EasySep™ Mouse Pan-ILC Enrichment Kit

For processing 1 x 10^9 cells

Catalog #19875

Negative Selection

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Description

Enrich untouched group 1, 2, and 3 innate lymphoid cells (ILC1, 2, and 3) from mouse lung or lymph node by immunomagnetic negative selection. When using single-cell suspensions from other tissue types, this kit may require optimization.

- 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 biotinylated antibodies recognizing specific cell surface markers. Unwanted cells are labeled with biotinylated 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 or cell sorting.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Mouse Pan-ILC Enrichment Cocktail	19875C	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.
EasySep™ Streptavidin RapidSpheres™ 50001	50001	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.

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

LUNG TISSUE

The following instructions are for processing 5 - 10 mouse lungs. If starting with more than 10 lungs, adjust volumes accordingly.

1. Prepare 10 mL of digestion medium by adding 1 mL of Collagenase/Hyaluronidase (Catalog #07912) and 1.5 mL of DNase I Solution (Catalog #07900) to 7.5 mL of RPMI 1640 Medium (Catalog #36750). Warm to room temperature (15 - 25°C).
2. Harvest lung tissue into a conical tube containing PBS with 2% fetal bovine serum (FBS).
3. Transfer lung tissue to a conical tube containing 10 mL of digestion medium and mince the tissue into small pieces using scissors. Incubate at 37°C for 20 minutes on a shaking platform.
4. Place a 70 µm nylon mesh strainer (Catalog #27260) over a 100 mm Petri Dish (Catalog #27110) and push the digested lung tissue through strainer with the rubber end of a syringe plunger to obtain a cell suspension.
5. Place a new 70 µm nylon mesh strainer over a 50 mL conical tube and filter the cell suspension through it. Rinse the strainer with recommended medium and collect in the same tube.
6. Centrifuge at 300 x g for 10 minutes at room temperature with the brake on low. Carefully remove and discard the supernatant.
7. Add 20 mL of Ammonium Chloride Solution (Catalog #07800) to the cell pellet. Incubate at room temperature for 5 minutes.
8. Top up to 50 mL with recommended medium. Centrifuge at 300 x g for 10 minutes at room temperature with the brake on low. Carefully remove and discard the supernatant.
9. Resuspend cells at 1 x 10^8 cells/mL in recommended medium.

LYMPH NODE

Harvest lymph node and transfer to a 70 µm nylon mesh strainer that is placed over a 100 mm Petri Dish (Catalog #27110) containing recommended medium. Push the lymph node tissue through strainer with the rubber end of a syringe plunger to obtain a cell suspension. Centrifuge at 300 x g for 10 minutes and resuspend at 1 x 10^8 cells/mL in recommended medium. Ammonium chloride treatment is not required when preparing the cells for separation.

Recommended Medium

EasySep™ Buffer (Catalog #20144), or PBS containing 2% FBS and 1 mM EDTA. Medium should be free of Ca++, Mg++, and biotin.

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.

Table 1. EasySep™ Mouse Pan-ILC Enrichment Kit Protocol

		EASYSEP™ MAGNET
STEP	INSTRUCTIONS	 **EasySep™ (Catalog #18000)**
1	Prepare sample within the volume range.	1 x 10^8 cells/mL 0.3 - 1 mL NOTE: If starting with fewer than 5 x 10^7 cells, resuspend cells in 0.3 mL.
	Add sample to required tube.	5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Catalog #38007)
2	Add Enrichment Cocktail to sample. NOTE: Do not vortex cocktail.	50 µL/mL of sample
	Mix and incubate.	RT for 5 minutes
3	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds
4	Add RapidSpheres™ to sample.	75 µL/mL of sample
	Mix and incubate.	RT for 5 minutes
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 2.5 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 3 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 add recommended medium to indicated volume. Mix by gently pipetting up and down 2 - 3 times.	Top up to 2.5 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 3 minutes
8	Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring off the enriched cell suspension.	Combine with poured-off fraction from step 6 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™ Mouse Pan-ILC Enrichment Kit Protocol

