



EasySep™ Human Naïve Pan T Cell Isolation Kit

Negative Selection
Catalog #17961

For processing 1 x 10⁹ cells



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Document #DX21693 | Version 1_0_0

Description

Isolate untouched and highly purified naïve pan 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 98% purity
- Isolated cells are untouched

This kit targets non-naïve pan 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 Naïve Pan T Cell Isolation Cocktail	17961C	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 TCR Gamma/Delta Depletion Cocktail	17863C	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 and 0.1% BSA. Includes an Fc receptor blocking antibody.
EasySep™ Dextran RapidSpheres™ 50102	50102	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.

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 and frozen samples, see www.stemcell.com/primarycells.

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 10⁷ 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 10⁷ 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 for 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% fetal bovine serum (FBS). Centrifuge at 300 x g for 10 minutes at room temperature. 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 the cells at 5 x 10⁷ 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 Naïve Pan 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	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 TCR Gamma/Delta Depletion Cocktail to sample.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
4	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
5	Add RapidSpheres™ to sample.	60 µL/mL of sample	60 µL/mL of sample
	Mix and incubate.	RT for 3 minutes	RT for 3 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	<ul style="list-style-type: none"> • 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 3 minutes	RT for 3 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 3 minutes	RT for 3 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 Naïve Pan T Cell Isolation Kit Protocol

STEP	INSTRUCTIONS	EASYSEP™ MAGNETS		
		 EasyEights™ (Catalog #18103)	 EasyEights™ (Catalog #18103)	 Easy 50 (Catalog #18002)
		5 mL tube	14 mL tube	
1	Prepare sample at the indicated cell concentration within the volume range.	5 x 10 ⁷ cells/mL 0.25 - 2 mL	5 x 10 ⁷ cells/mL 0.5 - 6 mL	5 x 10 ⁷ cells/mL 5 - 30 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 Isolation Cocktail to sample.	50 µL/mL of sample	50 µL/mL of sample	50 µL/mL of sample
3	Add TCR Gamma/Delta Depletion 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
4	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds	30 seconds
5	Add RapidSpheres™ to sample.	75 µL/mL of sample	90 µL/mL of sample	90 µL/mL of sample
	Mix and incubate.	RT for 3 minutes	RT for 3 minutes	RT for 3 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	<ul style="list-style-type: none"> • Top up to 5 mL for samples < 2 mL • Top up to 10 mL for samples ≥ 2 mL 	<ul style="list-style-type: none"> • 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
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	Use a new 50 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 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	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]).

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 Pan 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	Select protocol.	Human Naïve Pan T Cell Isolation 17961	
3	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	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.	Isolated cells are ready for use	

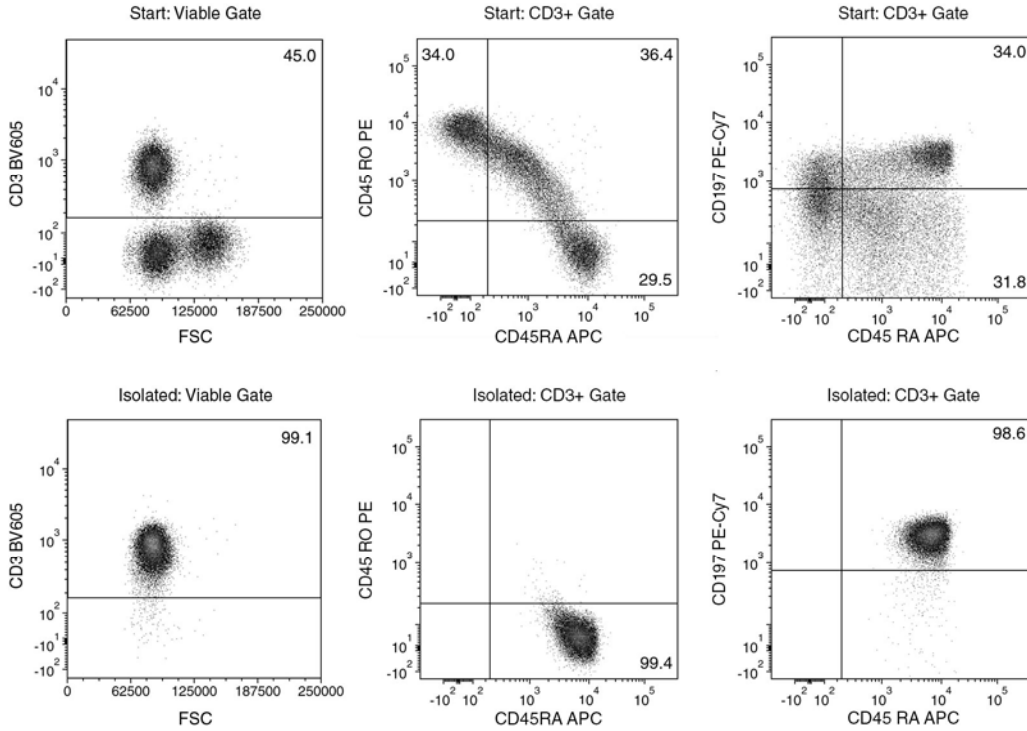
Notes and Tips

ASSESSING PURITY

For purity assessment of naïve pan T cells (CD3+CD45RA+CD45RO-CD197+) by flow cytometry use the following fluorochrome-conjugated antibody clones:

- Anti-Human CD3 Antibody, Clone UCHT1 (Catalog #60011) or Clone SK7 (Catalog #60127), and
- Anti-Human CD45RO Antibody, Clone UCHL1 (Catalog #60097), and
- Anti-human CD45RA antibody, and
- Anti-human CD197 antibody

Data



Starting with fresh PBMCs, the naïve pan T cell content (CD3+CD45RA+CD45RO-CD197+) of the isolated fraction is typically $96.1 \pm 2.3\%$ (mean \pm SD using the purple EasySep™ Magnet). In the above example, the purities of the start and final isolated fractions are 4.5% and 97%, respectively.

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