



EasySep™ Direct Human CD4+ T Cell Isolation Kit

Negative Selection

Catalog #19662

For processing 100 mL whole blood



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Document #28076 | Version 1_1_2

Description

Isolate highly purified CD4+ T cells directly from human whole blood by immunomagnetic negative selection.

The benefits of this kit include:

- > 99.9% RBC depletion without the need for density gradient centrifugation, sedimentation or lysis
- Up to 96% purity of isolated cells
- Fast, easy-to-use and column-free
- Isolated cells are untouched

This kit targets non-CD4+ T cells for removal with antibodies recognizing specific surface markers. Unwanted cells are labeled with antibodies and EasySep™ Direct RapidSpheres™, and separated using an EasySep™ magnet. Desired cells are simply collected into a new tube and 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™ Direct Human CD4+ T Cell Isolation Cocktail	19662C	2 x 2.5 mL	Store at 2 - 8°C.	Stable until expiry date (EXP) on label.	A combination of monoclonal antibodies in PBS.
EasySep™ Direct RapidSpheres™ 50300	50300	4 x 2.5 mL	Store at 2 - 8°C.	Stable until expiry date (EXP) on label.	A suspension of magnetic particles and monoclonal antibodies in PBS.

PBS - phosphate-buffered saline

Components may be shipped at room temperature (15 - 25°C) and should be refrigerated upon receipt.

Precipitate may be observed in the cocktail vial but will not affect performance.

Sample Preparation

For optimal RBC depletion, collect blood using heparin or acid citrate dextrose (ACD) as an anticoagulant. The use of K2EDTA or K3EDTA as an anticoagulant is not recommended.

For best recovery, use fresh unprocessed human whole blood. Recovery of the desired isolated cells decreases with samples that are older than 24 hours.

The volume of blood samples that can be processed depends on the EasySep™ magnet used for the isolation procedure. Blood samples must be placed in the required tube to properly fit into the appropriate EasySep™ magnet (see Tables 1 and 2).

Recommended Medium

PBS (Catalog #37350) that is 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™ Direct Human CD4+ T Cell Isolation Kit Protocol

		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS	 EasySep™ (Catalog #18000)	 “The Big Easy” (Catalog #18001)
1	Collect sample within the volume range.	0.5 - 1.5 mL	1.5 - 7.0 mL
	Add whole blood 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	Vortex RapidSpheres™.	30 seconds	30 seconds
3	Add Isolation Cocktail to sample.	50 µL/mL of sample	50 µL/mL of sample
4	Add RapidSpheres™ to sample.	50 µL/mL of sample	50 µL/mL of sample
5	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 double the volume for samples ≤ 5 mL • Top up to 10 mL for samples > 5 mL
7	Place the tube (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 5 minutes
8	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
9	Add RapidSpheres™ to the new tube containing the enriched cells.	Use same volume as in step 4	Use same volume as in step 4
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
10	Remove the tube from the magnet and place the tube from step 9 (without lid) into the magnet and incubate for a second separation.	RT for 5 minutes	RT for 5 minutes
11	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	Use a new 14 mL tube
12	Remove the tube from the magnet and place the new tube (without lid) into the magnet and incubate for a third separation.	---	RT for 5 minutes
13	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

RT; room temperature (15 - 25°C)

* Following the first magnetic separation the collected cells may contain a significant amount of RBCs and may look similar to the original unprocessed human whole blood sample.

** To minimize RBC contamination in the isolated cells, pour off the sample along a clean area of the tube (i.e. the opposite side to where the sample was poured in).