		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS	 EasyPlate™ (Catalog #18102)	EasyEights™ (Catalog #18103) 5 mL tube 
1	Prepare sample at the indicated cell concentration within the volume range.	1 x 10^8 cells/mL 0.025 - 0.2 mL	1 x 10^8 cells/mL 0.3 - 1 mL NOTE: If starting with fewer than 5 x 10^7 cells, resuspend cells in 0.3 mL.
	Add sample to required tube (or plate when using the EasyPlate™ EasySep™ Magnet).	Round-bottom, non-tissue culture-treated 96-well plate (e.g. Catalog #38018)	5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Catalog #38007)
2	Add Enrichment Cocktail to sample. NOTE: Do not vortex cocktail.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
3	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
4	Add RapidSpheres™ to sample.	75 µL/mL of sample	75 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
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 0.25 mL	Top up to 2.5 mL
	Place the tube or plate (without lid) into the magnet and incubate.	RT for 10 minutes	RT for 3 minutes
6	Carefully pipette** (do not pour) the enriched cell suspension into a new tube or plate.	Use a new 96-well plate Isolated cells are ready for use	Use a new 14 mL tube
7	Remove the tube from the magnet and add recommended medium to indicated volume. Mix by gently pipetting up and down 2 - 3 times.	---	Top up to 2.5 mL
	Place the tube (without lid) into the magnet and incubate.	---	RT for 3 minutes
8	Carefully pipette** (do not pour) the enriched cell suspension into a new tube or plate.	---	Combine with pipetted-off fraction from step 6 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]).

Notes and Tips

ASSESSING PURITY

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

NOTE: Subsets of ILCs are further characterized as follows: ILC1s are CD278-CD117-, ILC2s are CD278+CD117+/-, and ILC3s are CD278-CD117+.

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

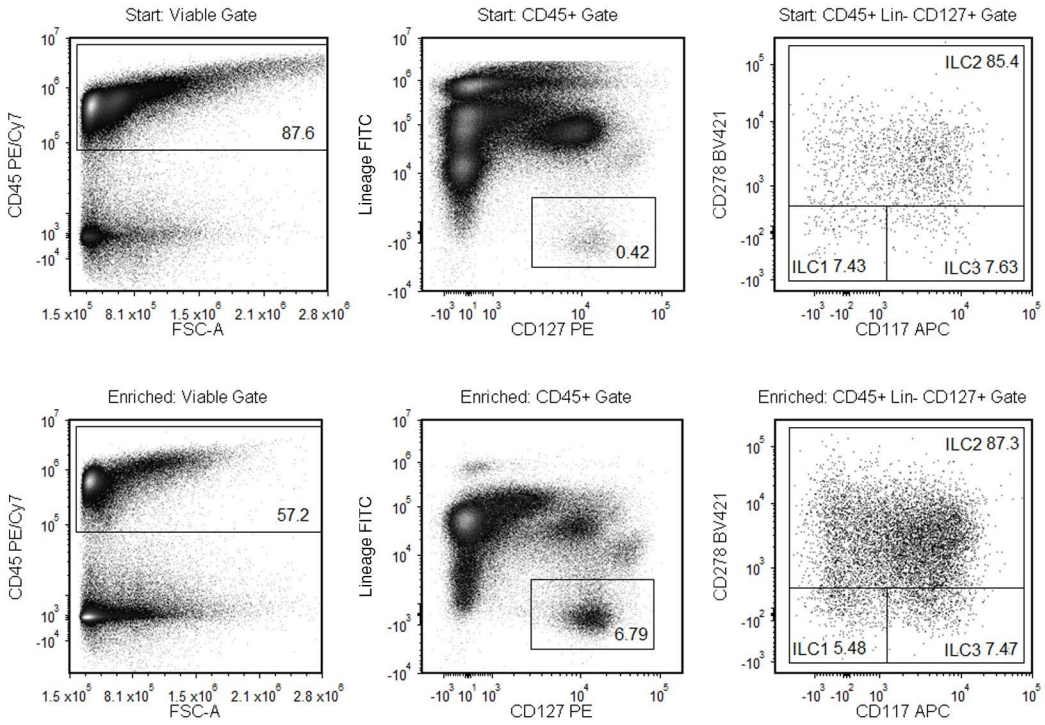
- Anti-Mouse CD45 Antibody, Clone 30-F11 (Catalog #60030), and
- Anti-mouse CD278 (ICOS) antibody, clone C3.98.4A, and
- Anti-mouse CD127 antibody, clone A7R34. and
- Anti-mouse CD117 (c-Kit) antibody, clone 2B8, and
- Anti-mouse lineage-specific antibodies (see below)

