

# PRE-ENRICH CELLS PRIOR TO SORTING

with EasySep™ or RosetteSep™

Starting your sort with pre-enriched populations allows you to obtain your rare cells faster than by using fluorescence-activated cell sorting (FACS) alone, saving you time and money. Pre-enrichment of your target population can be quickly achieved by depleting unwanted cells using either negative or positive selection strategies. Use EasySep™ or RosetteSep™ kits to pre-enrich cells that are then ready to be labeled with antibodies for immediate cell sorting by flow cytometry.

## Why Pre-Enrich Cells Before Sorting?

**FAST.** Reduce cell sorting time, especially when working with large sample volumes or rare cell types.

**EFFICIENT.** Increase sample throughput and reduce cell sorting-associated costs.

**IMPROVED RECOVERY.** Obtain high yields of rare cell populations.

## Cell Isolation Platforms for Enrichment



### EasySep™

#### Fast and Easy Immunomagnetic Cell Isolation

EasySep™ is an immunomagnetic cell isolation platform suitable for the enrichment of cells from virtually any type of sample, including spleen, bone marrow, lymph nodes, whole blood and bone marrow.

[www.EasySep.com](http://www.EasySep.com)

Most EasySep™ kits can be completely automated using RoboSep™, the fully automated cell isolation platform.

[www.RoboSep.com](http://www.RoboSep.com)



### RosetteSep™

#### Unique Immunodensity Cell Isolation

RosetteSep™ kits offer one-step enrichment of cells directly from human whole blood. By crosslinking unwanted cells to red blood cells (RBCs) present in the sample, target cells are purified during standard density gradient centrifugation.

[www.RosetteSep.com](http://www.RosetteSep.com)

RosetteSep™ can be easily combined with the specialized cell isolation tube, SepMate™, to standardize and minimize variability when isolating cells using density gradient centrifugation.

[www.SepMate.com](http://www.SepMate.com)

Find the right kit to enrich your desired cell population at [www.cellseparation.com](http://www.cellseparation.com)

## Case Study

# Rapid Isolation of Functional Innate Lymphoid Cells

Isolating rare cell types by flow sorting alone can be time-consuming. Here, the isolation of innate lymphoid cells (ILCs) is used as an example to show how pre-enrichment prior to fluorescence-activated cell sorting (FACS) can significantly reduce sorting times and increase final cell purities when working with rare and complex cell types.

## Background

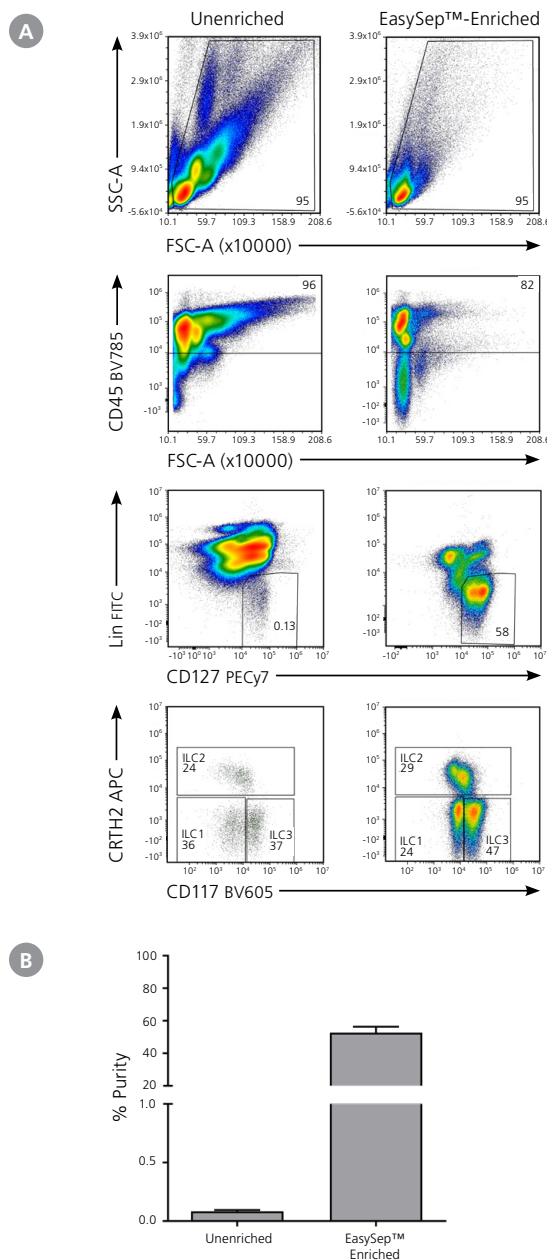
Innate lymphoid cells (ILCs) are important effector cells in innate immunity and have essential functions in immune regulation, inflammation and tissue homeostasis. In healthy individuals, ILCs are extremely rare, comprising <0.1% of CD45<sup>+</sup> leukocytes in mouse and human peripheral blood.<sup>1,2</sup> The low frequency and lack of unique surface markers for ILCs create challenges when it comes to their identification, isolation and characterization. Currently, ILCs are isolated using multicolor FACS but this method is time-consuming, expensive and can result in low cell recovery. Here we describe a method to drastically reduce the time required to isolate all ILC groups (ILC1, ILC2 and ILC3).

