EasySep™ Human CD3 Positive Selection Kit II

For processing 1 x 10^9 cells

Catalog #17851
Catalog #17851RF RoboSep™

Positive Selection

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STEMCELL™ TECHNOLOGIES
Scientists Helping Scientists™ | WWW.STEMCELL.COM
TOLL FREE PHONE 1 800 667 0322 • PHONE +1 604 877 0713
INFO@STEMCELL.COM • TECHSUPPORT@STEMCELL.COM
FOR GLOBAL CONTACT DETAILS VISIT OUR WEBSITE

Description

Isolate highly purified CD3+ cells from fresh or previously frozen human peripheral blood mononuclear cells (PBMCs) or washed leukapheresis samples in as little as 15 minutes by immunomagnetic positive selection.

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

This kit targets CD3+ cells for positive selection with antibodies 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™ Human CD3 Positive Selection Cocktail II	17851C	1 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™ 50100	50100	1 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.

PBS - phosphate-buffered saline

Components may be shipped at room temperature (15 - 25°C) but should be stored as indicated above.

Sample Preparation

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

PERIPHERAL BLOOD

Prepare a PBMC suspension from whole blood by centrifugation over a density gradient medium (e.g. Lymphoprep™, Catalog #07811). For more rapid PBMC preparation, use the SepMate™ RUO (Catalog #86450/86415) or SepMate™ IVD* (Catalog #85450/85415) cell isolation tube.

If using previously frozen PBMCs, incubate the cells with DNase I Solution (Catalog #07900) at a concentration of 100 µg/mL at room temperature (15 - 25°C) for at least 15 minutes prior to labeling and separation. Filter aggregated suspensions through a 37 µm cell strainer (Catalog #27250) for optimal results.

After preparation, resuspend cells at 1 x 10^8 cells/mL in recommended medium.

* SepMate™ IVD is available only in select regions where it is registered as an In Vitro Diagnostic (IVD) device for the isolation of mononuclear cells (MNCs) from whole blood or bone marrow by density gradient centrifugation. In all other regions, SepMate™ is available for research use only (RUO).

LEUKAPHERESIS

Wash the peripheral blood leukapheresis sample by adding an equivalent volume of recommended medium or PBS containing 2% fetal bovine serum (FBS). Centrifuge at 300 x g for 10 minutes at room temperature. If red blood cell (RBC) lysis is necessary, lyse with Ammonium Chloride Solution (Catalog #07800). If platelet removal is desired, centrifuge at 120 x g for 10 minutes with the brake off. Remove the supernatant and resuspend the cells at 1 x 10^8 cells/mL in recommended medium.

Recommended Medium

EasySep™ Buffer (Catalog #20144), RoboSep™ Buffer (Catalog #20104), or PBS containing 2% 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 for each magnet.

Table 1. EasySep™ Human CD3 Positive Selection Kit II Protocol

		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS	 **EasySep™ (Catalog #18000)**	**"The Big Easy" (Catalog #18001)** 
1	Prepare sample at the indicated cell concentration within the volume range.	1 x 10^8 cells/mL 0.1 - 2 mL	1 x 10^8 cells/mL 0.25 - 8 mL
	Add sample to required tube.	5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Catalog #38007)	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	Add Selection Cocktail to sample. NOTE: Do not vortex cocktail.	100 µL/mL of sample	100 µL/mL of sample
	Mix and incubate.	RT for 3 minutes	RT for 3 minutes
3	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
4	Add RapidSpheres™ to sample.	60 µL/mL of sample	60 µL/mL of sample
	Mix and incubate.	RT for 3 minutes	RT for 3 minutes
5	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 2.5 mL	• Top up to 3 mL for samples ≤ 2 mL • Top up to 10 mL for samples > 2 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 3 minutes	RT for 3 minutes
6	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
7	Repeat steps as indicated.	Steps 5 and 6, two more times (total of 3 x 3-minute separations)	Steps 5 and 6, two more times (total of 3 x 3-minute separations)
8	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.

