Easy Isolation of Particle-Free Human ILC2s from Peripheral Blood Mononuclear Cells

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Introduction

ILC2s are implicated in the development of allergic airway diseases, airway tissue repair, protection against helminth infection, and maintenance of metabolic homeostasis. To better understand their role in immunity, highly purified ILC2s are required. However, due to their low frequency the isolation of ILC2s can be challenging. Phenotypically ILC2 are identified as lineage$^-$ CRTH2$^+$ CD127$^+$ CD161$^+$ and comprise only 0.05% of peripheral blood mononuclear cells (PBMCs). To address this need, we have developed an efficient immunomagnetic selection method to obtain highly purified, particle-free ILC2s from human PBMCs.

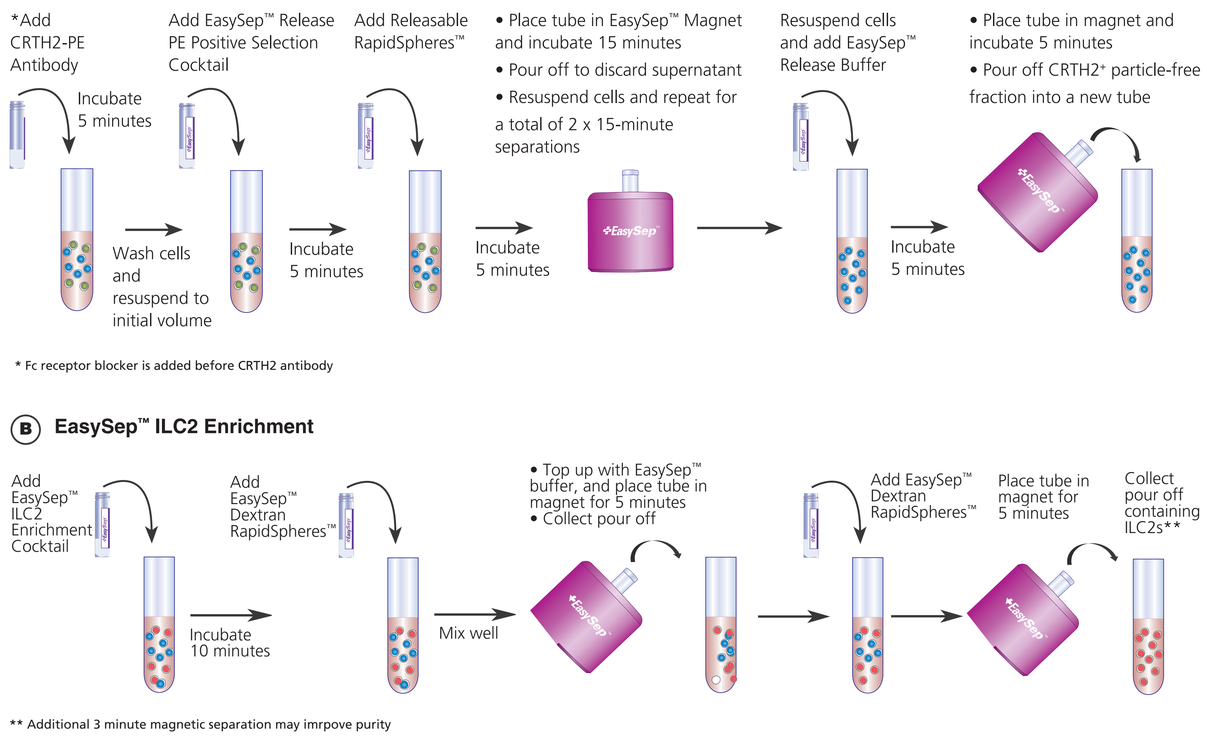
Methods

Samples:

Whole blood and human leukapheresis samples (Leuko Pak) were obtained from normal healthy donors. PBMCs from whole blood were obtained by density gradient centrifugation using Lymphoprep™ and SepMate™. Leuko Pak cells were washed twice with EasySep™ Buffer (1 x PBS, 1mM EDTA, 2% fetal bovine serum) prior to use.