Table 2. EasySep™ Direct Human CD4+ T Cell Isolation Kit Protocol

		EASYSEP™ MAGNETS		
STEP	INSTRUCTIONS	EasyEights™ (Catalog #18103)		Easy 50 (Catalog #18002)
		5 mL tube	14 mL tube	
1	Collect sample within the volume range.	0.5 - 1.5 mL	1.5 - 7.0 mL	7 - 30 mL
	Add whole blood 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	Vortex RapidSpheres™.	30 seconds	30 seconds	30 seconds
3	Add Isolation Cocktail to sample.	50 µL/mL of sample	50 µL/mL of sample	50 µL/mL of sample
4	Add RapidSpheres™ to sample.	50 µL/mL of sample	50 µL/mL of sample	50 µL/mL of sample
5	Mix and incubate.	RT for 5 minutes	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 double the volume for samples ≤ 5 mL - Top up to 10 mL for samples > 5 mL	- Top up to double the volume for samples ≤ 25 mL - Top up to 50 mL for samples > 25 mL
7	Place the tube (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 5 minutes	RT for 10 minutes
8	Carefully pipette*** (do not pour) the enriched cell suspension into a new tube. NOTE: Collect the entire clear fraction from top to bottom. For optimal recovery, also collect a small volume of RBCs (up to 10% of the starting sample volume).	Use a new 5 mL tube	Use a new 14 mL tube	Use a new 50 mL tube
9	Add RapidSpheres™ to the new tube containing the enriched cells.	Use same volume as in step 4	Use same volume as in step 4	Use same volume as in step 4
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes	RT for 5 minutes
10	Remove the tube from the magnet and place the tube from step 9 (without lid) into the magnet and incubate for a second round of separation.	RT for 5 minutes	RT for 5 minutes	RT for 5 minutes
11	Carefully pipette*** (do not pour) the enriched cell suspension into a new tube. NOTE: Collect only the clear fraction.	Use a new 5 mL tube	Use a new 14 mL tube	Use a new 50 mL tube
12	Remove the tube from the magnet and place the new tube (without lid) containing the enriched cells into the magnet and incubate for a third round of separation.	RT for 5 minutes	RT for 5 minutes	RT for 5 minutes
13	Carefully pipette*** (do not pour) the enriched cell suspension into a new tube. NOTE: 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°C)

*** Collect the entire enriched cell suspension, 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).

Notes and Tips

REMOVAL OF RESIDUAL RBCs IN THE ISOLATED CELLS

Typically, further RBC depletion is not required following cell isolation. If residual RBCs are visible in the isolated cell pellet following centrifugation after the end of the protocol, resuspend in a small volume (0.2 - 2.5 mL) of recommended medium or desired culture medium and place in a smaller EasySep™ magnet for an additional 5 minute separation. Collect the supernatant and the isolated cells are now ready for use in downstream applications. Residual RBCs may also be lysed using Ammonium Chloride Solution (Catalog #07800).

ASSESSING PURITY

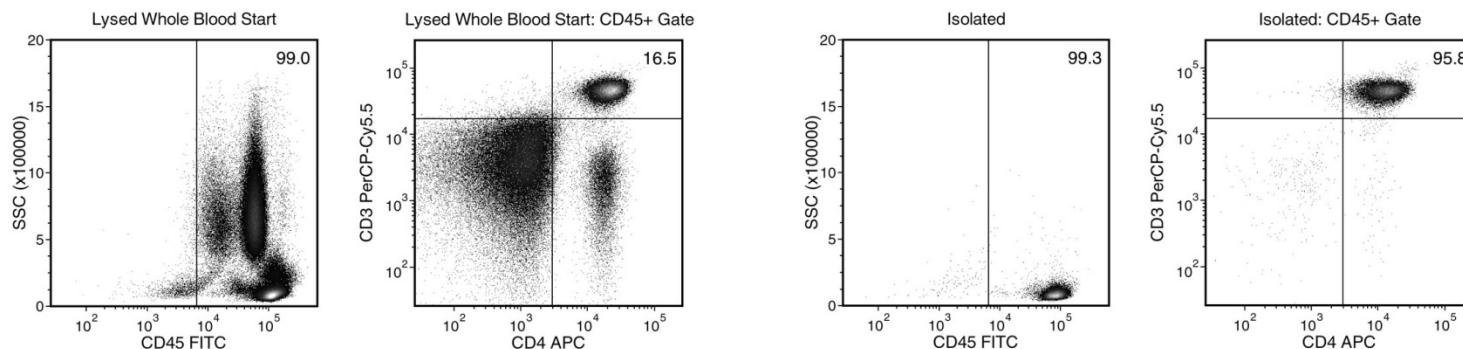
For purity assessment of CD4+ T cells (CD3+CD4+) by flow cytometry use fluorochrome-conjugated:

- Anti-Human CD3 Antibody, Clone UCHT1 (Catalog #60011), and
- Anti-Human CD4 Antibody, Clone OKT4 (Catalog #60016), and
- Anti-Human CD45 Antibody, Clone HI30 (Catalog #60018)

NOTE: It is recommended to assess purity on the CD45 positive cells to exclude debris, platelets and RBCs.

Data

Starting with human whole blood from normal healthy donors, the typical CD4+ T cell (CD3+CD4+) content of the non-lysed final isolated fraction is $93.6 \pm 2.5\%$ (gated on CD45) or $93.1 \pm 2.5\%$ (not gated on CD45).



In the above example, the CD4+ T cell (CD3+CD4+) content of the lysed whole blood start sample and non-lysed final isolated fraction is 16.5% and 95.8% (gated on CD45), respectively, or 16.3% and 95.1% (not gated on CD45), respectively. The starting frequency of CD4+ T cells in the non-lysed whole blood start sample is 0.016% (data not shown).

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