# EFFICIENT ENRICHMENT OF FUNCTIONAL ILC SUBSETS FROM HUMAN PBMCs BY IMMUNOMAGNETIC SELECTION

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## Abstract

Innate lymphoid cells (ILCs) are exceedingly rare but important regulators of homeostatic and disease-associated immune processes. The frequency of ILCs in peripheral blood of healthy humans is ~0.07% of CD45+ leukocytes. ILCs lack specific cell surface markers but can be divided into distinct subsets (ILC1, 2 and 3) based on their differential expression of effector cytokines and master transcription factors. Currently, cell sorting is the most widely used method to isolate ILCs, but it is time-consuming, expensive and often results in low purities and recoveries. Pre-enrichment of ILCs would allow for reduced sorting times and improved purities. Accordingly, we have developed a fast immunomagnetic negative selection method to pre-enrich all ILCs subsets from human leukapheresis samples. Briefly, unwanted cells are labeled with antibodies and magnetic particles and placed into an EasySep™ magnet. Unwanted cells are retained in the magnet and the enriched ILC fraction is simply poured off into a new tube. We find that total ILCs (defined as Lineage$^-$CD45$^+$CD127$^+$) are enriched from a frequency of 0.01 - 0.23% (n = 28) to a final frequency of 17 - 86%, an enrichment of 200 - 1500 fold with virtually no loss of ILCs. ILC1 were enriched from 0.01 - 0.2% to 4.5 - 14%. ILC2s were enriched from 0.01 - 0.1% to 5.8 - 51%, and ILC3s were enriched from 0.01 - 0.1% to 6 - 16%. This pre-enrichment drastically decreases sort time, allowing sorting over 3.7 x $10^5$ ILCs from 2 x $10^9$ PBMCs in only 12 minutes. Sorted cells maintained their functionality; when stimulated, ILC1s produced IFNγ, ILC2s secreted IL-13, and ILC3s produced IL-17A. Our newly developed method of ILC pre-enrichment should aid human ILC research by enabling their rapid isolation when combined with cell sorting.

## Methods

### Enrichment Strategies

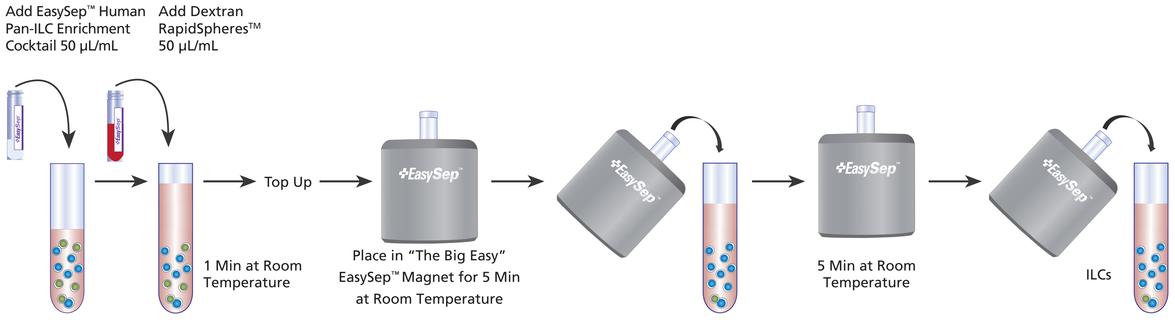



**FIGURE 1.** Human ILC Erichment from Leukapheresis by Negative Selection Using EasySep™

### Assessment of ILCs by Flow Cytometry

ILCs were identified as CD45$^+$, Lineage-negative (CD1a, CD3, CD11c, CD14, CD19, CD34, CD123, TCRαβ, TCRγδ, BDCA2, FcεR1, CD94, CD4, CD16), and CD127$^+$. ILC1s were defined as Lin$^-$CD127$^+$CRTH2$^-$CD117$^-$. ILC2s were defined as Lin$^-$CD127$^+$CRTH2$^+$CD117$^{+/-}$. ILC3s were defined as Lin$^-$CD127$^+$CRTH2$^-$CD117$^+$.

### Sorting of ILCs by FACS and Functional Analysis

The efficiency of ILC sorting from unenriched leukapheresis samples was compared. Sort time and ILC purity were analyzed. Sorted ILCs were stimulated in vitro, and their ability to produce cytokines was measured by ELISA.

