EasySep™ Human Th17 Cell Enrichment Kit

Positive Selection

Catalog #18162

For processing 1 x 10^9 cells

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Description

Isolate highly purified Th17 (CD4+CXCR3-CCR6+) T cells from fresh human peripheral blood mononuclear cells (PBMCs) or leukapheresis samples using a simple, two-step procedure.

- Fast and easy-to-use
- Up to 94% purity
- No columns required

First, CD4+CXCR3- cells are pre-enriched using the EasySep™ Human CD4+CXCR3- T Cell Pre-Enrichment Cocktail (19152C.1) with antibodies recognizing specific cell surface markers. Then, CCR6 positive cells are selected using the EasySep™ Human CCR6 Positive Selection Cocktail (18262C), which contains an antibody recognizing the CCR6 (CD196) surface marker. The EasySep™ cocktails label cells with antibodies that link to magnetic particles. The cells are separated without columns using an EasySep™ magnet. Isolated cells are immediately available for downstream applications such as flow cytometry, cell culture, or DNA/RNA extraction.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Human CD4+CXCR3- T Cell Pre-Enrichment Cocktail	19152C.1	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™ D2 Magnetic Particles	19650	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 TBS.
EasySep™ Human CCR6 Positive Selection Cocktail	18262C	1 x 0.5 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A combination of monoclonal antibodies in PBS. Includes an Fc receptor blocking antibody.
EasySep™ Magnetic Nanoparticles Positive Selection (● brown)	18150H	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; TBS - TRIS-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 peripheral 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 (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 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 (Catalog #07800) directly to the leukapheresis sample.

1. Add an equal volume of Ammonium Chloride Solution 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 Table 1 for detailed instructions regarding the EasySep™ procedure for each magnet.

Table 1. EasySep™ Human Th17 Cell Enrichment 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 0.5 - 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. Corning Catalog #352058)	14 mL (17 x 100 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352057)
2	Add T Cell Pre-Enrichment Cocktail to sample.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 10 minutes	RT for 10 minutes
3	Vortex D2 Magnetic Particles. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
4	Add D2 Magnetic Particles to sample.	100 µL/mL of sample NOTE: Two different particles are provided in this kit. Ensure D2 Magnetic Particles are used in this step.	100 µL/mL of sample NOTE: Two different particles are provided in this kit. Ensure D2 Magnetic Particles are used in this step.
	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 10 minutes	RT for 10 minutes
6	Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring the pre-enriched cells into a new tube.	Use a new 5 mL tube	Use a new 14 mL tube
7	Remove the original tube from the magnet. Place the new tube (without lid) containing pre-enriched cells 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 the pre-enriched cells into a new tube.	Use a new 5 mL tube	Use a new 14 mL tube
9	Centrifuge pre-enriched cells.	200 x g for 10 minutes at RT	200 x g for 10 minutes at RT
	Discard the supernatant and resuspend cell pellet at the indicated volume.	Resuspend in 0.25 mL	• Resuspend in 0.25 mL for samples with start volume ≤ 2.5 mL • Resuspend in 0.5 mL for samples with start volume > 2.5 - 4 mL • Resuspend in 1 mL for samples with start volume > 4 mL
Continue on to CCR6 Positive Selection Procedure.		See step 10, next page	See step 10, next page

STEP	INSTRUCTIONS (continued)	 EasySep™ (Catalog #18000)	"The Big Easy" (Catalog #18001) 
10	Add CCR6 Positive Selection Cocktail to sample.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 15 minutes	RT for 15 minutes
11	Mix Magnetic Nanoparticles (brown). NOTE: Particles should appear evenly dispersed.	Pipette up and down more than 5 times	Pipette up and down more than 5 times
12	Add Magnetic Nanoparticles (brown) to sample.	100 µL/mL of sample NOTE: Two different particles are provided in this kit. Ensure Magnetic Nanoparticles (● brown) are used in this step.	100 µL/mL of sample NOTE: Two different particles are provided in this kit. Ensure Magnetic Nanoparticles (● brown) are used in this step.
	Mix and incubate.	RT for 10 minutes	RT for 10 minutes
13	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 2.5 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 5 minutes
14	Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring off the supernatant. Remove the tube from the magnet; this tube contains the isolated cells.	Discard supernatant	Discard supernatant
15	Repeat steps as indicated.	Steps 13 and 14, two more times (For a total of 3 x 5-minute separations)	Steps 13 and 14, two more times (For a total of 3 x 5-minute separations)
16	Resuspend cells in desired medium. Be sure to collect cells from the sides of the 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.

Directions for Use – Fully Automated RoboSep™ Protocol

See page 1 for Sample Preparation and Recommended Medium. Refer to Table 2 for detailed instructions regarding the RoboSep™ procedure.

Table 2. RoboSep™ Human Th17 Cell Enrichment 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 - 8 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 CD4+CXCR3- T Cell Pre-Enrichment 19152
3	Vortex D2 Magnetic Particles. NOTE: Particles should appear evenly dispersed.	30 seconds
4	Load the carousel.	Follow on-screen prompts NOTE: Two different particles are provided in this kit. Ensure D2 Magnetic Particles are used in this step.
	Start the protocol.	Press the green “Run” button
5	Unload the carousel when the run is complete.	Remove the tube containing the isolated cells
6	Centrifuge the pre-enriched cells.	200 x g for 10 minutes at RT
	Discard the supernatant and resuspend cell pellet at the indicated volume.	• Resuspend in 0.25 mL for samples with start volume ≤ 2.5 mL • Resuspend in 0.5 mL for samples with start volume > 2.5 - 4 mL • Resuspend in 1 mL for samples with start volume > 4 mL
7	Add sample to required tube.	14 mL (17 x 100 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352057)
8	Select protocol.	Human CCR6 Positive Selection 18262
9	Mix Magnetic Nanoparticles (brown). NOTE: Particles should appear evenly dispersed.	Pipette up and down more than 5 times
10	Load the carousel.	Follow on-screen prompts NOTE: Two different particles are provided in this kit. Ensure Magnetic Nanoparticles Positive Selection (● brown) are used in this step.
	Start the protocol.	Press the green “Run” button
11	Unload the carousel when the run is complete. Remove the tube containing the isolated cells and resuspend in desired medium. Be sure to collect cells from the sides of the tube.	Isolated cells are ready for use

