EasySep™ Human Th17 Cell Enrichment Kit II

Positive Selection

Catalog #17862

For processing 2 x 10^9 cells

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Document #DX21834 | Version 1_1_0

Description

Isolate highly purified Th17 (CD4+CXCR3-CCR6+) 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 98% purity
- No columns required

First, CCR6+ cells are isolated by column-free immunomagnetic positive selection using antibody complexes and EasySep™ Releasable RapidSpheres™. Then, bound magnetic particles are removed from the EasySep™-isolated CCR6+ cells, and unwanted non-CD4+ T cells, CD45RA+ cells, and CXCR3+ cells are targeted for depletion using antibody complexes and EasySep™ Dextran RapidSpheres™. The final isolated fraction is enriched for IL-17-producing Th17 cells, which are immediately available for downstream applications. Following cell isolation with this EasySep™ Release kit, antibody complexes remain bound to the cell surface and may interact with Brilliant Violet™ antibody conjugates, polyethylene glycol-modified proteins, or other chemically related ligands.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Human CCR6 Positive Selection Cocktail II	17872C	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 and 0.1% BSA. Includes an Fc receptor blocking antibody.
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™ Releasable RapidSpheres™ 50201	50201	4 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.
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.
EasySep™ Release Buffer	20145	2 x 1 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A buffer for release of Releasable RapidSpheres™ from cells following positive selection.

BSA - bovine serum albumin; 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 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 1 x 10^8 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 1 x 10^8 cells/mL in recommended medium.

Recommended Medium

EasySep™ Buffer (Catalog #20144) 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 Th17 Cell Enrichment 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.	1 x 10^8 cells/mL 0.25 - 2 mL	1 x 10^8 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 CCR6 Positive Selection Cocktail to sample.	25 µL/mL of sample	25 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
3	Vortex Releasable RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
4	Add Releasable RapidSpheres™ to sample.	200 µL/mL of sample	200 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 supernatant.	Discard supernatant	Discard supernatant
7	Remove the tube from the magnet and 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 3 minutes
8	Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring off the supernatant.	Discard supernatant	Discard supernatant
9	Repeat steps as indicated.	Steps 7 and 8 (total of 1 x 5-minute and 2 x 3-minute separations)	Steps 7 and 8 (total of 1 x 5-minute and 2 x 3-minute separations)
Continue to step 10, next page		Continue to step 10, next page	Continue to step 10, next page

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.

		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS (CONTINUED)	 EasySep™ (Catalog #18000)	"The Big Easy" (Catalog #18001) 
10	Remove the tube from the magnet and add recommended medium to top up the sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times. Be sure to collect cells off the sides of the tube.	Half of the original starting sample volume§ (i.e. half of the volume used in step 1)	Half of the original starting sample volume§ (i.e. half of the volume used in step 1)
11	Add Release Buffer to sample.	200 µL/mL of resuspended sample	200 µL/mL of resuspended sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
12	Add CD4+CXCR3- T Cell Pre-Enrichment Cocktail to sample.	100 µL/mL of resuspended sample	100 µL/mL of resuspended sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
13	Vortex Dextran RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
	Add Dextran RapidSpheres™ to sample and mix.	50 µL/mL of resuspended sample NO INCUBATION, proceed immediately to next step	50 µL/mL of resuspended sample NO INCUBATION, proceed immediately to next step
14	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 start sample ≤ 4 mL • Top up to 10 mL for start sample > 4 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 5 minutes
15	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.

§ The resuspended sample volume should be used to determine the volume of reagents added in subsequent steps.

Table 2. EasySep™ Human Th17 Cell Enrichment Kit II Protocol

		EASYSEP™ MAGNETS		
STEP	INSTRUCTIONS	EasyEights™ (Catalog #18103) 5 mL tube	EasyEights™ (Catalog #18103) 14 mL tube	Easy 50 (Catalog #18002)
1	Prepare sample at the indicated cell concentration within the volume range.	1 x 10^8 cells/mL 0.25 - 2 mL	1 x 10^8 cells/mL 0.5 - 6 mL	1 x 10^8 cells/mL 5 - 40 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)	50 mL (30 x 115 mm) conical tube (e.g. Catalog #38010)
2	Add CCR6 Positive Selection Cocktail to sample.	25 µL/mL of sample	25 µL/mL of sample	25 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes	RT for 5 minutes
3	Vortex Releasable RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds	30 seconds
4	Add Releasable RapidSpheres™ to sample.	200 µL/mL of sample	200 µL/mL of sample	200 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 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	• Top up to 25 mL for samples ≤ 10 mL • Top up to 50 mL for samples > 10 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 10 minutes	RT for 10 minutes	RT for 10 minutes
6	Carefully pipette** (do not pour) off the supernatant.	Discard supernatant	Discard supernatant	Discard supernatant
7	Repeat steps as indicated.	Steps 5 and 6, two more times (total of 3 x 10-minute separations)	Steps 5 and 6, two more times (total of 3 x 10-minute separations)	Steps 5 and 6, two more times (total of 3 x 10-minute separations)
8	Remove the tube from the magnet and add recommended medium to top up the sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times. Be sure to collect cells off the sides of the tube.	Half of the original starting sample volume§ (i.e. half of the volume used in step 1)	Half of the original starting sample volume§ (i.e. half of the volume used in step 1)	Half of the original starting sample volume§ (i.e. half of the volume used in step 1)
Continue to step 9, next page		Continue to step 9, next page	Continue to step 9, next page	Continue to step 9, next page

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]).