## Results

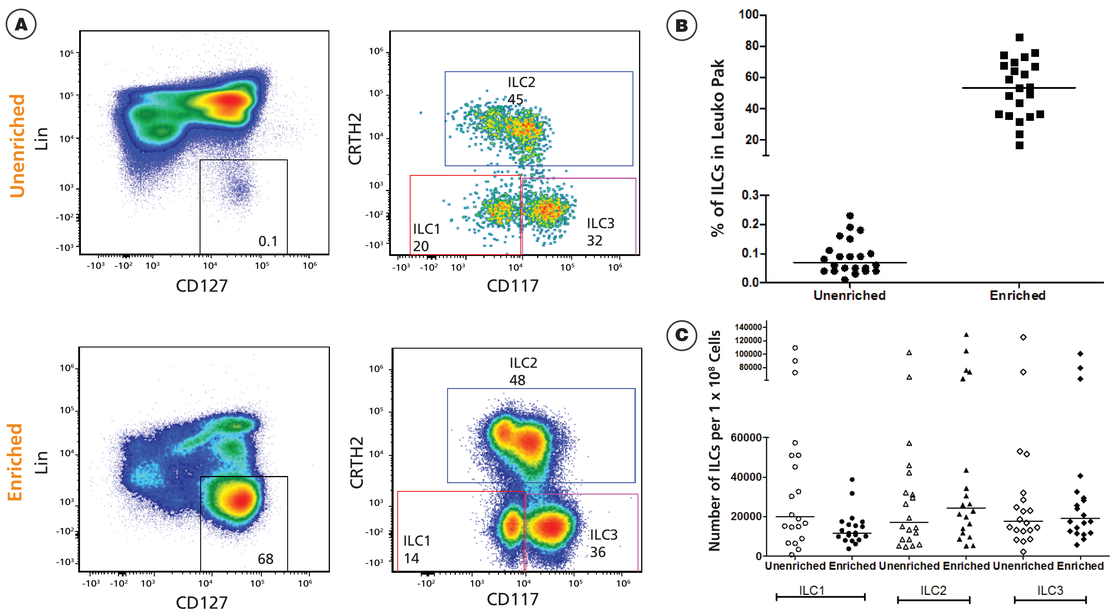
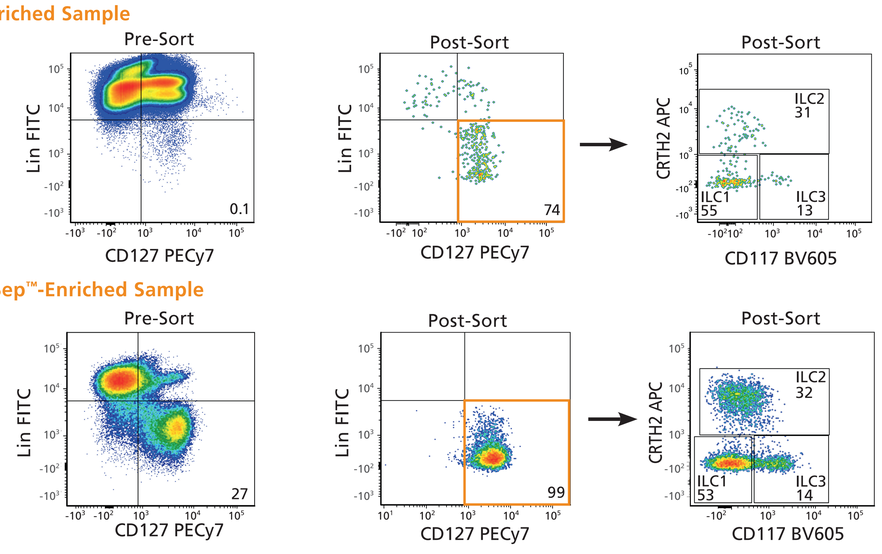



**FIGURE 2.** Percentage and Number of Pan ILCs Before and After Enrichment Using EasySep™ Negative Selection

**(A)** Cells were gated on WBC, viable cells, CD45$^+$, LIN$^-$, and CD127$^+$. **(B)** Percentage of Pan ILCs before and after enrichment. **(C)** Number of ILCs that can be obtained from 1x$10^8$ leukapheresis cells using the enrichment cocktail.

**FIGURE 3.** Pre-Enrichment with EasySep™ Significanly Reduces the Time Required to Obtain Pure ILCs

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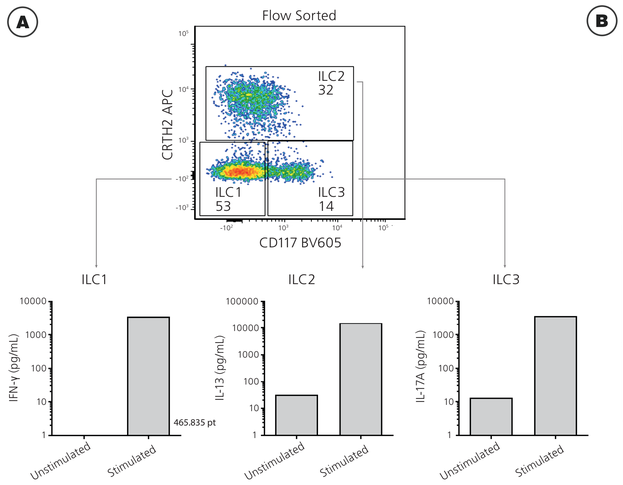
| | START | PRE-SORT | POST-SORT | | TIME (MIN) | |
|---|---|---|---|---|---|---|
| | # Starting Cells | Purity | Purity | # Total ILCs | Sample Prep Time | FACS Time |
| Unenriched | 2 x $10^9$ | 0.1% | 74% | 6.9 x $10^{5**}$ | 15 min | 3200 min (53 hrs)* |
| EasySep™-Enriched | 2 x $10^9$ | 27% | 99% | 3.7 x $10^5$ | 30 min | 12 min |

