EasySep™ HLA Chimerism Whole Blood CD3 Positive Selection Kit

For processing 30 mL leukoreduction system chamber

Catalog #17871

Positive Selection

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Description

Isolate highly purified CD3+ cells from a leukoreduction system chamber (LRSC; also known as an LRS cone) 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 CD3+ cells for positive selection with an antibody recognizing the CD3 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 CD3 Positive Selection Cocktail	17871C	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 and 2% HPCD. 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.	Stable until expiry date (EXP) on label.	A 10X concentrated red blood cell lysis reagent.

HPCD - 2-hydroxypropyl-β-cyclodextrin; 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

LEUKOREDUCTION SYSTEM CHAMBER

For optimal cell recovery, LRSC should be shipped and stored at 2 - 8°C. Immediate processing of the LRSC upon arrival is recommended.

Flush LRSC sample from the chamber into a 50 mL conical tube (e.g. Catalog #38010) using phosphate-buffered saline (PBS) containing 2% fetal bovine serum (FBS). Centrifuge at 800 x g for 10 minutes and resuspend the cell pellet in 10 mL of recommended medium. Store the cell suspension at 2 - 8°C until ready to start the cell separation protocol.

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 CD3 Positive Selection Kit Protocol for LRSC

		EASYSEP™ MAGNET
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.**	50 µL/mL of diluted sample
	Mix and incubate.	RT for 3 minutes
4	**Vortex RapidSpheres™.** **NOTE: Particles should appear evenly dispersed.**	30 seconds
5	**Add RapidSpheres™ to sample.**	50 µL/mL of diluted sample
	Mix and incubate.	RT for 3 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 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 3 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 3-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

RBC - red blood cell; 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 CD3 Positive Selection Kit Protocol for LRSC

		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.**	50 µL/mL of diluted sample
	Mix and incubate.	RT for 3 minutes
4	**Vortex RapidSpheres™.** **NOTE: Particles should appear evenly dispersed.**	30 seconds
5	**Add RapidSpheres™ to sample.**	50 µL/mL of diluted sample
	Mix and incubate.	RT for 3 minutes
6	**Add recommended medium to top up sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.**	• 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

RBC - red blood cell; 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).

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 CD3 Positive Selection Kit Protocol for LRSC

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 CD3 WB Positive Selection 17871 - LRSC 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

RBC - red blood cell

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.

*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

The EasySep™ HLA Chimerism Whole Blood CD3 Positive Selection Cocktail uses an anti-CD3 antibody clone that to our knowledge may fully or partially block other anti-CD3 antibody clones used to assess purity by flow cytometry. One of the following methods can be used to assess purity:

- Use an alternative marker such as fluorochrome-conjugated Anti-Human CD2 Antibody, Clone RPA-2.10 (Catalog #60007) to detect CD2+ cells.
- Use alternative markers such as fluorochrome-conjugated Anti-Human CD5 Antibody, Clone UCHT2 (Catalog #60082) and Anti-Human CD20 Antibody, Clone 2H7 (Catalog #60008) to detect CD5+CD20- cells.
- Use a fluorochrome-conjugated secondary antibody, such as Goat Anti-Mouse IgG (H+L) Antibody, Polyclonal (Catalog #60138).

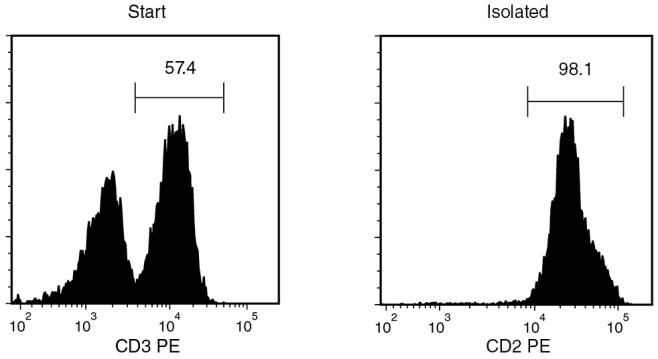
DONOR VARIABILITY

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 LRSC sample, the CD3+ cell content of the isolated fraction typically ranges from 97.8 - 99.6%. In the above example, the purities of the start and final isolated fractions are 57.4% and 98.1%, as assessed by staining the start and isolated fractions with anti-CD3 and anti-CD2 antibodies (gated on CD45), respectively.

NOTE: Red blood cells (RBCs) were removed from the start sample by lysis prior to flow cytometry.