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

- Anti-Mouse CD3e Antibody, Clone 145-2C11 (Catalog #60015), and
- Anti-Mouse CD11b Antibody, Clone M1/70 (Catalog #60001), and
- Anti-Mouse CD11c Antibody, Clone N418 (Catalog #60002), and
- Anti-Mouse CD19 Antibody, Clone 1D3 (Catalog #60112), and
- Anti-Mouse Gr-1 Antibody, Clone RB6-8C5 (Catalog #60028), and
- Anti-Mouse TER119 Antibody, Clone TER-119 (Catalog #60033), and
- Anti-mouse TCR beta chain antibody, clone H57-597, and
- Anti-Mouse TCR Gamma/Delta Antibody, Clone GL3 (Catalog #60104)

Data

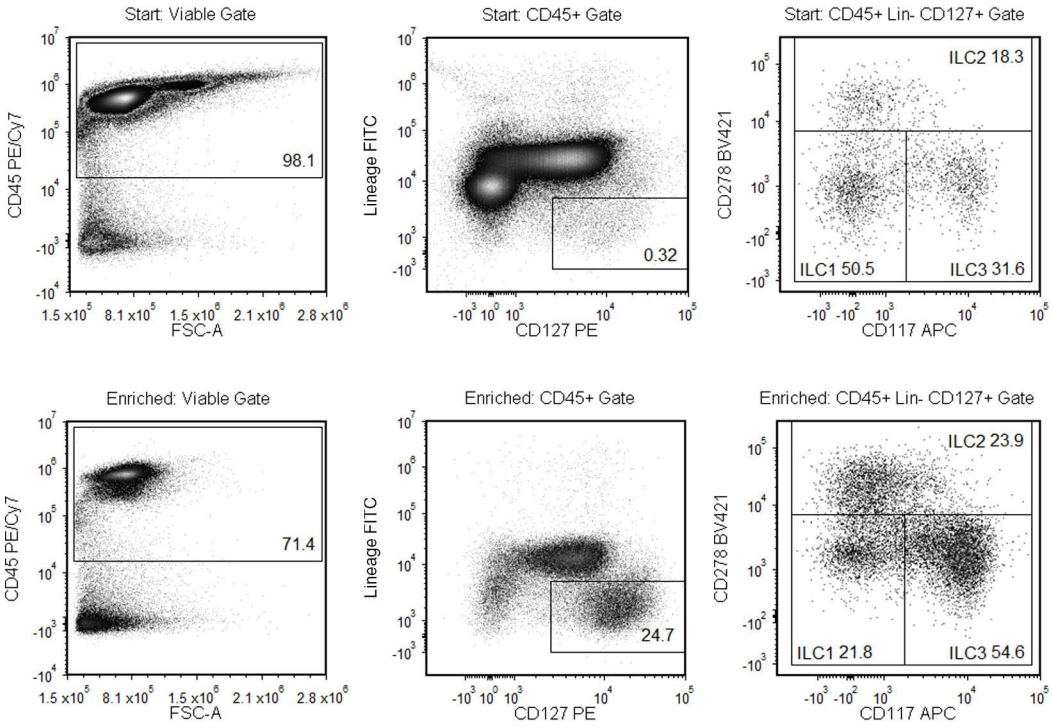
A Lung



Starting with a naïve mouse lung single-cell suspension, the total ILC content (CD45+Lin-CD127+) of the enriched fraction typically ranges from 3.1 - 7.6%. In the above example, the percentages of ILCs in the start and final enriched fractions are 0.4% and 3.9% (or 0.4% and 6.8% of CD45+ cells), respectively.

NOTE: The ILC content of the start fraction typically ranges from 0.3 - 0.6%.

B Lymph Node



Starting with a naïve mouse lymph node single-cell suspension, the total ILC content (CD45+Lin-CD127+) of the enriched fraction typically ranges from 21.1 - 45.2%. In the above example, the percentages of ILCs in the start and final enriched fractions are 0.3% and 17.6% (or 0.3% and 24.7% of CD45+ cells), respectively.

NOTE: The ILC content of the start fraction typically ranges from 0.3 - 0.4%.