EasySep™ Human Naïve CD4+ T Cell Isolation Kit

Negative Selection

Catalog #19555

For processing 1 x 10^9 cells

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Description

Isolate untouched and highly purified naïve CD4+ T cells from fresh or previously frozen human peripheral blood mononuclear cells (PBMCs) by immunomagnetic negative selection.

This kit targets non-naïve CD4+ T cells for removal with antibodies recognizing CD8, CD14, CD16, CD19, CD20, CD25, CD36, CD56, CD61, CD66b, CD123, HLA-DR, TCRγ/δ, and glycophorin A 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 CD4+ T Cell Isolation Cocktail	19555C	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™ Biotinylated Anti-CD45RO Antibody	19156C	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

‡ When using the Easy 50 EasySep™ Magnet, please contact Technical Support at techsupport@stemcell.com to request and additional vial of EasySep™ Dextran RapidSpheres™ 50103.

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 mononuclear cell (MNC) suspension from whole peripheral blood by density gradient centrifugation. For more rapid MNC preparation without the need for careful sample layering, use the SepMate™-15 (Catalog #15415) or SepMate™-50 (Catalog #15450) cell isolation tube.

If using previously frozen MNCs, incubate the cells with DNase I Solution (Catalog #07900) at a concentration of 100 µg/mL for at least 15 minutes at room temperature (15 - 25°C) prior to labeling and separation. Filter clumpy suspensions through a 40 µm Cell Strainer (Catalog #27305) for optimal results.

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

LEUKAPHERESIS (LEUKO PAK)

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 Leukopak 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 (Catalog #07800) directly to the leukapheresis sample.

1. Add an equal volume of Ammonium Chloride Solution to the leukapheresis sample.
2. Incubate for 15 minutes on ice.
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 (15 - 25°C) 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 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% FBS with 1 mM EDTA. Medium should be free of Ca++, Mg++, and biotin.

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 CD4+ T Cell Isolation Kit Instructions

		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 0.1 - 2 mL	5 x 10^7 cells/mL 0.25 - 8.5 mL
	Add sample to required tube.	5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352058)	14 mL (17 x 100 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352057)
2	**Add Biotinylated Anti-CD45RO Antibody to sample.**	50 µL/mL of sample	50 µL/mL of sample
	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™.**	30 seconds	30 seconds
4	**Add RapidSpheres™ to sample.**	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 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 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 5 minutes
6	**Pick up the magnet, and in one continuous motion invert the magnet and tube, pouring off 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 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 now ready for use	Isolated cells are now 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 CD4+ T Cell Isolation Kit Instructions

		EASYSEP™ MAGNETS		
		EasyEights™ (Catalog #18103)		
STEP	INSTRUCTIONS	5 mL tube	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 0.25 - 2 mL	5 x 10^7 cells/mL 2 - 8.5 mL	5 x 10^7 cells/mL 5 - 40 mL
	Add sample to required tube.	5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352058)	14 mL (17 x 100 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352057)	50 mL conical tube (e.g. Corning Catalog #352070)
2	Add Biotinylated Anti-CD45RO antibody to sample.	50 µL/mL of sample	50 µL/mL of sample	50 µL/mL of sample
	Add Isolation Cocktail to 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
3	Vortex RapidSpheres™.	30 seconds	30 seconds	30 seconds
4	Add RapidSpheres™ to 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
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 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 ≤ 10 mL • Top up to 50 mL for samples > 10 mL
	Place the plasticware (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 5 minutes	RT for 10 minutes
6	Carefully pipette** (do not pour) the enriched cell suspension into new plasticware.	Use a new 5 mL tube	Use a new 14 mL tube	Use a new 50 mL tube
7	Remove the plasticware from the magnet and place the new plasticware (without lid) into the magnet and incubate for a second round of separation.	RT for 5 minutes	RT for 5 minutes	RT for 10 minutes
8	Carefully pipette (do not pour) the enriched cell suspension into new plasticware. Collect only the clear fraction.	Isolated cells are now ready for use	Isolated cells are now ready for use	Isolated cells are now ready for use

RT; room temperature (15 - 25°)

‡ When using the Easy 50 EasySep™ Magnet, please contact Technical Support at techsupport@stemcell.com to request and additional vial of EasySep™ Dextran RapidSpheres™ 50103.

** It is recommended to collect the entire supernatant, all at once, into a single pipette (e.g. for the EasyEights™ 5 mL tube use a 2 mL serological pipette and 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™ Human Naïve CD4+ T Cell Isolation Kit Instructions

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 1 - 8.5 mL***
	Add sample to required tube.	14 mL (17 x 100 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352057)
2	Select protocol.	Human Naïve CD4+ T Cell Isolation 19555
3	Vortex RapidSpheres™.	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 now ready for use

*** For sample start volumes between 0.5 - 1 mL, resuspend cells to a final volume of 1 mL in RoboSep™ Buffer.

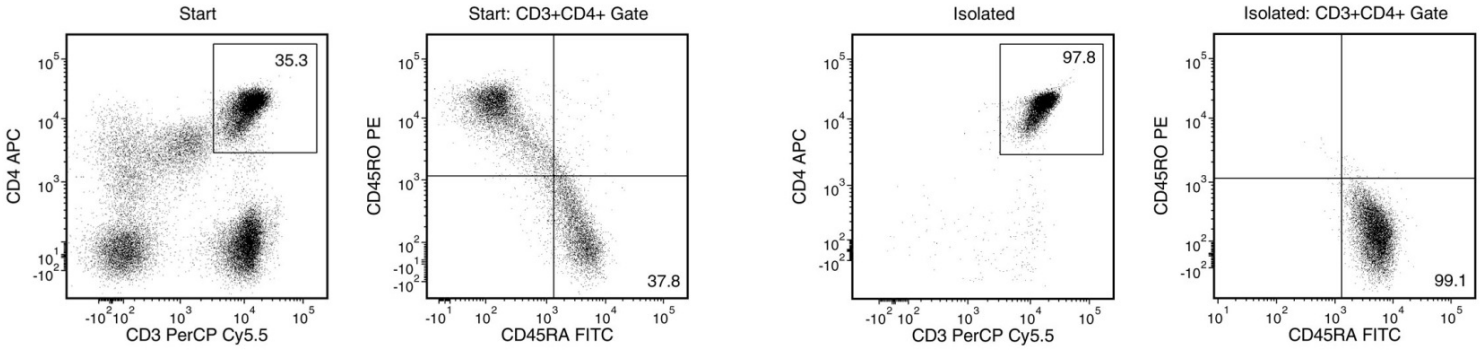
Notes and Tips

ASSESSING PURITY

For purity assessment of naïve CD4 T cells by flow cytometry use fluorochrome-conjugated:

- Anti-Human CD3 Antibody, Clone UCHT1 (Catalog #60011)
- Anti-Human CD4 Antibody, Clone OKT4 (Catalog #60016)
- Anti-Human CD45RO Antibody, Clone UCHL1 (Catalog #60097)
- anti-human CD45RA antibody

Data



Starting with a single-cell suspension of PBMCs, the naïve CD4+ T cell content (CD3+CD4+CD45RA+CD45RO-) of the isolated fraction typically ranges from 91.3% - 96.9%. In the above example, the purities of the start and final isolated fractions are 11.1% and 93.2%, respectively.