

EasySep™ HLA Chimerism Whole Blood CD33 Positive Selection Kit



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For processing 60 mL whole blood

Catalog #17885

Catalog #17885RF RoboSep™

Positive Selection

Document #10000003611 | Version 01

Description

Isolate highly purified CD33+ myeloid cells from fresh human whole blood or buffy coat by immunomagnetic positive selection.

- Fast and easy-to-use
- Up to 99% purity
- No columns required

This kit targets myeloid cells for positive selection with an antibody recognizing the CD33 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 lineage-specific chimerism analysis.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ HLA Chimerism Whole Blood CD33 Positive Selection Cocktail	17885C	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™ 50101	50101	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. Do not freeze.	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 the original component.

Sample Preparation

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

PERIPHERAL 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 Tables 1 - 3).


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 Tables 1 and 2 for detailed instructions regarding the EasySep™ procedure.


Table 1. EasySep™ HLA Chimerism Whole Blood CD33 Positive Selection Kit Protocol

		EASYSEP™ MAGNET	
STEP	INSTRUCTIONS	“The Big Easy” (Catalog #18001)	
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	
	Mix and incubate.	RT for 5 minutes	
3	Add Selection Cocktail to sample. NOTE: Do not vortex Cocktail.	25 µL/mL of diluted sample	
	Mix and incubate.	RT for 5 minutes	
4	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	
5	Add RapidSpheres™ to sample.	25 µL/mL of diluted sample	
	Mix and incubate.	RT for 5 minutes	
6	Add recommended medium to top up the sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.	<ul style="list-style-type: none"> • Top up to 5 mL for diluted samples < 4 mL • Top up to 10 mL for diluted samples ≥ 4 mL 	
	Place the tube (without lid) into the magnet and incubate.	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	
8	Add recommended medium to top up the sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.	<ul style="list-style-type: none"> • Top up to 5 mL for diluted samples < 4 mL • Top up to 10 mL for diluted samples ≥ 4 mL 	
	Place the tube (without lid) into the magnet and incubate.	RT for 15 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	
10	Resuspend cells in desired medium. Be sure to collect cells from the sides of the tube.	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.

Table 2. EasySep™ HLA Chimerism Whole Blood CD33 Positive Selection Kit Protocol

		EASYSEP™ MAGNET	
STEP	INSTRUCTIONS		EasyEights™ (Catalog #18103)
			14 mL tube
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
	Mix and incubate.		RT for 5 minutes
3	Add Selection Cocktail to sample. NOTE: Do not vortex cocktail.		25 µL/mL of diluted sample
	Mix and incubate.		RT for 5 minutes
4	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.		30 seconds
5	Add RapidSpheres™ to sample.		25 µL/mL of diluted sample
	Mix and incubate.		RT for 5 minutes
6	Add recommended medium to top up sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.		<ul style="list-style-type: none"> • Top up to 5 mL for diluted samples < 5 mL • Top up to 10 mL for diluted samples ≥ 5 mL
	Place the tube (without lid) into the magnet and incubate.		RT for 10 minutes
7	Carefully pipette** (do not pour) off the supernatant. Remove the tube from the magnet; this tube contains the isolated cells.		Discard supernatant
8	Add recommended medium to top up sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.		<ul style="list-style-type: none"> • Top up to 5 mL for diluted samples < 5 mL • Top up to 10 mL for diluted samples ≥ 5 mL
	Place the tube (without lid) into the magnet and incubate.		RT for 15 minutes
9	Carefully pipette** (do not pour) off the supernatant. Remove the tube from the magnet; this tube contains the isolated cells.		Discard supernatant
10	Resuspend cells in desired medium. Be sure to collect cells from the sides of the tube.		Isolated cells are ready for use


RT - room temperature (15 - 25°C)

** 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 3 for detailed instructions regarding the RoboSep™ procedure.

Table 3. RoboSep™ HLA Chimerism Whole Blood CD33 Positive Selection Kit Protocol

STEP	INSTRUCTIONS	RoboSep™ (Catalog #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	
	Mix and incubate.	RT for 5 minutes	
3	Select protocol.	HLA Chimerism CD33 WB Positive Selection 17885 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	

RT - room temperature (15 - 25°C)

Notes and Tips

EASYSEPTM 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

For purity assessment by flow cytometry, use the following fluorochrome-conjugated antibody clones:

- Anti-Human CD14 Antibody, Clone M5E2 (Catalog #60004) or Clone MoP9 (Catalog #60124), and
- Anti-Human CD66b Antibody, Clone G10F5 (Catalog #60086)

The following method can also be used:

- Use a fluorochrome-conjugated secondary antibody, such as Goat Anti-Mouse IgG (H+L) Antibody, Polyclonal (Catalog #60138).

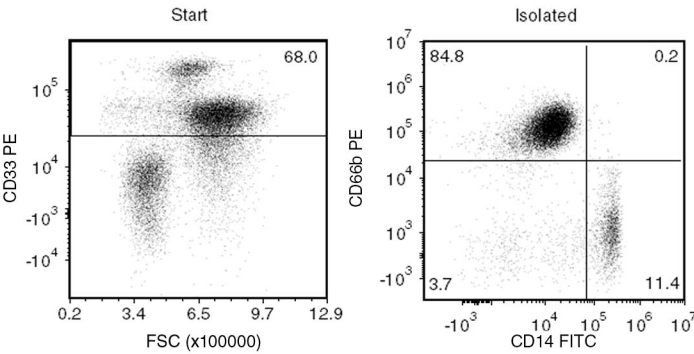
DONOR VARIABILITY

Certain donors express one or more soluble serum factors that can cause cross-linking with magnetic particles. This may result in visible aggregates in the enriched cell fraction following positive selection. These aggregates may appear as a distinct, high side-scatter population on FSC vs. SSC plots during flow cytometry analysis of the enriched fraction. This population consists solely of particles, with no cells or platelets present, as determined by staining with fluorescently-labeled antibodies against dextran, CD41, and CD45.

Potential aggregation can be avoided by washing away the donor plasma. Dilute the sample 2-fold in the recommended medium, and centrifuge at 300 x g for 10 minutes. Remove as much plasma as possible without disturbing the white and red blood cells, then resuspend the sample to the original volume with recommended medium before beginning the separation procedure.

If the samples have not been washed, any aggregates can be gated out during flow cytometry analysis of the enriched fraction based on their FSC vs. SSC characteristics, or by their lack of CD45 expression.

Data



Starting with fresh whole blood, the CD33+ cell content of the isolated fraction is typically 96 ± 2% (mean ± SD using “The Big Easy” EasySep™ Magnet). In the above example, the purities of the start (as assessed by labeling with anti-CD33) and the final isolated fractions are 68.0% and 96.2% (as assessed by labeling with anti-CD14 and anti-CD66b), respectively.

NOTE: RBCs were removed from the start sample by lysis prior to flow cytometry.

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