EasySep™

Negative Selection

Catalog #17962

EasySep™ Human Resting CD4+ T Cell Isolation Kit

For processing 1 x 10^9 cells

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

Document #DX20671 | Version 1_0_1

Description

Isolate untouched and highly purified resting CD4+ T cells from fresh or previously frozen human peripheral blood mononuclear cells (PBMCs) or washed leukapheresis samples.

- Fast, easy-to-use and column-free
- Up to 99% purity
- Isolated cells are untouched

This kit targets non-resting CD4+ 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 Resting CD4+ T Cell Isolation Cocktail	17962C	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™ Human CD25 Depletion Cocktail	17862C	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	2 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.
RoboSep™ Empty Vial	27401	1	Not applicable	Not applicable	Not applicable

PBS - phosphate-buffered saline

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

Sample Preparation

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.

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 40 µm Cell Strainer (Catalog #27305) for optimal results. After preparation, resuspend cells at 5 x 10^7 cells/mL in PBS containing 2% fetal bovine serum (FBS) without EDTA.

* 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 as research use only (RUO).

LEUKAPHERESIS (LEUKO PAK)

Wash the peripheral blood leukapheresis sample by adding an equivalent volume of recommended medium or PBS containing 2% FBS. Centrifuge at 300 x g for 10 minutes at room temperature (15 - 25°C). If red blood cell (RBC) lysis is desired, 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 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 and 1 mM EDTA, unless otherwise noted. 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 Resting CD4+ 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 NOTE: If starting with fewer than 1.25 x 10^7 cells, resuspend cells in 0.25 mL	5 x 10^7 cells/mL 0.5 - 6 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
3	**Add CD25 Depletion Cocktail to sample.** **NOTE: Recovery can be improved by adding depletion cocktail at a decreased concentration. This will improve recovery but may reduce purity. See Notes and Tips.**	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 10 minutes	RT for 10 minutes
4	**Vortex RapidSpheres™.** **NOTE: Particles should appear evenly dispersed.**	30 seconds	30 seconds
5	**Add RapidSpheres™ to sample.**	75 µL/mL of sample	75 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
6	**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 < 2 mL • Top up to 10 mL for samples ≥ 2 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 5 minutes
7	**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
8	**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
9	**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 Resting CD4+ T Cell Isolation Kit Protocol

		EASYSEP™ MAGNETS	
		EasyEights™ (Catalog #18103)	
STEP	INSTRUCTIONS	5 mL tube	14 mL tube
1	Prepare sample at the indicated cell concentration within the volume range.	5 x 10^7 cells/mL 0.5 - 2 mL	5 x 10^7 cells/mL 0.5 - 6 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
3	Add CD25 Depletion Cocktail to sample. NOTE: Recovery can be improved by adding depletion cocktail at a decreased concentration. This will improve recovery but may reduce purity. See Notes and Tips.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 10 minutes	RT for 10 minutes
4	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
5	Add RapidSpheres™ to sample.	75 µL/mL of sample	75 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
6	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 < 2 mL • Top up to 10 mL for samples ≥ 2 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 10 minutes	RT for 10 minutes
7	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
8	Remove the tube from the magnet and place the new tube (without lid) into the magnet and incubate for a second separation.	RT for 10 minutes	RT for 10 minutes
9	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 (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 Resting CD4+ T Cell Isolation Kit 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 - 6 mL
	Add sample to required tube.	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	**Add CD25 Depletion Cocktail (diluted if necessary) to the RoboSep™ Empty Vial provided.** **NOTE: Recovery can be improved by adding depletion cocktail at a decreased concentration. This will improve recovery but may reduce purity. See Notes and Tips.**	50 µL/mL of sample[§]
3	**Select protocol.**	Human Resting CD4+ T Cell Isolation 17962
4	**Vortex RapidSpheres™.** **NOTE: Particles should appear evenly dispersed.**	30 seconds
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.**	Isolated cells are ready for use

[§] RoboSep™ requires an extra 100 µL to run properly (compared to manual protocols). Follow on-screen prompts for the minimum volume to load into the RoboSep™ Empty Vial.
[‡] When using RoboSep™-S and the provided RoboSep™ Empty Vial to dilute EasySep™ Human CD25 Depletion Cocktail, the barcode reader may return an error message. This error message can be overridden without affecting the successful completion of the protocol.