Cell Isolation Strategy:

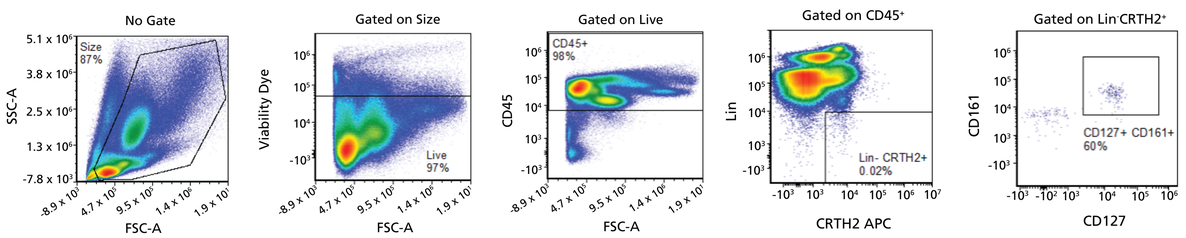
Figure 1. EasySep™ ILC2 Isolation Procedure



Assessment of ILC2s by Flow Cytometry

Human ILC2s were identified as CD45$^+$, Lineage (CD1a, CD3, CD4, CD14, CD16, CD19, CD34, CD94, CD123, CD11c, TCRαβ, TCRγδ, BDCA-2, FcεR1) negative, CRTH2$^+$ CD161$^+$ CD127$^+$.

Figure 2. Flow Cytometry Gating Strategy for ILC2s from Human PBMCs

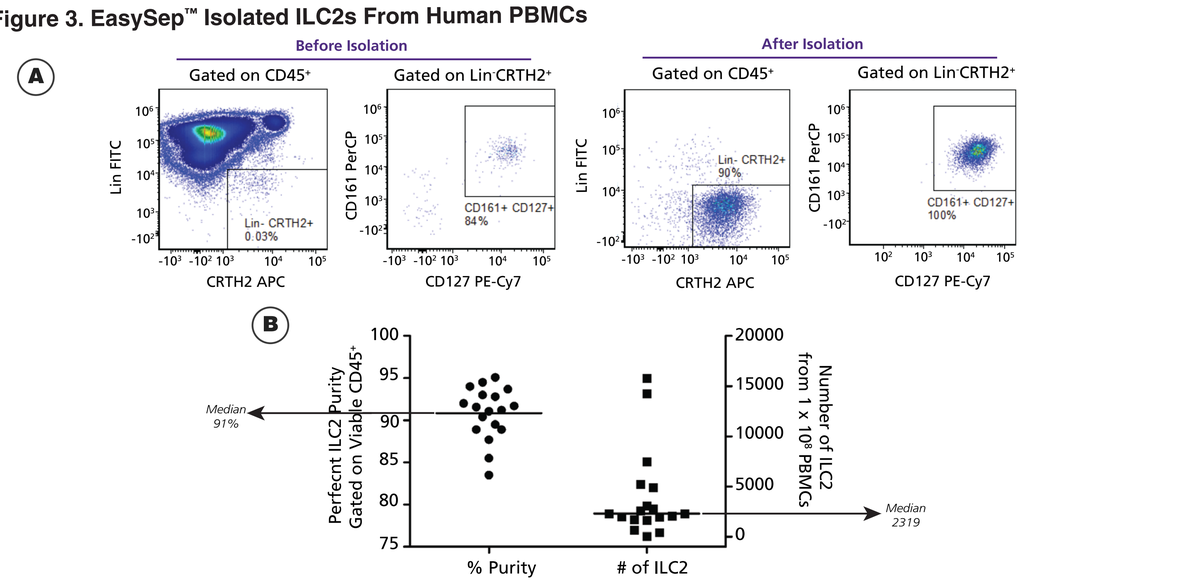


ILC2 Functional Analysis

500 - 1000 EasySep™ isolated ILC2s were cultured in 200 µL of ImmunoCult™-XF T Cell Expansion Medium (#10981) on 96 well U-bottom plate with or without IL-2 and IL-33.

