EASY ISOLATION OF PARTICLE-FREE HUMAN ILC2s FROM PERIPHERAL BLOOD MONONUCLEAR CELLS

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Abstract

Group 2 innate lymphoid cells (ILC2s) have important roles in type-2 immune responses and are implicated in allergies, asthma, helminth infections and other metabolic diseases. To better understand their role in immunity, highly purified ILC2s are required. ILC2s are phenotypically defined as CD45+ lineage-negative CRTH2+ CD127+ CD161+, and comprise approximately 0.05% of peripheral blood mononuclear cells (PBMCs). Fluorescence activated cell sorting is the most common method to isolate ILC2s but it is both time-consuming and expensive because the cells are so rare. To address this issue, we have developed an immunomagnetic isolation method to obtain highly pure, particle-free ILC2s from human PBMCs in 2.5 hours. First, CRTH2 positive cells are labelled with a CRTH2-PE monoclonal antibody. These cells are then positively selected using an anti-PE antibody complex, EasySep™ Releasable RapidSpheres™ and an EasySep™ magnet. After separation, the magnetic particles are released from the positively selected cells and a second cocktail of antibody complexes and EasySep™ Dextran RapidSpheres™ is added to label non-ILC2s within the CRTH2 positive population. After a second separation, the released particles and unwanted cells are retained in the tube within the magnet, whereas the ILC2s are simply poured off and ready for use. With this method, the purity of ILC2s isolated from PBMCs was 84-95% (median 91%, n=18) with a recovery of 0.4-14.3x10^3 ILC2s per 10^8 starting PBMCs (median 2.46x10^3, n=18). The isolated ILC2s are functional, producing IL-13 upon in vitro stimulation with IL-33, and IL-2. Overall, EasySep™ Human ILC2 Isolation Kit allows researchers to easily and efficiently isolate highly pure and functional ILC2s.

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. Suspend cells at 2 x 10^8 cells/mL and add FC Receptor blocker to sample before cell separation. If starting with fewer than 2 x 10^8 cells, resuspend cells in 1 mL.

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+ CD127+ CD161+.

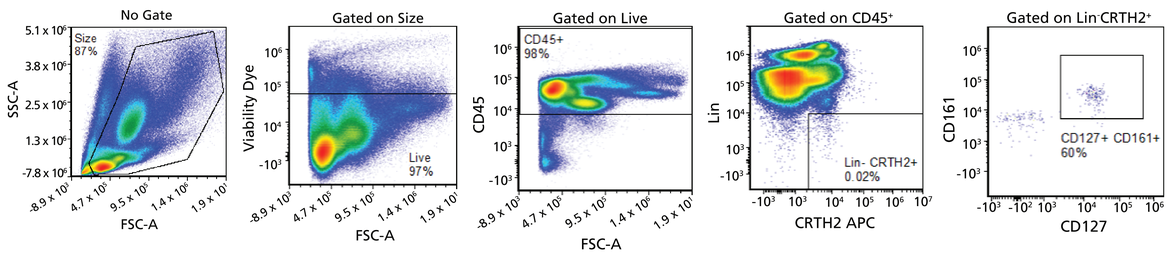
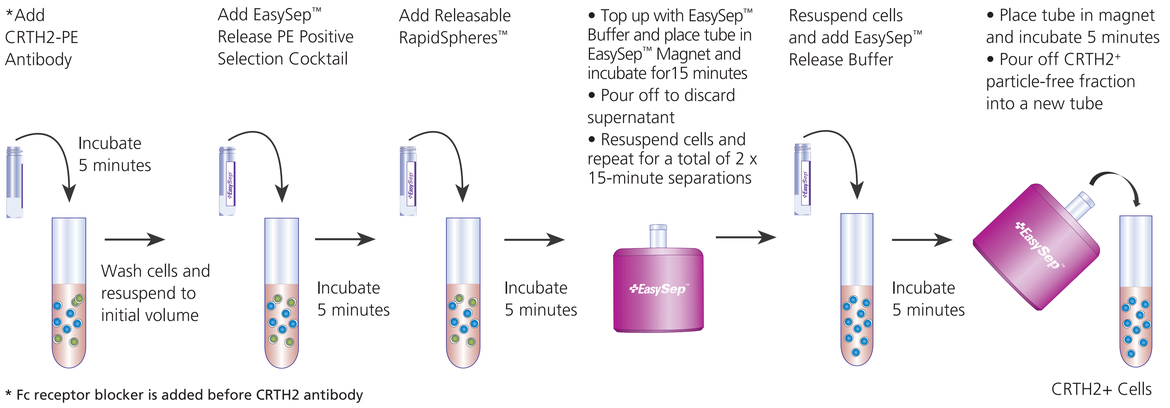