Notes and Tips

ASSESSING PURITY

Resting CD4+ T cells are described as CD3+CD4+CD8-CD25-CD69-HLA-DR-. It is recommended to assess purity gated on the CD45-positive cells to exclude debris, platelets, and RBCs.

For optimal purity assessment of resting CD4+ T cells by flow cytometry use the following fluorochrome-conjugated antibody clones:

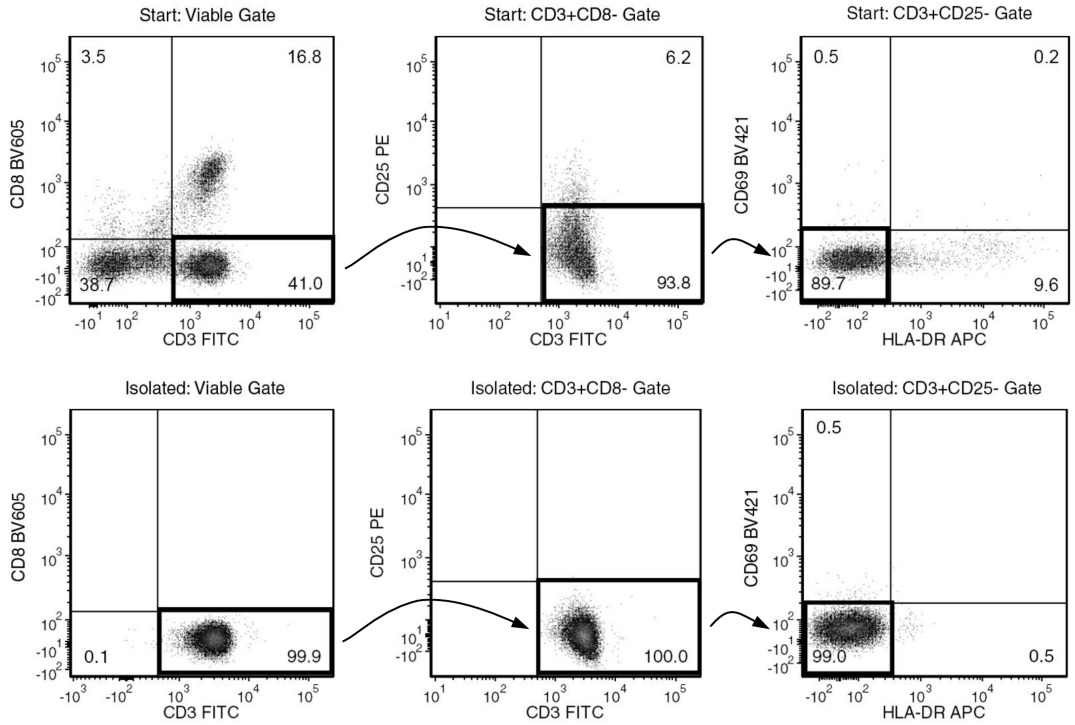
- Anti-Human CD3 Antibody, Clone UCHT1, FITC (Catalog #60011FI), and
- Anti-human CD4 antibody, clone RPA-T4, APC-Cy7, and
- Anti-human CD8a antibody, clone RPA-T8, BV605, and
- Anti-Human CD25 Antibody, Clone 2A3, PE (Catalog #60153PE), and
- Anti-human CD45 antibody, clone HI30, PE-Cy7, and
- Anti-human CD69 antibody, clone FN50, BV421, and
- Anti-human HLA-DR antibody, clone LN3, APC