## Methods

Single-cell suspensions were prepared by washing unprocessed leukapheresis samples twice with PBS containing 2% fetal bovine serum (FBS). Following staining of the single-cell suspensions with fluorescently labeled antibodies, ILCs were isolated by FACS. Alternatively, single-cell suspensions were pre-enriched for target cells using the EasySep™ Human Pan-ILC Enrichment Kit (Catalog #17975) prior to staining and FACS. In brief, ILCs were enriched by immunomagnetic negative selection where unwanted cells were targeted for removal by antibody complexes and magnetic particles. Final purities, recoveries and protocol times were compared between the two isolation methods.

## Results

To evaluate the effect of pre-enrichment with EasySep™ on ILC isolation, unenriched and EasySep™-enriched samples were stained with fluorescently labeled antibodies, and ILCs were identified as CD45<sup>+</sup>, lineage-negative (CD1a, CD3, CD11c, CD14, CD19, CD34, CD123, TCRαβ, TCRγδ, BDCA-2, FcεR1α, CD94, CD4, CD16), and CD127<sup>+</sup> by flow cytometry. ILC1, ILC2 and ILC3 populations were further defined based on CRTH2 and CD117 expression. As shown in Figure 1, the EasySep™ kit significantly increased the purity of ILCs prior to FACS.



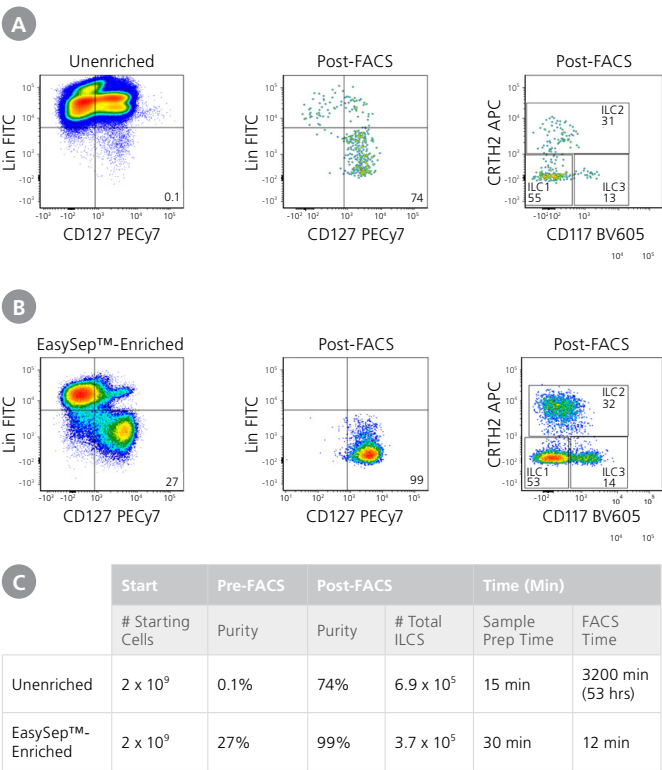
**Figure 1. Pre-Enrichment with EasySep™ Improves ILC Starting Purity Prior to FACS**

