample volume.
4	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds
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. Remove the tube containing the isolated cells and resuspend in desired medium. Be sure to collect cells from the sides of the tube.	Isolated cells are ready for use

Notes and Tips

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.

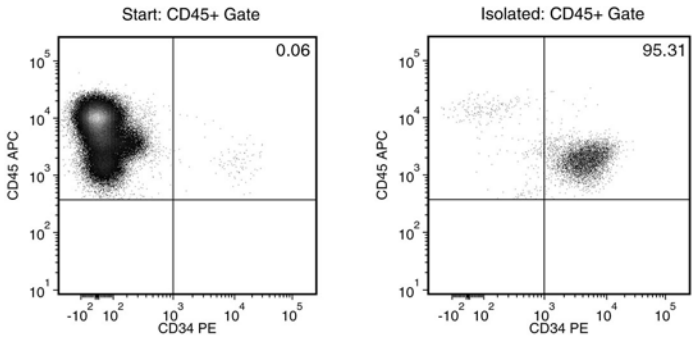
ASSESSING PURITY

EasySep™ Human 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 of CD34+ cells by flow cytometry, use one of the following class III fluorochrome-conjugated antibody clones:

- 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)

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

Data



Starting with whole blood, the CD34+ cell content of the isolated fraction is typically 90.4 ± 7.0% (gated on CD45+ cells; mean ± SD using RoboSep™-S). In the above example, the purities of the start and final isolated fractions are 0.06% and 95.31%, respectively.

NOTE: RBCs were removed by lysis prior to flow cytometry.