FIGURE 1. Flow Cytometry Gating Strategy for ILC2s from Human PBMCs

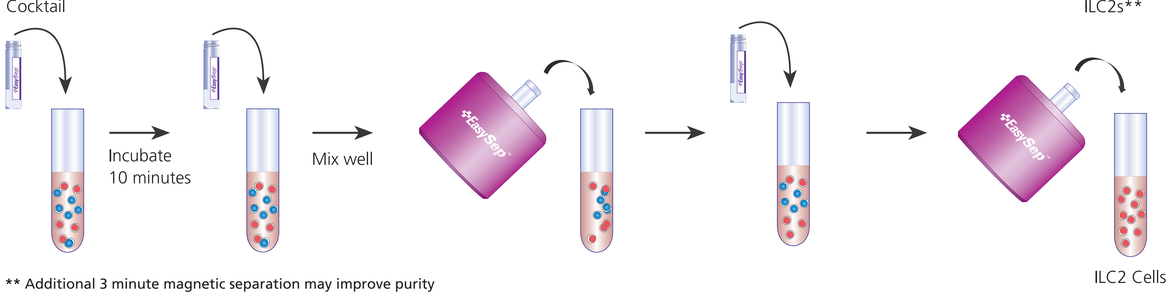
Cell Isolation Strategy

(A) CRTH2 PE Positive Selection



* Fc receptor blocker is added before CRTH2 antibody

(B) EasySep™ ILC2 Enrichment



** Additional 3 minute magnetic separation may improve purity

FIGURE 2. EasySep™ ILC2 Isolation Procedure

ILC2 Functional Analysis

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

Results

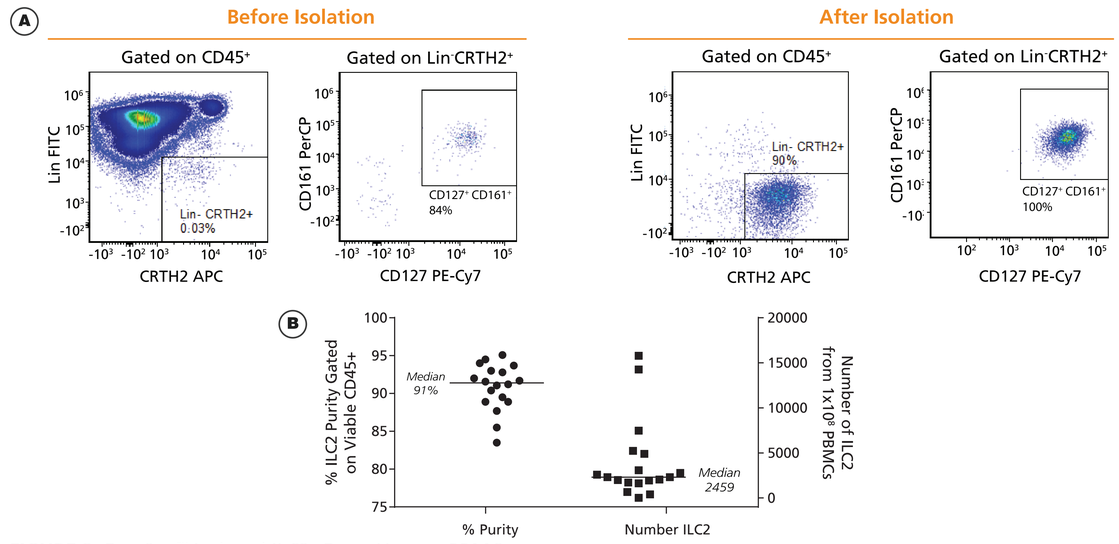


FIGURE 3. EasySep™ Isolated ILC2s From Human PBMCs

(A) Representative plots of ILC2 (Lin- CRTH2+ CD127+ CD161+) 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.

