EasySep™ Positive Selection

Catalog #18086

EasySep™ Human Whole Blood CD34 Positive Selection Kit

For labeling 75 mL of 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.

To isolate CD34+ cells from whole blood without the use of lysis buffer, we recommend using the Complete Kit for Human Whole Blood CD34+ Cells (Catalog #15086). If isolating CD34+ cells from fresh cord blood, we recommend using the EasySep™ Human Cord Blood CD34 Positive Selection Kit (Catalog #18096). If isolating CD34+ cells from any other sample type, we recommend using the EasySep™ Human CD34 Positive Selection Kit (Catalog #18056).

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Human Whole Blood / Buffy Coat CD34 Positive Selection Cocktail	18086C	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.
EasySep™ Magnetic Nanoparticles Positive Selection	18150	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 the expiry date (EXP) of original component.

Sample Preparation

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

WHOLE BLOOD

Collect whole blood in a blood collection tube containing anticoagulant.

BUFFY COAT

1. Add 1 part recommended medium to 1 part whole blood.
2. Centrifuge at 200 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.
4. Transfer a maximum of 4.5 mL of buffy coat to the required tube (see Table 1).

Alternatively, HetaSep™ (Catalog #07906) RBC sedimentation can be used to concentrate leukocytes. Please contact Technical Support for further information.

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++.

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 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 100 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352057)	14 mL (17 x 100 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352057)
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	Mix Magnetic Particles.	Pipette up and down more than 5 times	Pipette up and down more than 5 times
5	Add Magnetic Particles to sample.	20 µL/mL of sample	40 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 5 mL for samples < 2.5 mL • Top up to 10 mL for samples ≥ 2.5 mL	• Top up to 5 mL for samples < 2.5 mL • Top up to 10 mL for samples ≥ 2.5 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	Remove the tube from the magnet and add recommended medium to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.	• Top up to 5 mL for samples < 2.5 mL • Top up to 10 mL for samples ≥ 2.5 mL	• Top up to 5 mL for samples < 2.5 mL • Top up to 10 mL for samples ≥ 2.5 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 5 minutes
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	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 3 for detailed instructions regarding the RoboSep™ procedure.

Table 2. RoboSep™ Human Whole Blood CD45 Positive Selection Kit 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 100 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352057)
2	Add 1X EasySep™ RBC Lysis Buffer to sample.	Equal volume to sample
3	Select protocol.	Human CD34 Buffy Coat Positive Selection 18086-high purity
4	Mix Magnetic Particles.	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. 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.

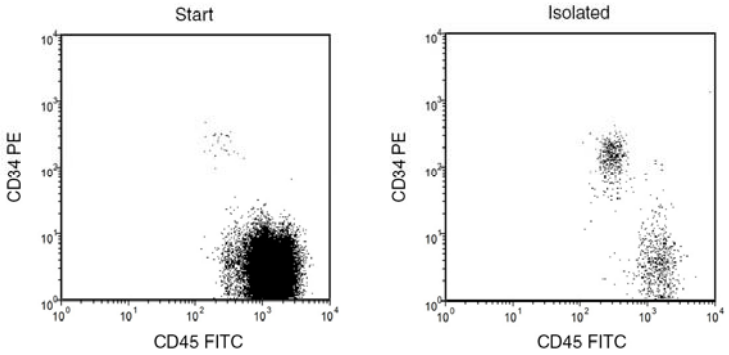
ASSESSING PURITY

The EasySep™ Human Whole Blood/Buffy Coat CD34 Positive Selection Cocktail uses a class II anti-CD34 antibody 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), or clone 8G12, AC136, or 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 enriched fraction typically ranges from 26 - 41% (95% CI; reported as a percentage of viable CD45+ cells). In the above example, the purities of the start and final isolated fractions are 0.09% and 40%, respectively. RBCs were lysed prior to flow cytometry.