

EasySep™ Human ILC2 Enrichment Kit

For processing 1×10^9 cells

Catalog #17972

Negative Selection

Document #1000005318 | Version 04



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Description

Enrich untouched Group 2 Innate Lymphoid Cells (ILC2s) from washed leukapheresis samples by immunomagnetic negative selection.

- Fast, easy-to-use, and column-free
- Untouched, viable cells
- Facilitates rapid flow sorting of ILC2s

This kit targets non-ILC2s 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 and cell sorting.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Human ILC2 Enrichment Cocktail	17972C	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	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

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.

LEUKAPHERESIS

Wash the peripheral blood leukapheresis sample (e.g. Human Peripheral Blood Leukopak, Fresh*, Catalog #70500) 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 (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 the cells at 1×10^8 cells/mL in recommended medium.

PERIPHERAL BLOOD

Prepare a peripheral blood mononuclear cell (PBMC) suspension from whole blood (e.g. Human Whole Peripheral Blood*, Catalog #70507) 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 1×10^8 cells/mL in recommended medium.

* Some primary cell products are available only in select regions. Contact us at techsupport@stemcell.com for further information.

** SepMate™ IVD is available only 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).

Recommended Medium

EasySep™ Buffer (Catalog #20144) or PBS containing 2% 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 ILC2 Enrichment Kit Protocol

		EASYSEP™ MAGNET
STEP	INSTRUCTIONS	<p>“The Big Easy” (Catalog #18001)</p> 
1	Prepare sample within the volume range.	1 x 10 ⁸ cells/mL 1 - 5 mL
	Add sample to required tube.	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	Add Enrichment Cocktail to sample. NOTE: Do not vortex cocktail.	100 µL/mL of sample
	Mix and incubate.	RT for 10 minutes
3	Vortex Magnetic Particles. NOTE: Particles should appear evenly dispersed.	1 minute
4	Add Magnetic Particles to sample.	100 µL/mL of sample
	Mix and incubate.	RT for 1 minute
5	Add recommended medium to top up the sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.	<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 5 minutes
6	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 14 mL tube
7	Remove the tube from the magnet; place the new tube (without lid) into the magnet and incubate for a second separation.	RT for 5 minutes
8	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

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 ILC2 Enrichment Kit Protocol

		EASYSEP™ MAGNET	
STEP	INSTRUCTIONS	Easy 50 (Catalog #18002)	
1	Prepare sample at the indicated cell concentration within the volume range.	1 x 10 ⁸ cells/mL 5 - 25 mL	
	Add sample to required tube.	50 mL (30 x 115 mm) conical tube (e.g. Catalog #38010)	
2	Add Enrichment Cocktail to sample. NOTE: Do not vortex cocktail.	100 µL/mL of sample	
	Mix and incubate.	RT for 10 minutes	
3	Vortex Magnetic Particles. NOTE: Particles should appear evenly dispersed.	1 minute	
4	Add Magnetic Particles to sample.	100 µL/mL of sample	
	Mix and incubate.	RT for 1 minute	
5	Add recommended medium to top up sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.	<ul style="list-style-type: none"> • Top up to 22 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 15 minutes	
6	Carefully pipette** (do not pour) off the enriched cell suspension into a new tube.	Use a new 50 mL tube	
7	Remove the tube from the magnet; place the new tube (without lid) into the magnet and incubate for a second separation.	RT for 15 minutes	
8	Carefully pipette** (do not pour) off the enriched cell suspension into a new tube.	Isolated cells are ready for use	

RT - room temperature (15 - 25°C)

** Collect the entire supernatant, all at once, into a single pipette.

Notes and Tips

ASSESSING PURITY

ILC2s are described as lineage-negative, CD45-positive, CD127-positive, CD161-positive, and CD294-positive. For purity assessment of ILC2s by flow cytometry, use the following fluorochrome-conjugated antibodies:

- Anti-Human CD45 Antibody, Clone HI30 (Catalog #60018), and
- Anti-human CD127 (IL-7R α) antibody, clone A019D5, and
- Anti-human CD161 (KLRB1) antibody, clone HP-3G10, and
- Anti-human CD294 (CRTH2) antibody, clone BM16, and
- Anti-human lineage-specific antibodies (see below)