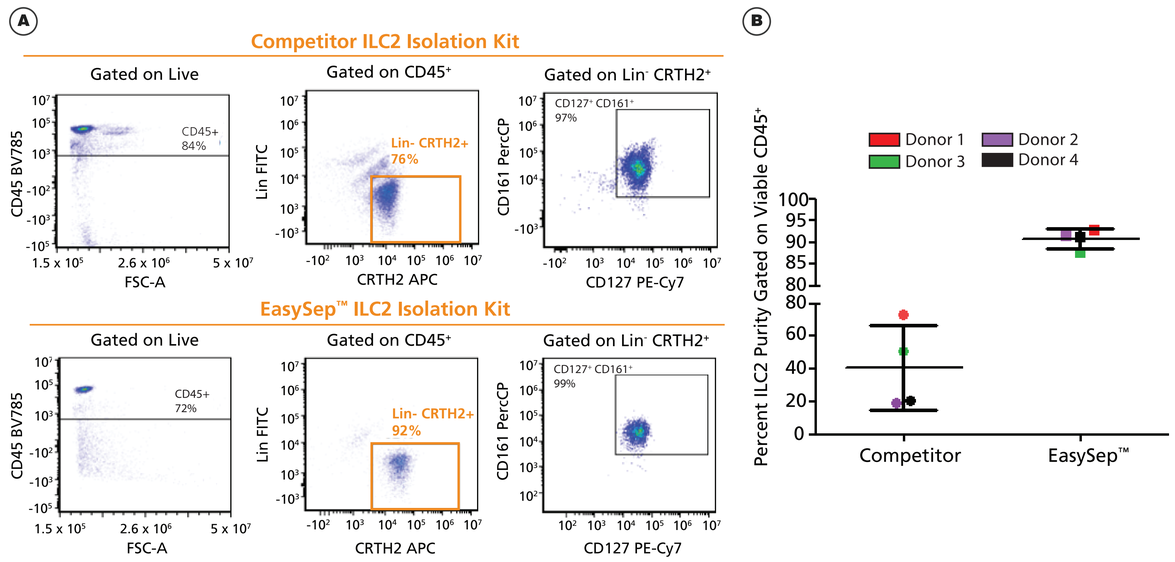


FIGURE 4. Comparative Analysis of Competitor vs. EasySep™ ILC2 Isolation Kit

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+) and lines represent median, with standard deviation, across four independent experiments. Each color indicates head-to-head comparison.

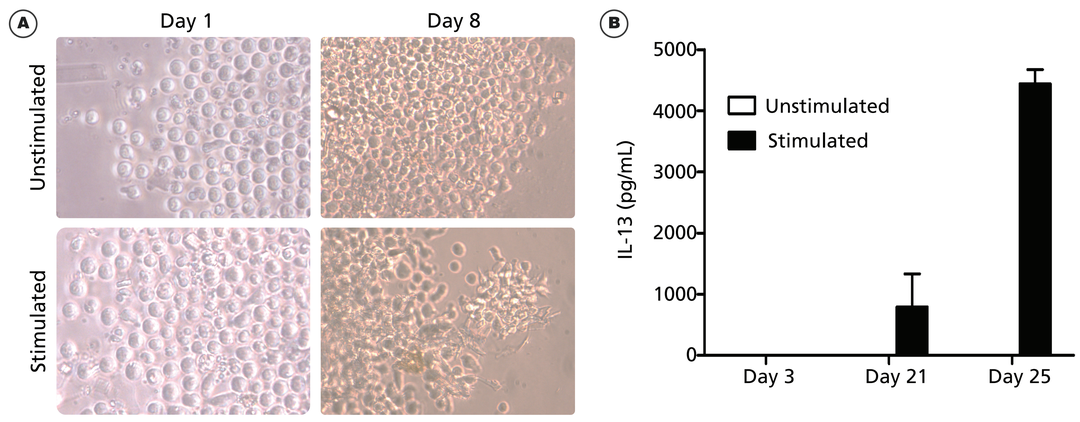


FIGURE 5. EasySep™ Isolated ILC2s are Functional

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 of 91% and cell recoveries of 2459 ILC2s per 1 x 10^8 starting PBMCs can be achieved (median, n = 18)
- 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

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