EasySep™ Serology Whole Blood CD3 Positive Selection Kit

For processing 60 mL of whole blood

Catalog #18981

Positive Selection

Document #10000005326 | Version 01

STEMCELL™ TECHNOLOGIES

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Description

Isolate highly purified CD3+ cells from fresh human whole blood by immunomagnetic positive selection.

- Fast and easy-to-use
- Up to 99% purity
- No columns required
- Compatible with HLA serological applications

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 HLA serology, flow cytometry, or DNA/RNA extraction.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Serology Whole Blood CD3 Positive Selection Cocktail	18981C.1	2 x 0.65 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™ Releasable RapidSpheres™ 50201*	50201	6 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.

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.

* NOTE: Particles remain attached to isolated cells.

Sample Preparation

Collect whole blood in a blood collection tube containing anticoagulant.

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™ Protocol

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™ Serology Whole Blood CD3 Positive Selection Kit Protocol

		EASYSEP™ MAGNETS
STEP	INSTRUCTIONS	**"The Big Easy" (Catalog #18001)** 
1	**Add whole blood to required tube.**	1 - 4.5 mL
	Required tube.	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	**Dilute whole blood with recommended medium (e.g. EasySep™ Buffer).***	Equal volume to sample
3	**Add EasySep™ Serology Whole Blood CD3 Positive Selection Cocktail to sample.** **NOTE: Do not vortex cocktail.**	10 µL/mL of diluted whole blood
	Mix and incubate.	RT for 5 minutes
4	**Vortex EasySep™ Releasable RapidSpheres™ 50201.** **NOTE: Particles should appear evenly dispersed.**	30 seconds
5	**Add EasySep™ Releasable RapidSpheres™ 50201 to sample.**	50 µL/mL of diluted whole blood
	Mix and incubate.	RT for 5 minutes
6	**Add recommended medium (e.g. EasySep™ Buffer) to top up the sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.**	Top up to 10 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)
* NOTE: Do NOT use 1X RBC Lysis Buffer to dilute whole blood.
** 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™ Serology Whole Blood CD3 Positive Selection Kit Protocol

		EASYSEP™ MAGNET
STEP	INSTRUCTIONS	 EasyEights™ (Catalog #18103) 14 mL tube
1	Prepare sample within the volume range.	1 - 4.5 mL
	Add sample to required tube.	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	Dilute whole blood with recommended medium (e.g. EasySep™ Buffer).*	Equal volume to sample
3	Add EasySep™ Serology Whole Blood CD3 Positive Selection Cocktail to sample. NOTE: Do not vortex cocktail.	10 µL/mL of diluted sample
	Mix and incubate.	RT for 5 minutes
4	Vortex EasySep™ Releasable RapidSpheres™ 50201. NOTE: Particles should appear evenly dispersed.	30 seconds
5	Add EasySep™ Releasable RapidSpheres™ 50201 to sample.	50 µL/mL of diluted sample
	Mix and incubate.	RT for 5 minutes
6	Add recommended medium (e.g. EasySep™ Buffer) to top up sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.	Top up to 10 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 5 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 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)
* NOTE: Do NOT use 1X RBC Lysis Buffer to dilute whole blood.
** 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™ Serology Whole Blood CD3 Positive Selection Kit Protocol

STEP	INSTRUCTIONS	RoboSep™ (Catalog #20000 and #21000) 
1	**Add whole blood to required tube.**	1 - 4.5 mL
	Required tube.	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	**Dilute whole blood with recommended medium (e.g. EasySep™ Buffer).***	Equal volume to sample
3	**Vortex EasySep™ Releasable RapidSpheres™ 50201.** **NOTE: Particles should appear evenly dispersed.**	30 seconds
4	**Select protocol.** **NOTE: Enter volume.**	HLA Serology Whole Blood CD3 Positive Selection 18981 v2 NOTE: Enter diluted sample volume.
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 from the magnet and resuspend in desired medium. Be sure to collect cells from the sides of the tube.**	Isolated cells are ready for use

* NOTE: Do NOT use 1X RBC Lysis Buffer to dilute whole blood.

