EasySep™ Human Pan-ILC Enrichment Kit

Negative Selection

Catalog #17975

For processing 1 x 10^9 cells

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Description

Enrich untouched Group 1, 2, and 3 Innate Lymphoid Cells (ILC1, 2, and 3) from washed leukapheresis samples by immunomagnetic negative selection.

- Fast, easy-to-use and column-free
- Isolated cells are untouched
- Facilitates rapid flow sorting of ILCs

This kit targets non-ILCs 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 Pan-ILC Enrichment Cocktail	17975C	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.
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 (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 (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 x 10^8 cells/mL in recommended medium.

Recommended Medium

EasySep™ Buffer (Catalog #20144), RoboSep™ Buffer (Catalog #20104), 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 Pan-ILC Enrichment Kit Protocol

		EASYSEP™ MAGNET
STEP	INSTRUCTIONS	**"The Big Easy" (Catalog #18001)** 
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.	50 µL/mL of sample
	Mix and incubate.	RT for 10 minutes
3	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds
4	Add RapidSpheres™ to sample.	50 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.	Top up to double the original sample volume
	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 and 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 Pan-ILC Enrichment Kit Protocol

		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS	**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 1 - 5 mL	1 x 10^8 cells/mL 10 - 25 mL
	Add sample to required tube.	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 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 RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
4	Add RapidSpheres™ to sample.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 1 minutes	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.	Top up to double the original sample volume	Top up to double the original sample volume
	Place the tube (without lid) into the magnet and incubate.	RT for 15 minutes	RT for 15 minutes
6	Carefully pipette** (do not pour) the enriched cell suspension into a new tube.	Use a new 14 mL tube	Use a new 50 mL tube
7	Remove the tube from the magnet and place the new tube (without lid) into the magnet and incubate for a second separation.	RT for 15 minutes	RT for 15 minutes
8	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

RT - room temperature (15 - 25°C)
** Collect the entire supernatant, all at once, into a single pipette.

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 Pan-ILC Enrichment Kit Protocol

STEP	INSTRUCTIONS	RoboSep™ (Catalog #20000 and #21000) 
1	Prepare sample at the indicated cell concentration 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	Select protocol.	Human Pan-ILC Negative Selection 17975
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

ILCs are defined as lineage-negative (see below for lineage-specific labeling), CD45-positive, and CD127-positive.

NOTE: Subsets of ILCs are further characterized as follows: ILC1s are CD161+/-CD294-CD117-, ILC2s are CD161+CD294+CD117+/-, and ILC3s are Lin-CD161+CD294-CD117+.

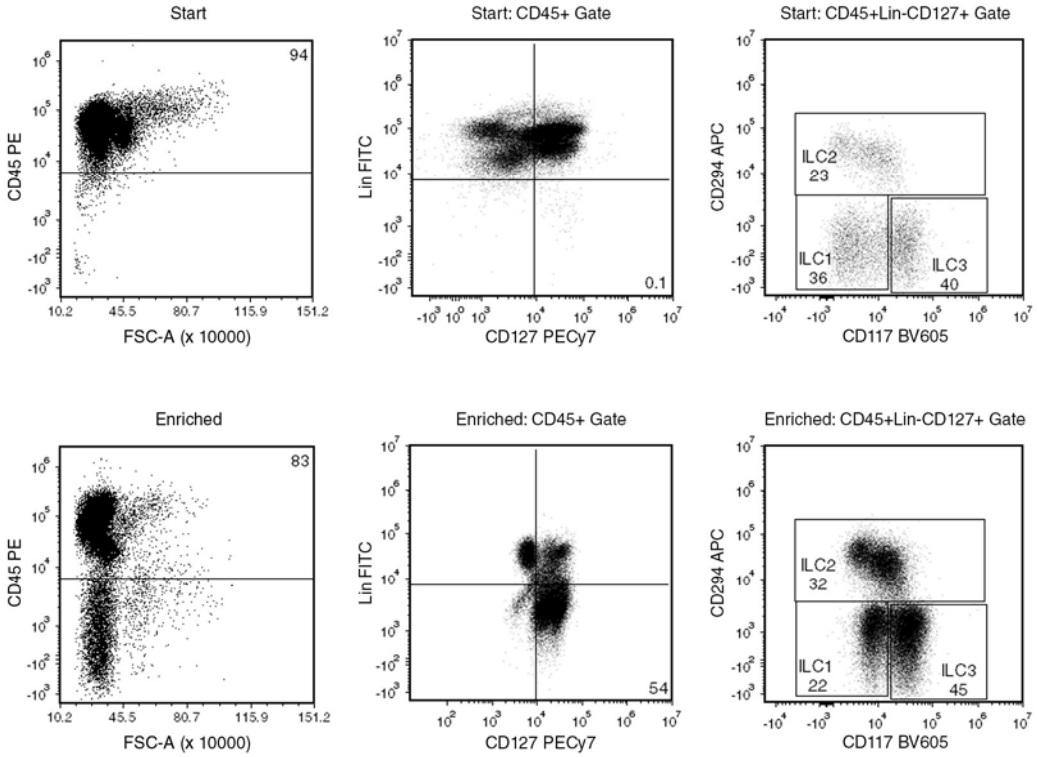
For purity assessment of ILCs by flow cytometry use the following fluorochrome-conjugated antibody clones:

- Anti-Human CD45 Antibody, Clone HI30 (Catalog #60018),
- Anti-human CD127 (IL-7Ra) antibody, clone A019D5,
- Anti-human CD161 (KLRB1) antibody, clone HP-3G10,
- Anti-human CD294 (CRTH2) antibody, clone BM16,
- Anti-Human CD117 (c-Kit) Antibody, Clone 104D2 (Catalog #60087), 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, 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-3Ra) Antibody, Clone 6H6 (Catalog #60110), and
- Anti-human CD303 antibody, clone 201A, and
- Anti-human FceR1a antibody, clone AER-37, and
- Anti-human TCR alpha/beta antibody, clone IP26, and
- Anti-human TCR gamma/delta antibody, clone B1

Data



Starting with fresh leukapheresis samples, the total ILC content (Lin-CD45+CD127+) of the enriched fraction typically ranges from 17 - 85%. In the above example, the percentages of ILCs in the start and final enriched fractions are 0.09% and 45% (or 0.1% and 54% of CD45+ cells), respectively.