For lineage-specific antigen labeling, use the following fluorochrome-conjugated antibodies:

- Anti-human CD1a antibody, clone HI149, and
- Anti-Human CD3 Antibody, Clone UCHT1 (Catalog #60011), and
- Anti-Human CD4 Antibody, Clone RPA-T4 (Catalog #100-0298), and
- Anti-human CD11c antibody, clone 3.9, and
- Anti-Human CD14 Antibody, Clone M5E2 (Catalog #60004), and
- Anti-Human CD16 Antibody, Clone 3G8 (Catalog #60041), and
- Anti-Human CD19 Antibody, Clone HIB19 (Catalog #60005), and
- Anti-Human CD34 Antibody, Clone 581 (Catalog #60013), and
- Anti-human CD94 antibody, clone DX22, and
- Anti-Human CD123 (IL-3R α) Antibody, Clone 6H6 (Catalog #60110), and
- Anti-human CD303 antibody, clone 201A, and
- Anti-human Fc ϵ R1a antibody, clone AER-37, and
- Anti-human TCR alpha/beta antibody, clone IP26, and
- Anti-human TCR gamma/delta antibody, clone B1

Data

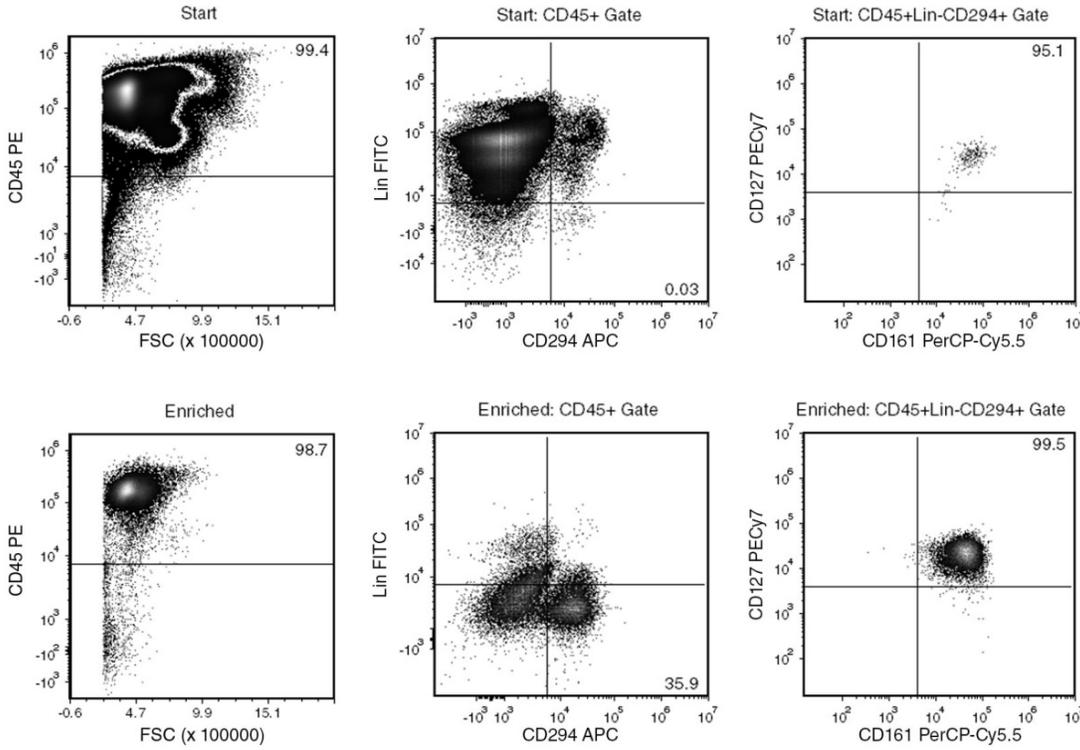


Figure 1. Typical EasySep™ Human ILC2 Enrichment Profile

Starting with washed leukapheresis sample, the ILC2 content (Lin-CD45+CD294+CD127+CD161+) of the enriched fraction typically ranges from 13 - 78%. In the above example, the percentages of ILC2s in the start and final enriched fractions are 0.03% and 35.3%, respectively.

NOTE: The ILC2 content of the start fraction typically ranges from 0.001 - 0.16%.

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