Notes and Tips

ASSESSING PURITY

The EasySep™ Serology Whole Blood CD3 Positive Selection Cocktail uses an anti-CD3 antibody clone that to our knowledge blocks all 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, or
- 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).

NOTE: It is recommended to assess purity on CD45+ cells to exclude debris, platelets, and RBCs.

- Anti-Human CD45 Antibody, Clone HI30 (Catalog #60018).

DONOR VARIABILITY

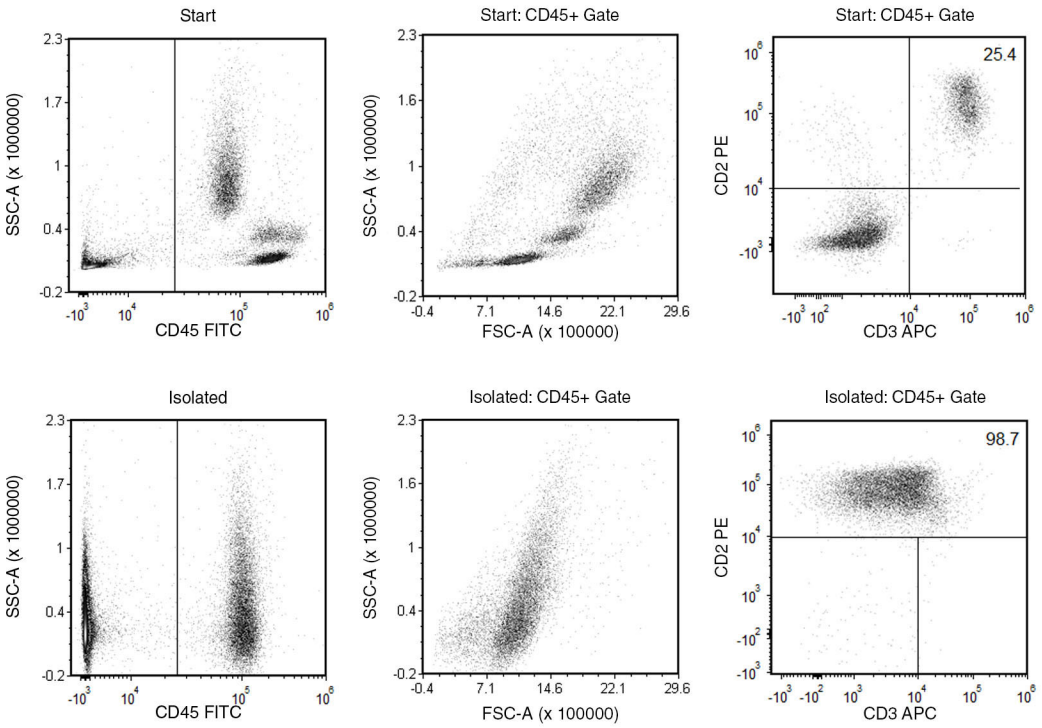
Certain donors express one or more soluble serum factors that can cause cross-linking with magnetic particles. This may result in visible aggregates in the enriched cell fraction following positive selection. These aggregates may appear as a distinct, high side-scatter population on FSC vs. SSC plots 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 CD41 and CD45.

Potential aggregation can be avoided by washing away the donor plasma. Dilute the sample 2-fold in 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.

NOTE: Particles remain attached to the cell surface following cell isolation. The magnetic particles present on the surface of the isolated CD3 cells will cause a large FSC/SSC shift when the samples are run on a flow cytometer. This will make it difficult to gate on a specific lymphocyte population based on FSC/SSC. Instead, we recommend first gating the cells on CD45 and examining the lymphocyte populations based on the CD45+ gate.

Data



Starting with human whole blood, the CD3+ cell content of the isolated fraction typically ranges from 96.7 - 99.6% (as assessed by labeling with CD2; gated on CD45). In the above example, the purities of the start and final isolated fractions are 25.4% and 98.7%, respectively (gated on CD45+).

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