EasySep™ Human CD8+ T Cell Isolation Kit

For processing 1 x 10^9 cells

Catalog #17953
Catalog #17953RF RoboSep™

Negative Selection

Document #10000005310 | Version 03

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Description

Isolate untouched and highly purified CD8+ T cells from fresh or previously frozen human peripheral blood mononuclear cells (PBMCs) or washed leukapheresis samples in as little as 8 minutes by immunomagnetic negative selection.

- Fast, easy-to-use, and column-free
- Up to 91% purity with high recovery
- Untouched, viable cells

This kit targets non-CD8+ T cells for removal with antibodies recognizing specific cell surface markers. Unwanted cells are labeled with antibodies and magnetic particles and separated without columns using an EasySep™ magnet. Desired cells are simply poured off into a new 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 CD8+ T Cell Isolation Cocktail	17953C	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.
EasySep™ Dextran RapidSpheres™ 50103	50103	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 (e.g. Human Whole Peripheral Blood*, Catalog #70507) 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, or source fresh PBMCs (e.g. Human Peripheral Blood Mononuclear Cells, Fresh*, Catalog #200-0077).

If using previously frozen PBMCs (e.g. Human Peripheral Blood Mononuclear Cells, Frozen*, Catalog #70025), 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. It is recommended to wash the cells at least twice with a medium or buffer of choice (e.g. DMEM, IMDM, RPMI, or PBS containing 10% fetal bovine serum [FBS]) prior to labeling and separation. Filter aggregated suspensions through a 37 µm cell strainer (Catalog #27250) for optimal results.

After preparation, resuspend cells at 5 x 10^7 cells/mL in recommended medium.

LEUKAPHERESIS

Wash the peripheral blood leukapheresis sample (e.g. Human Peripheral Blood Leukopak, Fresh*, Catalog #70500) by adding an equivalent volume of recommended medium or PBS with 2% FBS and centrifuging at 300 x g for 10 minutes at room temperature (15 - 25°C). If red blood cell lysis is necessary, lyse with Ammonium Chloride Solution (Catalog #07800). If platelet removal is necessary, centrifuge at 120 x g for 10 minutes with the brake off. Remove the supernatant and resuspend the cells at 5 x 10^7 cells/mL in recommended medium.

* Some primary cell products are available only in select regions. Contact us at techsupport@stemcell.com for further information.

** 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).

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.

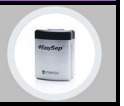
Table 1. EasySep™ Human CD8+ T Cell Isolation Kit Protocol

		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS	 **EasySep™ (Catalog #18000)**	**"The Big Easy" (Catalog #18001)** 
1	Prepare sample at the indicated cell concentration within the volume range.	5 x 10^7 cells/mL 0.25 - 2 mL	5 x 10^7 cells/mL 1 - 8.5 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 Isolation Cocktail to sample. NOTE: Do not vortex cocktail.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
3	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
4	Add RapidSpheres™ to sample and mix.	50 µL/mL of sample	50 µL/mL of sample
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 5 mL for samples ≤ 4 mL • Top up to 10 mL for samples > 4 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 the enriched cell suspension into a new 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 CD8+ T Cell Isolation Kit 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.	5 x 10^7 cells/mL 0.1 - 0.2 mL	5 x 10^7 cells/mL 0.5 - 2 mL	5 x 10^7 cells/mL 1 - 8.5 mL	5 x 10^7 cells/mL 10 - 45 mL
	Add sample to required tube (or plate when using the EasySep™ EasyPlate™ 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 Isolation Cocktail to sample. NOTE: Do not vortex cocktail.	50 µL/mL of sample	50 µL/mL of sample	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes	RT for 5 minutes	RT for 5 minutes
3	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds	30 seconds	30 seconds
4	Add RapidSpheres™ to sample and mix.	50 µL/mL of sample	50 µL/mL of sample	50 µL/mL of sample	50 µL/mL of sample
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 ≤ 4 mL • Top up to 10 mL for samples > 4 mL	• Top up to 25 mL for samples ≤ 20 mL • Top up to 50 mL for samples > 20 mL
	Place the tube or plate (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 5 minutes	RT for 10 minutes	RT for 10 minutes
6	Carefully pipette** (do not pour) the enriched cell suspension into new tube or plate.	Use a new well in the 96-well plate	Use a new 5 mL tube	Use a new 14 mL tube	Use a new 50 mL tube
7	Remove the tube or plate, containing the isolated cells, from the magnet; Place the new tube or plate (without lid) into the magnet and incubate for a second separation.	RT for 5 minutes	RT for 5 minutes	RT for 10 minutes	RT for 10 minutes
8	Carefully pipette** (do not pour) the enriched cell suspension into new tube or plate.	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 (e.g. 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 CD8+ T Cell Isolation Kit Protocol

STEP	INSTRUCTIONS	RoboSep™ (Catalog #21000) 
1	Prepare sample at the indicated cell concentration within the volume range.	5 x 10^7 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 CD8+ T Cell Isolation 17953
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.	Isolated cells are ready for use

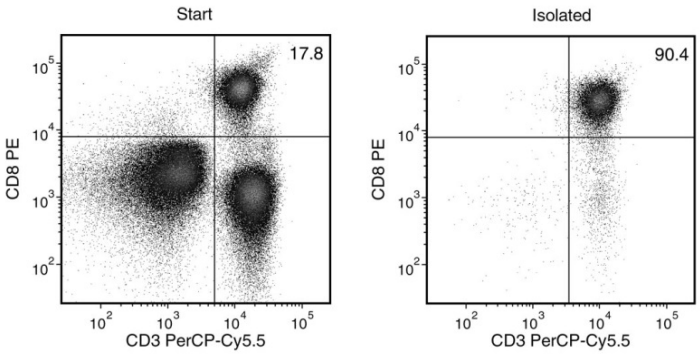
Notes and Tips

ASSESSING PURITY

For purity assessment of CD8+ T cells (CD3+CD8+) by flow cytometry, use the following fluorochrome-conjugated antibodies:

- Anti-Human CD3 Antibody, Clone UCHT1 (Catalog #60011), and
- Anti-Human CD8a Antibody, Clone RPA-T8 (Catalog #60022)

Data



Starting with human PBMCs, the CD8+ T cell content (CD3+CD8+) of the isolated fraction is typically 85.6 ± 4.9% (mean ± SD using the purple EasySep™ Magnet). In the above example, the purities of the start and final isolated fractions are 17.8% and 90.4%, respectively.