EasySep™ Human Naïve CD8+ T Cell Isolation Kit II

For processing 1 x 10^9 cells

Catalog #17968

Negative Selection

Document #10000000733 | Version 01

STEMCELL™ TECHNOLOGIES

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Description

Isolate untouched and highly purified naïve CD8+ T cells (CD8+CD45RA+CCR7+ and CD45RO-CD57-CD56-) from fresh human peripheral blood mononuclear cells (PBMCs) by immunomagnetic negative selection.

- Fast, easy-to-use and column-free
- Up to 97% purity
- Untouched, viable cells

This kit targets non-naïve 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 Naïve CD8+ T Cell Isolation Cocktail	19258C	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 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 #07801). For more rapid PBMC preparation, use the SepMate™ RUO (Catalog #86450/86415) or SepMate™ IVD* (Catalog #85450/85415) cell isolation tube.

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

* SepMate™ IVD is only available 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

1. Add an equal volume of Ammonium Chloride Solution (Catalog #07800) to the leukapheresis sample.
   NOTE: If working with large volumes (> 150 mL), concentrate leukapheresis sample first by centrifuging at 500 x g for 10 minutes. Remove the supernatant and resuspend the cells in 1/10th of the original leukapheresis volume with recommended medium (e.g. for 300 mL of cells, resuspend in 30 mL of recommended medium). For small volumes (≤ 150 mL), add Ammonium Chloride Solution directly to the leukapheresis sample.
2. Incubate on ice for 15 minutes.
3. Centrifuge at 500 x g for 10 minutes at room temperature (15 - 25°C). Remove the supernatant.
4. Wash the cells by topping up the tube with recommended medium. Centrifuge the cells at 150 x g for 10 minutes at room temperature with the brake off. Carefully remove the supernatant.
5. Repeat step 4 one or more times until most of the platelets have been removed (indicated by a clear supernatant).
6. Resuspend the cells at 5 x 10^7 cells/mL in recommended medium.

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 for each magnet.

Table 1. EasySep Human Naïve CD8+ T Cell Isolation 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.**	5 x 10^7 cells/mL 0.25 - 2 mL	5 x 10^7 cells/mL 0.5 - 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 Isolation Cocktail to sample.**	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.**	50 µL/mL of sample No incubation, IMMEDIATELY proceed to next step	50 µL/mL of sample No incubation, IMMEDIATELY proceed to next step
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 5 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.**	Use a new 5 mL tube	Use a new 14 mL tube
7	**Remove the tube from the magnet and place the new tube (without lid) into the magnet and incubate for a second separation.**	RT for 3 minutes	RT for 5 minutes
8	**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 Naïve CD8+ T Cell Isolation Kit II Protocol

		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS	EasyEights™ (Catalog #18103)	
		5 mL tube	14 mL tube
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 0.5 - 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 Isolation Cocktail to sample.	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 No incubation, IMMEDIATELY proceed to next step	50 µL/mL of sample No incubation, IMMEDIATELY proceed to next step
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 5 minutes	RT for 10 minutes
6	Carefully pipette (do not pour) the enriched cell suspension** into a new tube.	Use a new 5 mL tube	Use a new 14 mL tube
7	Remove the tube from the magnet and place the new tube (without lid) into the magnet and incubate for a second separation.	RT for 5 minutes	RT for 10 minutes
8	Carefully pipette (do not pour) 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)
** 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 Naïve CD8+ T Cell Isolation Kit II Protocol

STEP	INSTRUCTIONS	RoboSep™ (Catalog #20000 and #21000) 
1	Prepare sample at the indicated cell concentration within the volume range.	5 x 10^7 cells/mL 0.5 - 8 mL
	Add sample to required tube.	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	Select protocol.	Human Naive CD8+ T Cell Isolation Kit II 17968
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.	Isolated cells are ready for use

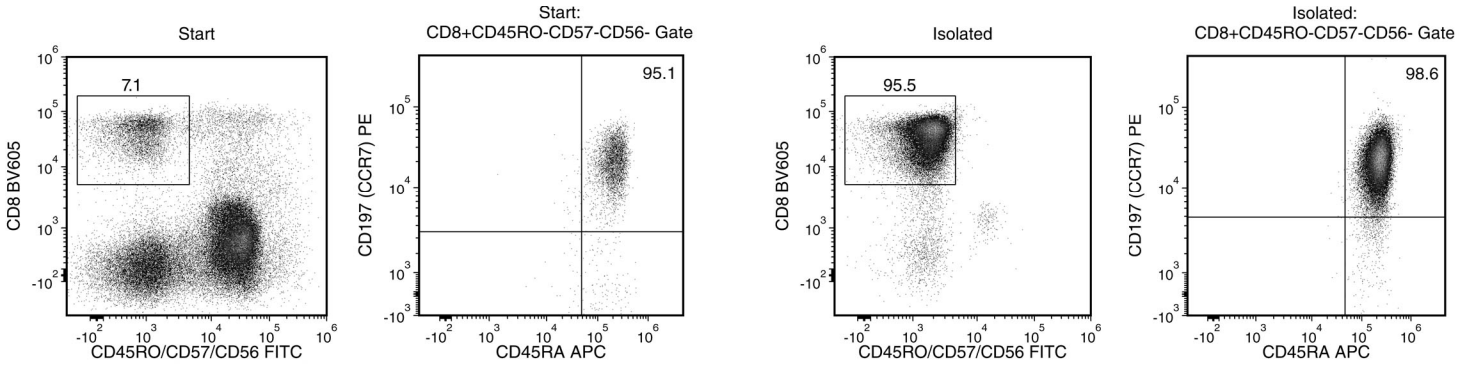
Notes and Tips

ASSESSING PURITY

For purity assessment of naïve CD8+ T cells (CD8+CD45RA+CCR7+ and CD45RO-CD57-CD56-) by flow cytometry, use the following fluorochrome-conjugated antibodies:

- Anti-Human CD8a Antibody, Clone RPA-T8 (Catalog #60022), and
- Anti-human CD45RA antibody, and
- Anti-human CD197 (CCR7) antibody, and
- Anti-Human CD45RO Antibody, Clone UCHL1 (Catalog #60097), and
- Anti-human CD57 antibody, and
- Anti-Human CD56 (NCAM) Antibody, Clone HCD56 (Catalog #60021)

Data



Starting with fresh PBMCs, the naïve CD8+ T cell content (CD8+CD45RA+CCR7+ and CD45RO-CD57-CD56-) of the isolated fraction is typically 93.7 ± 2.4% (mean ± SD using the purple EasySep™ Magnet). In the above example, the purities of the start and final isolated fractions are 6.8% and 94.2%, respectively.