

EasySep™ HLA Chimerism Whole Blood CD19 Positive Selection Kit

For processing 60 mL of buffy coat or whole blood

Catalog #17874

Positive Selection

Document #10000003596 | Version 05



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Description

Isolate highly purified CD19+ cells from fresh human whole blood, buffy coat, or bone marrow by immunomagnetic positive selection.

- Fast and easy-to-use
- Up to 99% purity
- No columns required
- Compatible across EasySep™ platforms "The Big Easy", EasyEights™, and RoboSep™-S

This kit targets CD19+ cells for positive selection with an antibody recognizing the CD19 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.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ HLA Chimerism Whole Blood CD19 Positive Selection Cocktail	17874C	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 with 2% HPCD and 0.09% rHA. 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	10 mL	Store at 15 - 25°C.	Stable until expiry date (EXP) on label.	A 10X concentrated red blood cell lysis reagent.

HPCD - 2-hydroxypropyl- β -cyclodextrin; PBS - phosphate-buffered saline; rHA - recombinant human albumin

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.

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 a maximum of 4.5 mL of buffy coat to the required tube (see Tables 1 - 3).

BONE MARROW

The bone marrow sample should be processed as soon as possible and within 72 hours after collection.

1. Dilute the sample 5- to 10-fold in D-PBS (Without Ca⁺⁺ and Mg⁺⁺; Catalog #37350) and mix gently by pipetting up and down.
2. OPTIONAL (recommended): Pre-wet a 70 µm strainer with D-PBS. Filter the sample through the pre-wetted strainer to remove bone fragments, cell aggregates, and debris. Rinse the strainer with D-PBS.
3. Centrifuge cells at 300 x g for 10 minutes with the brake off.
4. Using a pipette, carefully remove and discard the plasma, without disturbing the cell pellet. Do not pour.

OPTIONAL: For bone marrow samples > 24 hours old, add DNase I Solution (1 mg/mL; Catalog #07900) at 50 µL per sample or up to 100 µg/mL of the original sample volume to help reduce cell clumping. DNase I Solution can be added directly to the pelleted cells with gentle mixing. Incubate at room temperature (15 - 25°C) for 15 - 30 minutes prior to beginning the EasySep™ protocol.

NOTE: Avoid repeated freeze-thaw cycles of DNase I Solution.

5. Resuspend the cell pellet with EasySep™ Buffer:
 - If the sample has low cellularity, or if the sample volume is ≥ 2.5 mL, resuspend to the original sample volume.
 - If the sample volume is < 2.5 mL and has high cellularity or cellularity is unknown, resuspend to twice the original sample volume.

LEUKOREDUCTION SYSTEM CHAMBER

If processing leukoreduction system chamber (LRSC) samples, refer to the applicable PIS (Document #10000006453).


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 pages 1 and 2 for Sample Preparation and Recommended Medium. Refer to Tables 1 and 2 for detailed instructions regarding the EasySep™ procedure for each magnet.


Table 1. EasySep™ HLA Chimerism Whole Blood CD19 Positive Selection Kit Protocol

		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS	“The Big Easy” (Catalog #18001)	
1	Prepare sample within the volume range.	0.5 - 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	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 ≤ 2.5 mL • Top up to 10 mL for diluted samples > 2.5 mL 	
	Place the tube (without lid) into the magnet and incubate.	RT for 5 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	Repeat steps as indicated.	Steps 6 and 7, two more times (total of 3 x 5-minute separations)	
9	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 CD19 Positive Selection Kit Protocol

		EASYSEP™ MAGNET	
STEP	INSTRUCTIONS		EasyEights™ (Catalog #18103)
			14 mL tube
1	Prepare sample within the volume range.		0.5 - 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	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 ≤ 2.5 mL • Top up to 10 mL for diluted samples > 2.5 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	Repeat steps as indicated.		Steps 6 and 7, two more times (total of 3 x 10-minute separations)
9	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 the EasyEights™ 14 mL tube use a 10 mL serological pipette [Catalog #38004]).

Directions for Use – Fully Automated RoboSep™ Protocol

See pages 1 and 2 for Sample Preparation and Recommended Medium. Refer to Table 3 for detailed instructions regarding the RoboSep™ procedure.

Table 3. RoboSep™ HLA Chimerism Whole Blood CD19 Positive Selection Kit Protocol

STEP	INSTRUCTIONS	RoboSep™ (Catalog #21000)	
1	Prepare sample within the volume range.	0.5 - 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.	HLA Chimerism CD19 WB Positive Selection 17874 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

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.

*Type I water refers to ultrapure water suitable for use in analytical procedures. It is defined by the American Society for Testing and Materials (ASTM) as having a resistivity of > 18 MΩ-cm, a conductivity of < 0.056 μS/cm, and < 50 ppb of total organic carbons (TOC).

ASSESSING PURITY

For purity assessment of CD19+ cells by flow cytometry, use one of the following fluorochrome-conjugated antibody clones:

- Anti-Human CD19 Antibody, Clone H1B19 (Catalog #60005; partially blocked), or
- Anti-human CD19 antibody, clone 4G7 or FMC63 (partially blocked)

One of the following methods can also be used:

- Use an alternative marker such as fluorochrome-conjugated Anti-Human CD20 Antibody, Clone 2H7 (Catalog #60008). This may underestimate the CD19+ purity by up to 15%.
- Use a fluorochrome-conjugated secondary antibody, such as Goat Anti-Mouse IgG (H+L) Antibody, Polyclonal (Catalog #60138).

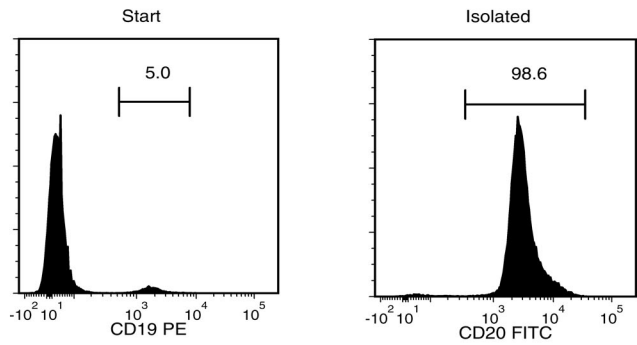
DONOR VARIABILITY

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 human whole blood, the CD19+ cell content of the isolated fraction typically ranges from 94.3 - 99.6% (as assessed by staining the start and isolated fractions with anti-CD19 or anti-CD20 antibodies, respectively). In the above example, the purities of the start and the final isolated fractions are 5.0% and 98.6%, respectively (gated on CD45).

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

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