OPTIMIZING PERFORMANCE

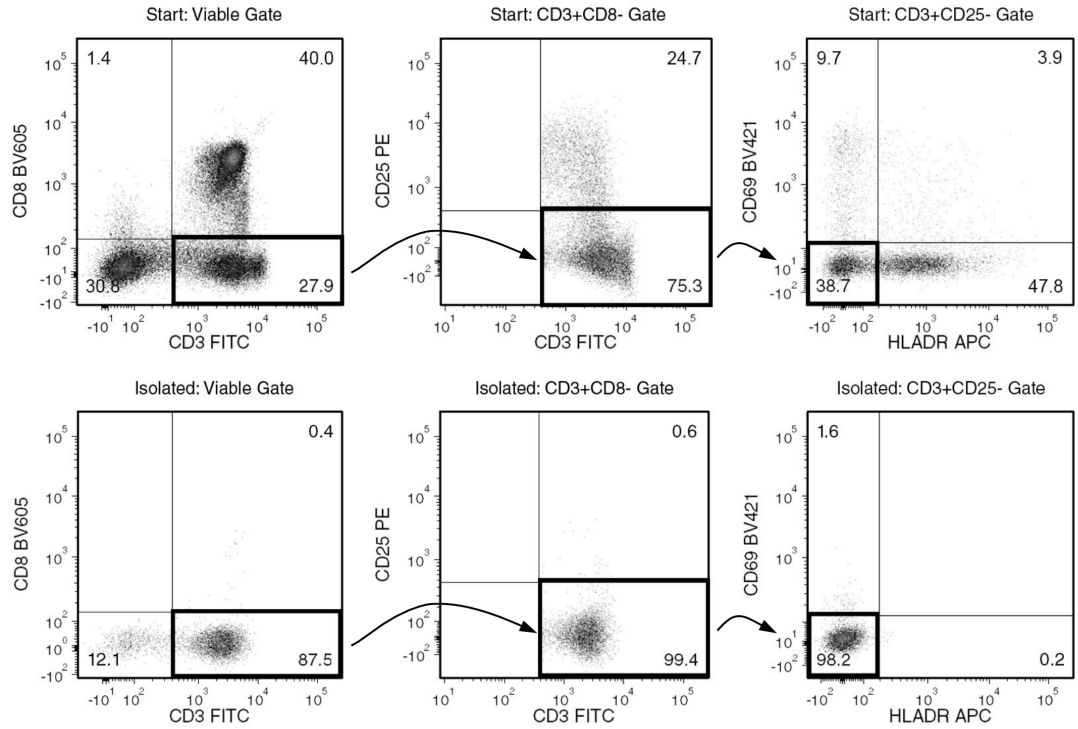
- For low starting cell numbers (e.g. fewer than 5 x 10^6 cells), a titration of EasySep™ Dextran RapidSpheres™ may be required for optimal performance.
- Addition of EasySep™ Human CD25 Depletion Cocktail at the recommended 50 µL/mL removes both $CD25^{high}$ and $CD25^{mid}$ cell populations. A dilution of EasySep™ Human CD25 Depletion Cocktail 1 in 5 to 1 in 10 in D-PBS (Without Ca++ and Mg++; Catalog #37350) may be required to retain the $CD25^{mid}$ cell population. Use the diluted EasySep™ Human CD25 Depletion Cocktail within 24 hours of preparation.

Data

A PBMCs



B PBMCs with Elevated CD25/CD69/HLA-DR Expression



(A) Starting with fresh PBMCs, the resting CD4+ T cell content (CD3+CD4+CD8-CD25-CD69-HLA-DR-) of the isolated fraction is typically 95.5 ± 6.5% (mean ± SD using the purple EasySep™ Magnet). In the above example, the purities of the start and final isolated fractions are 35% and 99%, respectively.
(B) Starting with fresh PBMCs that have elevated CD25/CD69/HLA-DR expression, the resting CD4+ T cell content of the isolated fraction is typically 88.0 ± 7.0% (mean ± SD using the purple EasySep™ Magnet). In the above example, the purities of the start and final isolated fractions are 8% and 85%, respectively.