EasySep™ Mouse ILC2 Enrichment Kit

Negative Selection

Catalog #19842

For processing 1 x 10^9 cells

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Description

Enrich untouched group 2 innate lymphoid cells (ILC2s) from mouse lungs 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
- Enriched cells are untouched
- Facilitates rapid flow sorting of ILC2s

This kit targets non-ILC2s 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. Enriched ILC2s 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 ILC2 Enrichment Cocktail	19842C	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 PBS.

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. For optimal recovery, it is recommended to process enough lung tissue to obtain at least 1.5 x 10^7 cells.

1. Prepare 10 mL of digestion medium by adding 1 mL of Collagenase/Hyaluronidase (Catalog #07912) and 1.5 mL of DNase I Solution (1 mg/mL; Catalog #07900) to 7.5 mL of RPMI 1640 Medium (Catalog #36750). Warm to room temperature (15 - 25°C).
   NOTE: If starting with more than 10 lungs, adjust volumes accordingly.
2. Harvest lung tissue into a tube containing PBS with 2% fetal bovine serum (FBS).
3. Transfer lung tissue into a dish without medium. Mince into a homogenous paste (< 1 mm in size) using a razor blade or scalpel.
4. Transfer minced lung tissue into a tube containing 10 mL of digestion medium and incubate at 37°C for 20 minutes on a shaking platform.
5. Place a 70 µm nylon mesh strainer 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.
6. Place a new 70 µm nylon mesh strainer over a 50 mL conical tube (e.g. Catalog #38010) and filter the cell suspension. Rinse the strainer with recommended medium.
7. Centrifuge at 300 x g for 6 minutes at room temperature with the brake on low. Carefully remove and discard the supernatant.
8. Add 20 mL of Ammonium Chloride Solution (Catalog #07800) to the cell pellet. Incubate at room temperature for 5 minutes.
9. Top up to 50 mL with recommended medium. Centrifuge at 300 x g for 6 minutes at room temperature with the brake on low. Carefully remove and discard the supernatant.
10. Resuspend cells at 1 x 10^8 cells/mL in recommended medium.
    NOTE: If starting with fewer than 3 x 10^7 cells, resuspend cells at 5 x 10^7 cells/mL.

Recommended Medium

EasySep™ Buffer (Catalog #20144), or PBS with 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 Table 1 for detailed instructions regarding the EasySep™ procedure.

Table 1. EasySep™ Mouse ILC2 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 3 x 10^7 cells, resuspend cells at 5 x 10^7 cells/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.	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)
* Starting cell concentration lower than 5 x 10^7 cells/mL is not recommended.
** 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.

Notes and Tips

ASSESSING PURITY

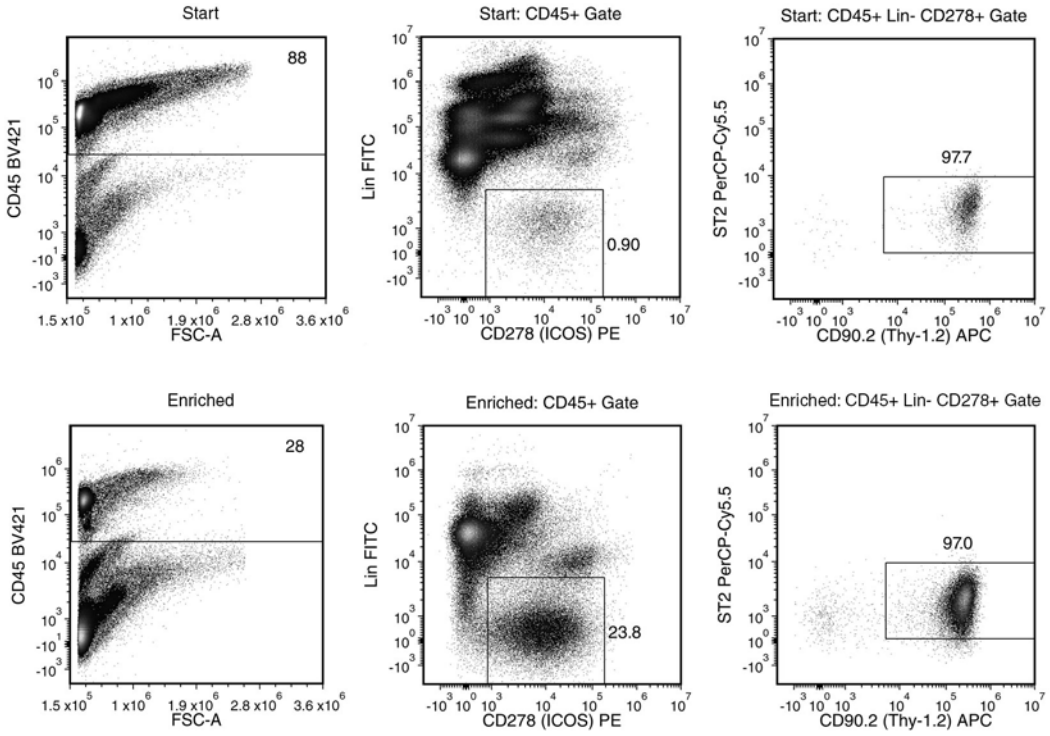
ILC2s are described as CD45-positive, lineage (CD3, CD4, CD11b, CD11c, CD19, NK1.1, Gr-1, TER119, TCR beta, TCR gamma/delta)-negative, CD278 (ICOS)-positive, CD90.2 (Thy-1.2)-positive, CD127-positive, and ST2-positive. For purity assessment of ILC2s by flow cytometry, use the following fluorochrome-conjugated antibodies:

- Anti-Mouse CD45 Antibody, Clone 30-F11 (Catalog #60030), and
- Anti-Mouse CD90.2 (Thy-1.2) Antibody, Clone 53-2.1 (Catalog #60115), and
- Anti-mouse CD278 (ICOS) antibody, clone C3.98.4A, and
- Anti-mouse ST2 antibody, clone DIH9, and
- Anti-mouse lineage-specific antibodies (see below)

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

- Anti-Mouse CD3e Antibody, Clone 145-2C11 (Catalog #60015), and
- Anti-mouse CD4 antibody, clone H129.19, 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 NK1.1 (CD161) Antibody, Clone PK136 (Catalog #60103), 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



Starting with a naïve mouse lung single-cell suspension, the ILC2 content (CD45+Lin-CD278+CD90.2+ST2+) of the final enriched fraction typically ranges from 2.2 - 7.1%. In the above example, the percentage of ILC2s in the start and final enriched fractions are 0.8% and 6.5% (or 0.9% and 22.3% of CD45+ cells), respectively.

NOTE: The ILC2 content of the start fraction typically ranges from 0.1 - 1%.