



## EasySep™ Mouse ILC2 Enrichment Kit

Negative Selection

Catalog #19842

For processing  $1 \times 10^9$  cells



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Document #DX20661 | Version 1\_0\_0

## 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.<br>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.<br>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  $1.5 \times 10^7$  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 (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 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  $1 \times 10^8$  cells/mL in recommended medium.  
NOTE: If starting with fewer than  $3 \times 10^7$  cells, resuspend cells at  $5 \times 10^7$  cells/mL.

## Recommended Medium

EasySep™ Buffer (Catalog #20144), or PBS containing 2% FBS and 1 mM EDTA. Medium should be free of  $\text{Ca}^{++}$ ,  $\text{Mg}^{++}$ , 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**

|             |                                                                                                                                      | <b>EASYSEP™ MAGNET</b>                                                            |                                                                                                                                                             |
|-------------|--------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>STEP</b> | <b>INSTRUCTIONS</b>                                                                                                                  |  | <b>EasySep™<br/>(Catalog #18000)</b>                                                                                                                        |
| <b>1</b>    | Prepare sample within the volume range.                                                                                              |                                                                                   | 1 x 10 <sup>8</sup> cells/mL<br>0.3 - 1 mL<br>NOTE: If starting with fewer than 3 x 10 <sup>7</sup> cells, resuspend cells at 5 x 10 <sup>7</sup> cells/mL* |
|             | Add sample to required tube.                                                                                                         |                                                                                   | 5 mL (12 x 75 mm) polystyrene round-bottom tube<br>(e.g. Corning Catalog #352058)                                                                           |
| <b>2</b>    | Add Enrichment Cocktail to sample.                                                                                                   |                                                                                   | 50 µL/mL of sample                                                                                                                                          |
|             | Mix and incubate.                                                                                                                    |                                                                                   | RT for 5 minutes                                                                                                                                            |
| <b>3</b>    | Vortex RapidSpheres™.<br>NOTE: Particles should appear evenly dispersed.                                                             |                                                                                   | 30 seconds                                                                                                                                                  |
| <b>4</b>    | Add RapidSpheres™ to sample.                                                                                                         |                                                                                   | 75 µL/mL of sample                                                                                                                                          |
|             | Mix and incubate.                                                                                                                    |                                                                                   | RT for 5 minutes                                                                                                                                            |
| <b>5</b>    | 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                                                                                                                                            |
| <b>6</b>    | 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                                                                                                                                        |
| <b>7</b>    | 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                                                                                                                                            |
| <b>8</b>    | 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<br>Isolated cells are ready for use                                                                            |

RT - room temperature (15 - 25°C)

\* Starting cell concentration lower than 5 x 10<sup>7</sup> 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

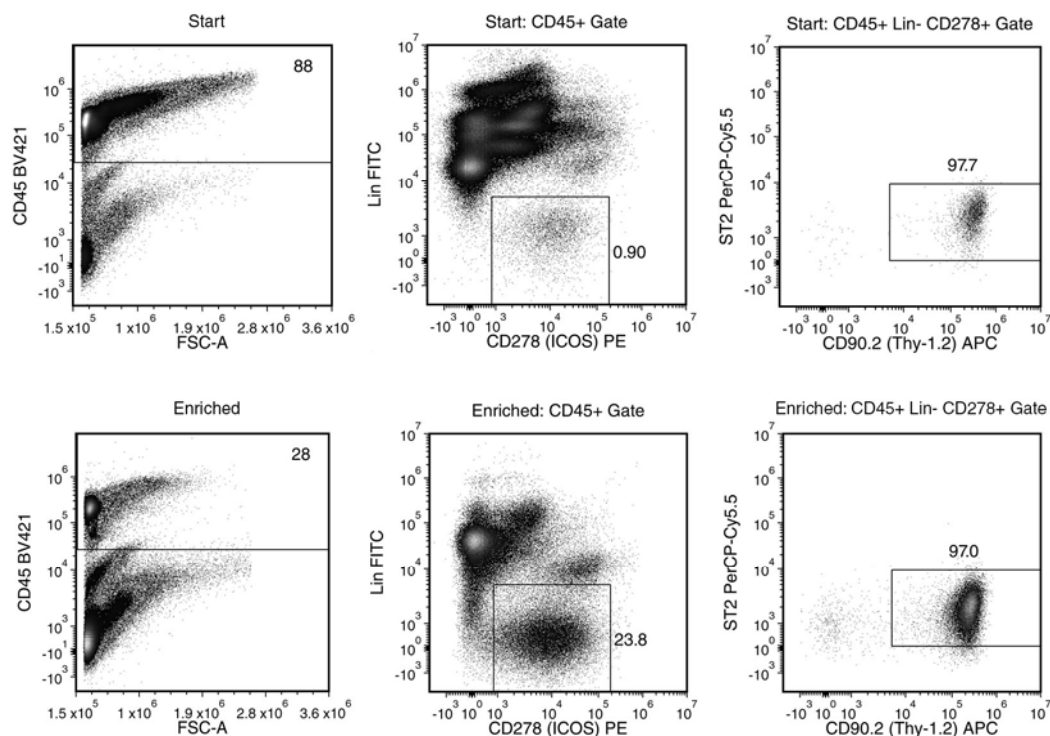
ILC2s are described as lineage (CD3, CD4, CD11b, CD11c, CD19, NK1.1, Gr-1, TER119, TCR beta, TCR gamma/delta)-negative, CD278 (ICOS)-positive, CD90.2 (Thy-1.2)-positive, CD127-positive, and ST2-positive. For purity assessment of ILC2s by flow cytometry use the following fluorochrome-conjugated antibodies:

- Anti-Mouse CD45 Antibody, Clone 30-F11 (Catalog #60030; optional), 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%.

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