(A) Starting with a fresh leukapheresis sample, unenriched and EasySep™-enriched cells were stained and gated on WBC, viable cells, CD45<sup>+</sup>, Lin<sup>-</sup> and CD127<sup>+</sup>. ILC1, ILC2, and ILC3 subsets were identified based on expression of CRTH2 and CD117. Representative FACS plots are shown. (B) The ILC frequency of the unenriched and EasySep™-enriched sample was 0.07% and 53.6% of CD45<sup>+</sup> cells, respectively (Unenriched n=20, EasySep™-Enriched n=22; data represents the median ± SEM).

Results Continued

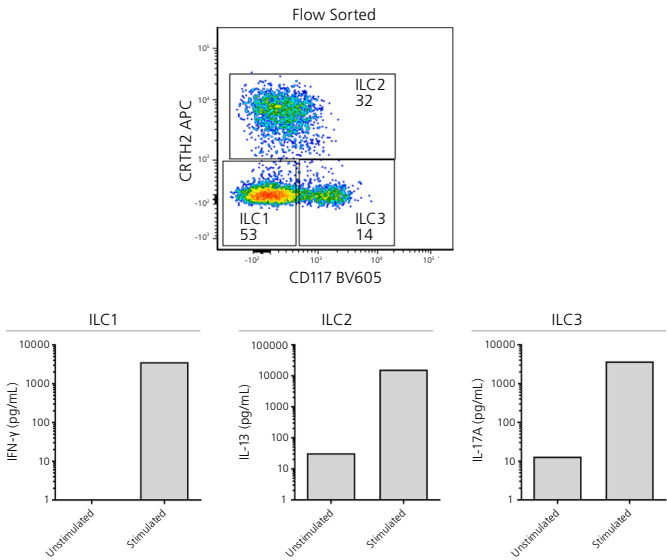
To evaluate the efficiency of ILC sorting from unenriched and EasySep™-enriched leukapheresis samples, FACS time and ILC purities were compared (Figure 2).

Starting with  $7.5 \times 10^7$  total cells and a pre-FACS purity of 0.1%, isolation by FACS alone resulted in a final ILC purity of 74% and took approximately 120 minutes of sort time. A second round of sorting was required in order to obtain a final purity of 97% (Data not shown). Extrapolating from these results, it would take approximately 3,200 minutes of sort time to process  $2 \times 10^9$  cells. Conversely, starting with  $2 \times 10^9$  cells from the same donor, pre-enrichment by EasySep™ increased the pre-FACS ILC frequency from 0.1% to 27%. Flow sorting time for this EasySep™-enriched sample was reduced to 12 minutes, with a final Lin<sup>-</sup>CD127<sup>+</sup> purity of 99%. As shown in Figure 3, sorted ILC1, ILC2 and ILC3 subsets from EasySep™-enriched samples remain functional as assessed by cytokine secretion.



**Figure 2.** Pre-Enrichment with EasySep™ Significantly Reduces the Time Required to Obtain Pure ILCs

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 FACS. (C) Corresponding purities and FACS times at each stage are reported.



**Figure 3.** EasySep™-Enriched ILCs are Functional

ILC1, ILC2 and ILC3s sorted from EasySep™-enriched samples were cultured independently in a serum-free expansion 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.