Table 2. EasySep™ Human CD3 Positive Selection Kit II Protocol

		EASYSEP™ MAGNETS			
STEP	INSTRUCTIONS	EasyPlate™ (Catalog #18102)	EasyEights™ (Catalog #18103) 5 mL tube	EasyEights™ (Catalog #18103) 14 mL tube	Easy 50 (Catalog #18002)
1	Prepare sample at the indicated cell concentration within the volume range.	1 x 10^8 cells/mL 0.05 - 0.2 mL	1 x 10^8 cells/mL 0.25 - 2 mL	1 x 10^8 cells/mL 1 - 8 mL	1 x 10^8 cells/mL 5 - 40 mL
	Add sample to required tube (or plate if using the EasyPlate™ EasySep™ Magnet).	Round-bottom, non-tissue culture-treated 96-well plate (e.g. Catalog #38018)	5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Catalog #38007)	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)	50 mL (30 x 115 mm) conical tube (e.g. Catalog #38010)
2	Add Selection Cocktail to sample. NOTE: Do not vortex cocktail.	100 µL/mL of sample	100 µL/mL of sample	100 µL/mL of sample	100 µL/mL of sample
	Mix and incubate.	RT for 3 minutes	RT for 3 minutes	RT for 3 minutes	RT for 3 minutes
3	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds	30 seconds	30 seconds
4	Add RapidSpheres™ to sample.	60 µL/mL of sample	100 µL/mL of sample	100 µL/mL of sample	100 µL/mL of sample
	Mix and incubate.	RT for 3 minutes	RT for 3 minutes	RT for 3 minutes	RT for 3 minutes
5	Add recommended medium to top up sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.	Top up to 0.25 mL	Top up to 2.5 mL	• Top up to 5 mL for samples ≤ 3 mL • Top up to 10 mL for samples > 3 mL	Top up to: • 10 mL for samples ≤ 5 mL • 20 mL for samples > 5 - 10 mL • 30 mL for samples > 10 - 15 mL • 40 mL for samples > 15 - 20 mL • 50 mL for samples > 20 - 40 mL
	Place the tube or plate (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 10 minutes	RT for 10 minutes	RT for 10 minutes
6	Carefully pipette** (do not pour) off the supernatant. Remove the tube or plate, containing the isolated cells, from the magnet.	Discard supernatant	Discard supernatant	Discard supernatant	Discard supernatant
7	Add recommended medium to top up sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.	Top up to 0.25 mL	Top up to 2.5 mL	• Top up to 5 mL for samples ≤ 3 mL • Top up to 10 mL for samples > 3 mL	Top up to: • 10 mL for samples ≤ 5 mL • 20 mL for samples > 5 - 10 mL • 30 mL for samples > 10 - 15 mL • 40 mL for samples > 15 - 20 mL • 50 mL for samples > 20 - 40 mL
	Place the tube or plate (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 5 minutes	RT for 5 minutes	RT for 5 minutes
8	Carefully pipette** (do not pour) off the supernatant. Remove the tube or plate, containing the isolated cells, from the magnet.	Discard supernatant	Discard supernatant	Discard supernatant	Discard supernatant
9	Repeat steps as indicated.	Steps 7 and 8 (total of 3 x 5-minute separations)	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)	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	Isolated cells are ready for use	Isolated cells are ready for use

RT - room temperature (15 - 25°C)

** Collect the entire supernatant, all at once, into a single pipette (for EasyEights™ 5 mL tube, use a 2 mL serological pipette [Catalog #38002]; 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™ Human CD3 Positive Selection Kit II Protocol

STEP	INSTRUCTIONS	RoboSep™ (Catalog #21000) 
1	Prepare sample at the indicated cell concentration within the volume range.	1 x 10^8 cells/mL 0.5 - 8.5 mL
	Add sample to required tube.	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	Select protocol.	Human CD3 Positive Selection II 17851
3	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds
4	Load the carousel.	Follow on-screen prompts
	Start the protocol.	Press the green “Run” button
5	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

ASSESSING PURITY

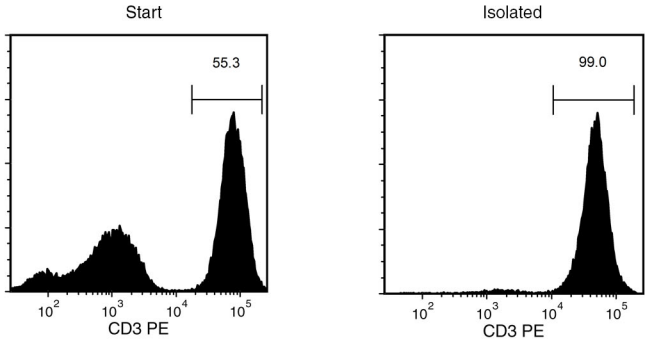
The EasySep™ Human CD3 Positive Selection Cocktail uses an anti-CD3 antibody clone that to our knowledge fully or partially blocks all anti-CD3 antibody clones used to assess purity by flow cytometry. For purity assessment of CD3+ cells by flow cytometry, use the following fluorochrome-conjugated antibody clone:

- Anti-Human CD3 Antibody, Clone UCHT1 (Catalog #60011; partially blocked)

One of the following methods can also be used:

- Use alternative markers such as fluorochrome-conjugated Anti-Human CD4 Antibody, Clone OKT4 (Catalog #60016) and Anti-Human CD8a Antibody, Clone RPA-T8 (Catalog #60022).
- Use a fluorochrome-conjugated secondary antibody, such as Goat Anti-Mouse IgG (H+L) Antibody, Polyclonal (Catalog #60138).

Data



Starting with a single-cell suspension of human PBMCs, the CD3+ cell content of the isolated fraction is typically 99.2 ± 0.2% (mean ± SD using the purple EasySep™ Magnet). In the above example, the purities of the start and final isolated fractions are 55.3% and 99.0%, respectively.