§ The resuspended sample volume should be used to determine the volume of reagents added in subsequent steps.

		EASYSEP™ MAGNETS		
STEP	INSTRUCTIONS (CONTINUED)	EasyEights™ (Catalog #18103) 5 mL tube	EasyEights™ (Catalog #18103) 14 mL tube	Easy 50 (Catalog #18002)
9	Add Release Buffer to sample.	200 µL/mL of resuspended sample	200 µL/mL of resuspended sample	200 µL/mL of resuspended sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes	RT for 5 minutes
10	Add CD4+CXCR3- T Cell Pre-Enrichment Cocktail to sample.	100 µL/mL of resuspended sample	100 µL/mL of resuspended sample	100 µL/mL of resuspended sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes	RT for 5 minutes
11	Vortex Dextran RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds	30 seconds
12	Add Dextran RapidSpheres™ to sample.	50 µL/mL of resuspended sample	50 µL/mL of resuspended sample	50 µL/mL of resuspended sample
	Mix and incubate.	RT for 3 minutes	RT for 3 minutes	RT for 3 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 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 tube (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 5 minutes	RT for 10 minutes
14	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	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]).

Notes and Tips

ASSESSING PURITY

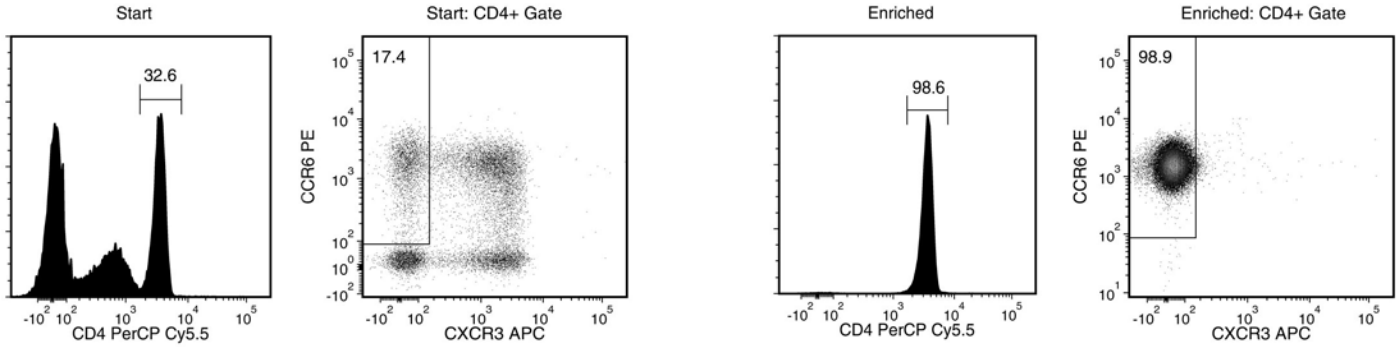
For purity assessment of Th17 (CD4+CXCR3-CCR6+) cells by flow cytometry use the following fluorochrome-conjugated antibody clone:

- Anti-Human CD4 Antibody, Clone OKT4 (Catalog #60016), and
- Anti-Human CD183 (CXCR3) Antibody, Clone G025H7 (Catalog #60088), and
- Anti-Human CD196 (CCR6) Antibody, Clone G034E3 (Catalog #60090)

NOTE: Brilliant Violet™ antibody conjugates should be carefully titrated on EasySep™ Release-isolated cells prior to analysis by flow cytometry or fluorescence microscopy. For purity assessment with Brilliant Violet™ antibody conjugates, use of BD Horizon Brilliant™ Stain Buffer is recommended to reduce non-specific interactions. For more information, refer to the manufacturer's instructions or contact us at techsupport@stemcell.com.

In addition, intracellular staining of IL-17 cytokine may be assessed after stimulation of cells with Phorbol 12-myristate 13-acetate (PMA; Catalog #74042) and Ionomycin (Catalog #73722).

Data



Starting with fresh PBMCs, the Th17 cell content (CD4+CXCR3-CCR6+) of the isolated fraction typically ranges from 96 - 98%. Following overnight stimulation with PMA-Ionomycin, 6 - 19% of the isolated cells are IL-17+ by intracellular flow cytometry. These values vary widely among donors. IFN-γ-producing cells are typically < 5% of the enriched fraction.