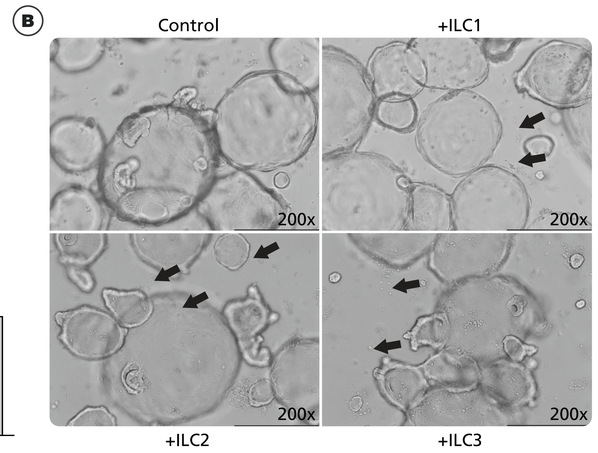
*estimated based on extrapolated values **extrapolated from a 53 hour sort starting with 2 x $10^9$ cells

Starting with a fresh leukapheresis sample, ILCs were isolated in parallel from an unenriched or an EasySep™-enriched sample. **(A)** In an unenriched sample, ILC frequency was assessed by flow cytometry at the start and after one round of FACS. **(B)** In an EasySep™-enriched sample, ILC frequency was assessed immediately after EasySep™ enrichment, and again after one round of cell sorting. **(C)** Corresponding purities and cell sorting times at each stage are reported.

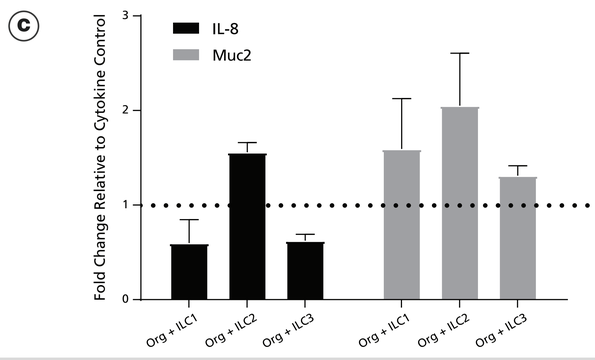
**FIGURE 4.** EasySep™-Enriched and Sorted ILCs are Functional and Impact the Gene Expression of Intestinal Organoids




**(A)** ILC1, ILC2 and ILC3s sorted from EasySep™-enriched samples were cultured independently in ImmunoCult™ medium with or without stimuli. After 6 days, supernatants from ILC1 (IL-12 and IL-15), ILC2 (IL-2 and IL-33) and ILC3 (IL-2, IL-1β and IL-23) cultures were collected and analyzed for the secretion of IFNγ, IL-13, and IL-17A, respectively, by ELISA.




**(B)** ILC1, ILC2 and ILC3 were co-cultured with intestinal organoids for 7 days. The co-culture medium, Intesticult™ was supplemented with cytokines to support the growth and proliferation of the ILCs as indicated above. Representative images of ILCs and intestinal organoid co-cultures at day 7 (200x magnification). The scale bar is 500µm.




**(C)** Organoid epithelial gene expression was assessed by qPCR. The data shows relative expression of IL-8 and Muc2 by organoids in co-culture, normalized to organoids alone, but in the presence of supplemented cytokines. Representative images of ILCs and intestinal organoid co-cultures at day 7 (200x magnification).

## Summary

- Pan ILCs show enrichment from 0.01 - 0.23% to 17- 86% (n = 22).
- Cell sorting from these pre-enriched samples was faster, and yielded higher ILC purity than cell sorting from non-enriched controls.
- Sorted ILCs from enriched samples were stimulated and cultured. They secreted high levels of IFNγ (ILC1), IL-13 (ILC2) and IL-17A (ILC3) as assessed by ELISA, indicating that these cells are functional.
- Enrichment of ILCs prior to FACS increases purity, shortens sorting time and maintains ILC functionality.
- Sorted ILCs in co-culture with intestinal organoids modulate gene expression of the intestinal cells.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