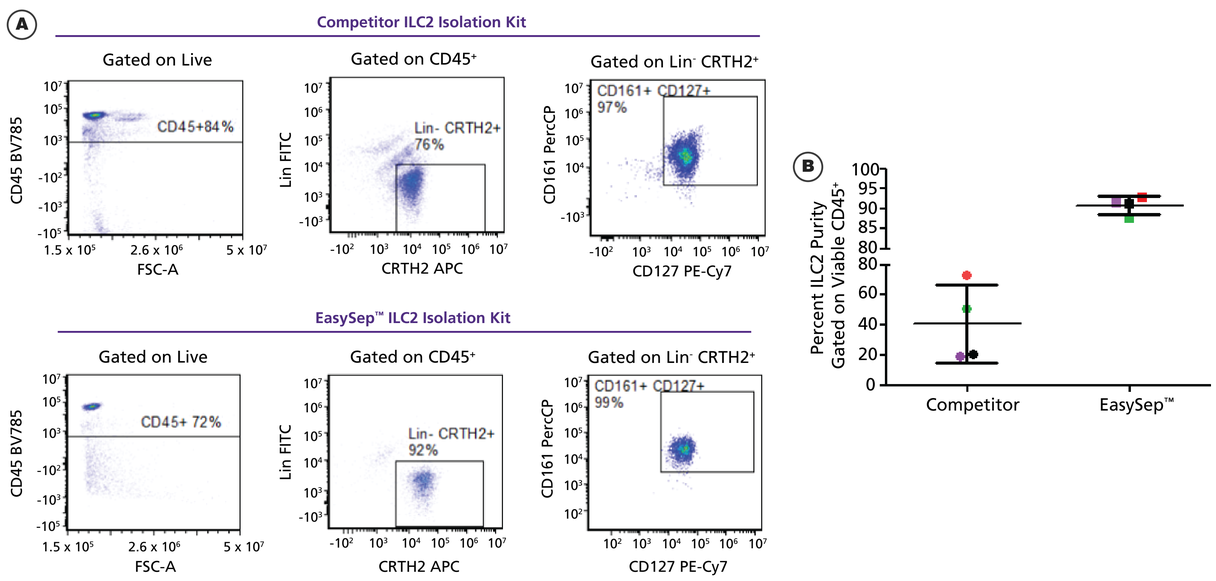
Results

Figure 3. EasySep™ Isolated ILC2s From Human PBMCs



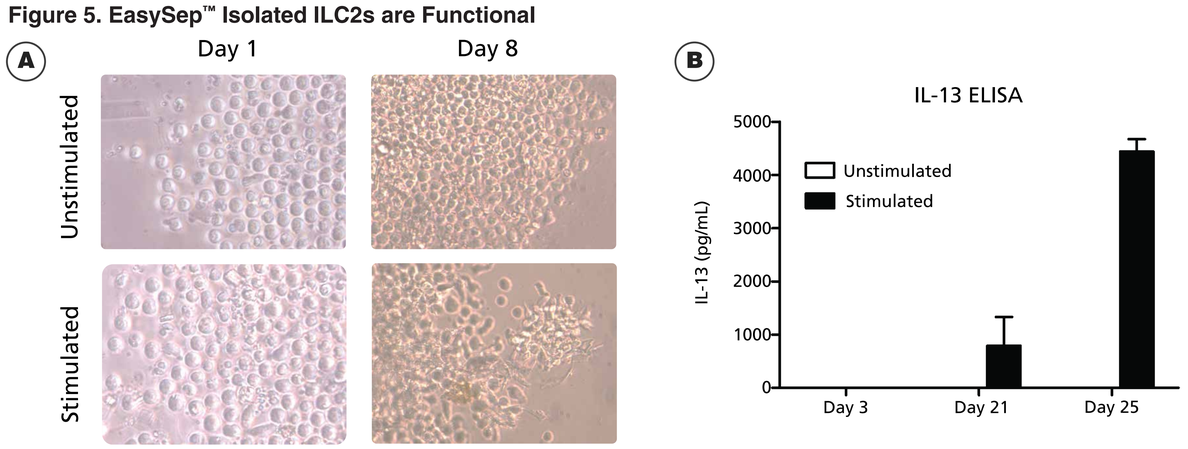
(A) Representative plots of ILC2 (Lin$^-$ CRTH2$^+$ CD161$^+$ CD127$^+$) frequency before and after EasySep™ isolation. **(B)** Summary of ILC2 purity (gated on viable CD45$^+$) and the number of ILC2s obtained from 1x10^8 PMBCs across 18 donors. Lines represent median.

Figure 4. Comparative Analysis of Competitor vs. EasySep™ ILC2 Isolation Kit Indicating Median Values



ILC2s were isolated from normal healthy donors using EasySep™ or competitor cell isolation kit according to manufacturers instructions. **(A)** Flow cytometry purity assessment of isolated ILC2s. **(B)** Summary of ILC2 purity (gated on viable CD45$^+$) across four experiments. Each color indicates head-to-head comparison.

Figure 5. EasySep™ Isolated ILC2s are Functional



EasySep™ isolated ILC2s were stimulated and cultured. **(A)** Morphological changes to ILC2s upon activation on day 1 and 8 of culture. **(B)** IL-13 concentration in supernatants of ILC2 culture was assessed by ELISA at the indicated time points (n=2, mean +/- SD of duplicate wells).

Summary

- Highly pure and functional ILC2s can be isolated in 2.5 hours using the EasySep™ Human ILC2 Isolation Kit
- Purities and proportional cell recoveries of 91% and 2319 (median, n = 18) ILC2s from 1 x 10^8 starting PBMCs can be achieved, respectively
- EasySep™ isolated ILC2s are functional, as shown by their ability to expand and produce IL-13 upon in vitro stimulation with IL-33 and IL-2