Summary

By using EasySep™ to pre-enrich for ILCs, it is possible to process large sample sizes to isolate distinct ILC subsets. The short protocol times of EasySep™ and RosetteSep™ kits provide an easy means of streamlining the cell isolation step in a given assay. In this case study, we demonstrate how a negative selection protocol can quickly deplete unwanted cells to enrich for rare untouched ILCs that are ready for analysis or cell sorting. By combining FACS with EasySep™, the overall cell isolation process resulted in greater purity in a significantly shorter period of time.

References

1. Mjösberg JM et al. (2011) Human IL-25- and IL-33-responsive type 2 innate lymphoid cells are defined by expression of CRTH2 and CD161. *Nat Immunol* 12(11): 1055-62.

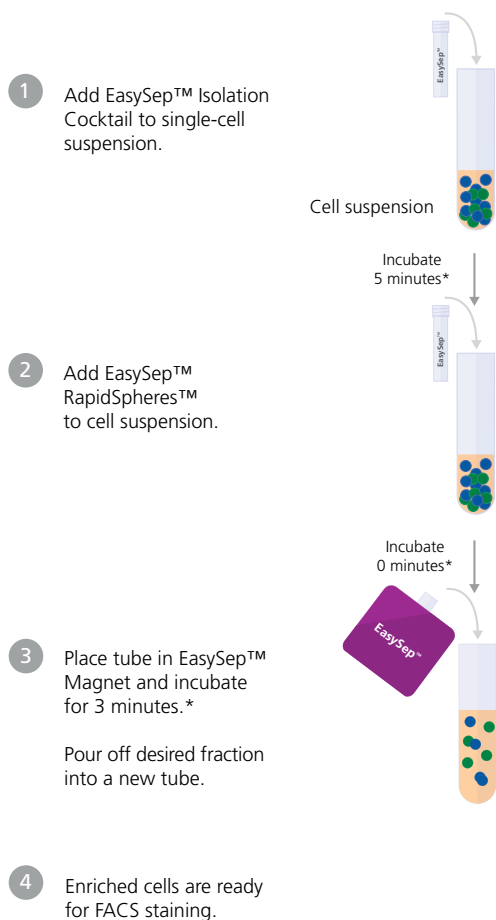
2. Halim TYF & Takei F. (2014) Isolation and characterization of mouse innate lymphoid cells. *Curr Protoc Immunol* 106(5): 3.25.1-13.

ILC Product Listing

Product	Source	Catalog #
EasySep™ Human Pan-ILC Enrichment Kit	Leukopak	17975 17975RF
RosetteSep™ Human ILC2 Enrichment Kit	Whole Blood	15382
EasySep™ Mouse ILC2 Enrichment Kit	Single-Cell Suspension from Mouse Tissues	19842

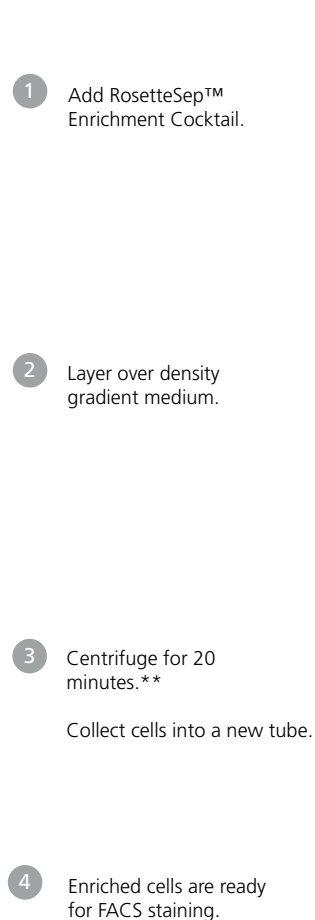
## Pre-Enrichment Workflow

### Typical EasySep™ Cell Isolation Protocol (Negative Selection)



\*Times and steps shown are typical for next-generation EasySep™ human negative selection kits. Time for each kit will vary depending on the exact isolation protocol and magnet used.

### Typical RosetteSep™ Protocol (Negative Selection)



\*\*Use SepMate™ to reduce centrifugation time to 10 minutes with brake on.



Perform FACS to isolate your specific cells of interest.

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