RT - room temperature (15 - 25°C)

Notes and Tips

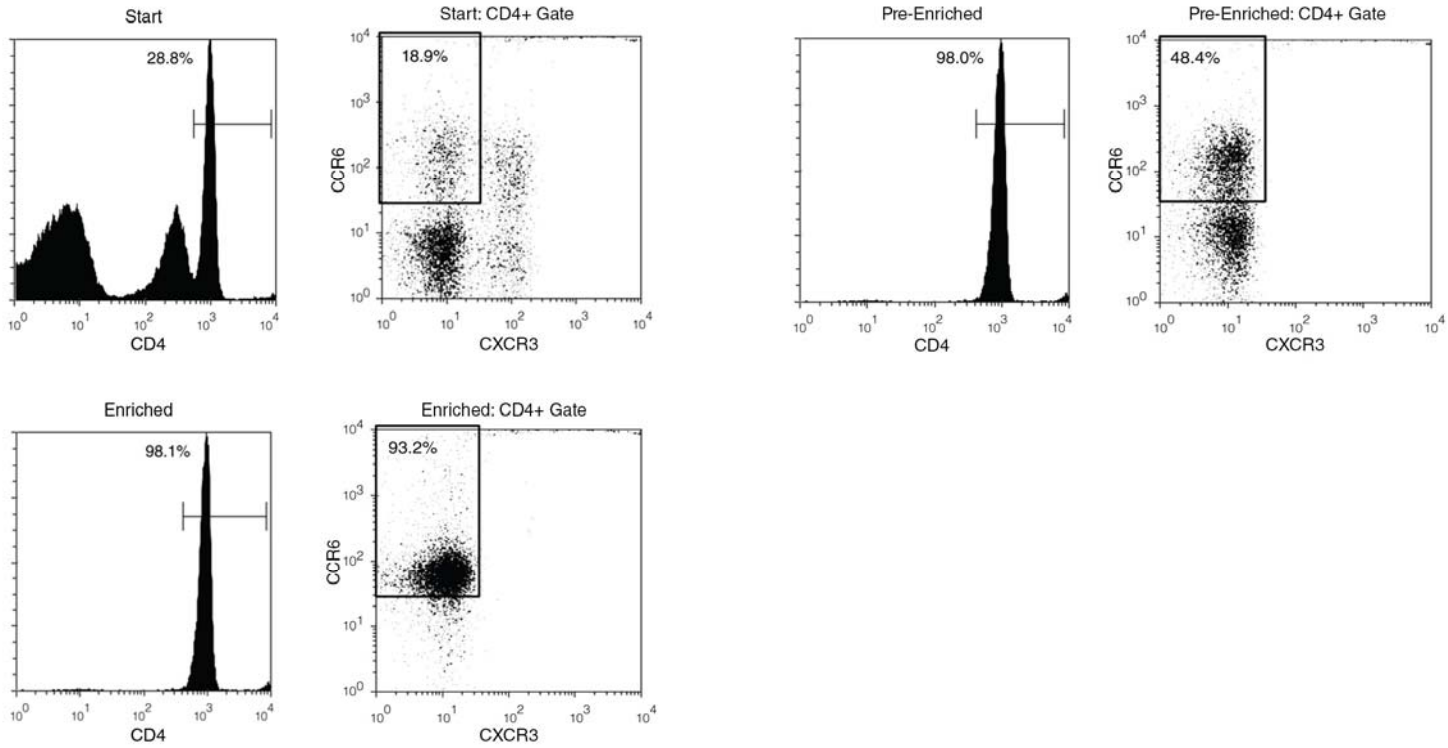
ASSESSING PURITY

For purity assessment of Th17 (CD4+CXCR3-CCR6+) T cells by flow cytometry use the following fluorochrome-conjugated antibody clones:

- Anti-Human CD4 Antibody, Clone OKT4 (Catalog #60016), and
- Anti-Human CD196 (CCR6) Antibody, Clone G034E3 (Catalog #60090), recommended at a concentration of 0.25 µg/mL, and
- Anti-Human CXCR3 Antibody, Clone G025H7 (Catalog #60088), recommended at a concentration of 4.0 µg/mL

In addition, intracellular staining of IL-17 cytokine may be assessed after stimulation of cells with PMA-Ionomycin.

Data



Starting with fresh PBMCs, the Th17 cell (CD4+CXCR3-CCR6+) content of the isolated fraction is typically 85 - 94%. In the above example, the purities of the start, pre-enriched, and final isolated fractions are 5.4%, 47.4%, and 91.4%, respectively. Intracellular staining for IL-17-producing cells typically ranges from 5 - 20% IL-17+ cells. These values vary widely among donors. IFN-γ-producing cells are typically < 5% of the enriched fraction.

References

Crome SQ et al. (2010) Inflammatory effects of ex vivo human Th17 cells are suppressed by regulatory T cells. J Immunol 185(6): 3199–208.

Rossi RL et al. (2011) Distinct microRNA signatures in human lymphocyte subsets and enforcement of the naive state in CD4+ T cells by the microRNA miR-125b. Nat Immunol 12(8): 796–803.