Improve the Efficiency and Quality of Your Cell Sorting by Pre-Enriching Cells Prior to FACS

In this document, we highlight a method to improve pre-FACS purity and maintain high recoveries of complex immune cell subsets and rare cells, while drastically reducing downstream cell subset sorting time.

Background

Using fluorescence-activated cell sorting (FACS) alone to isolate large numbers of complex or rare cell types can be time-consuming, expensive, and lead to reduced viability of sorted cells. Starting your FACS with a cell population that has been pre-enriched for your cells of interest enables you to more quickly and efficiently isolate complex or rare immune cell subsets than can be achieved using FACS alone, helping you accomplish your research goals faster.

EasySep™, a column-free immunomagnetic cell isolation technology, is one of the fastest and easiest ways to pre-enrich cells prior to FACS. With both negative and positive selection protocols, you can perform upfront bulk removal of unwanted cells from your sample in as little as 8 minutes. The case studies below outline how pre-enrichment of dendritic and innate lymphoid cells using EasySep™ can result in improved purity and higher recovery, thereby drastically reducing cell subset sorting times.

Case Study: Reducing FACS Time for Dendritic Cell Subset Isolation

Dendritic cells (DCs) are a group of innate immune cells that recognize pathogen-associated molecular patterns (PAMPs) and respond by processing associated extracellular and intracellular proteins to present as antigens in the context of MHC molecules. Through antigen presentation to naïve T cells, DCs bridge the innate and adaptive immune systems. DCs can be further characterized into two separate subsets—myeloid or conventional DCs (cDCs) and plasmacytoid DCs (pDCs).[1]

Some immune cell subsets, such as cDCs and pDCs, are found at a low frequency in samples. Isolating these cells can require the design of complex multicolor flow cytometry experiments where multiple cell surface markers are fluorescently labeled and used for FACS. Due to their complexity, these FACS experiments can often take hours, which can adversely affect the quality of the sorted cells. Not only can these lengthy experiments directly cause reduced cell viability, but the presence of significant numbers of dead cells in your sample can increase autofluorescence and non-specific binding to

Why Pre-Enrich Cells Before FACS?

GENTLE. Obtain viable and functional cells that are immediately ready for FACS.

FAST. Increase sample throughput by reducing downstream FACS time.

HIGHER STARTING FREQUENCY. Increase the purity of complex or rare cell types.

fluorescently labeled antibodies, thereby altering scatter properties and compromising the purity of your isolated cells.[2,3] Starting your FACS with a pre-enriched cell population increases the frequency of your target cells, helping you achieve faster isolation of complex cell types and reducing the risk of FACS-related cell death that could impact your downstream experiments. Here, we describe a method for pre-enriching a pan-DC population that improves the pre-FACS purity of cDCs and pDCs while maintaining high cell recoveries, thereby drastically reducing the time required for downstream FACS.

Methods

To create a single-cell suspension, mouse splenocytes were digested at 37°C using Spleen Dissociation Medium (Catalog #07915). Single-cell suspensions were first pre-enriched for pan-DCs using immunomagnetic negative selection with EasySep™ Mouse Pan-DC Enrichment Kit II (Catalog #19863) prior to staining with fluorescently labeled antibodies. DC subsets were then analyzed using flow cytometry. Alternatively, red blood cells were first lysed prior to staining and flow cytometry. Lineage-negative markers were used to identify DCs (CD3e, CD19, Ly-6G, F4/80, NK1.1 (CD161), TER119, and IgM) by flow cytometry. cDCs were defined as Lin$^-$CD11c$^+$PDCA-1$^-$ and pDCs were defined as Lin$^-$CD11c^{low}PDCA-1$^+$. Final purities were compared between the two isolation methods, and the percentage recoveries of cDCs and pDCs were calculated in EasySep™-enriched samples. To calculate protocol times, each sample was run on the flow cytometer for an equivalent amount of time with a consistent flow rate and the data was used to extrapolate the total time required to sort 1 million pan-DCs, cDCs, or pDCs using FACS.

A

B

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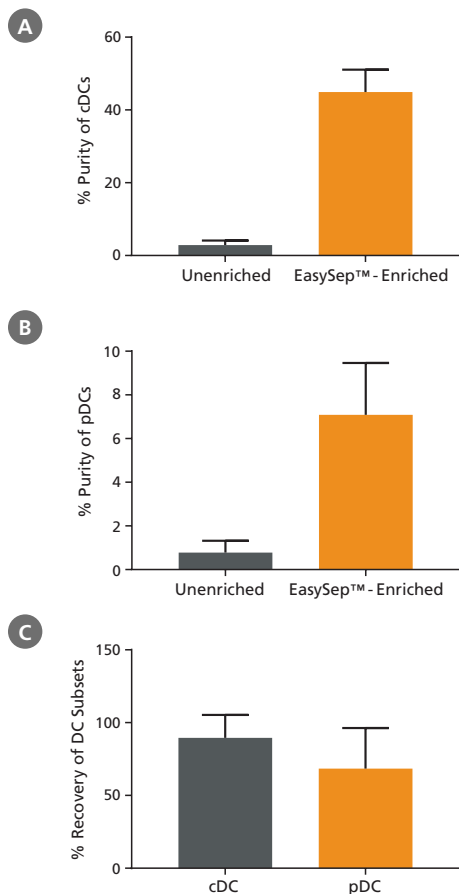
Figure 1. Pre-Enrichment Results in Improved Purity and High Recovery of DC Subsets

Following mouse splenocyte digestion and red blood cell lysis, unenriched samples were stained with fluorescently labeled antibodies for flow cytometric analysis of conventional DCs (cDCs) or plasmacytoid DCs (pDCs). Alternatively, pan-DCs were first enriched using EasySep™ prior to being run on the flow cytometer. Cell purity of (A) cDCs and (B) pDCs was measured using flow cytometry in unenriched and EasySep™-enriched samples. (C) The % recovery of DC subsets was calculated for EasySep™-enriched samples. Data shown as mean ± SD; n = 5 - 9.
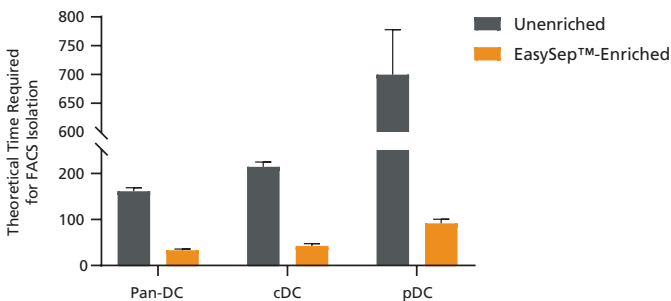
Figure 2. Pre-Enrichment Reduces the Time Required to Obtain DC Subsets by FACS

Starting with a single-cell suspension of mouse splenocytes, pan-DCs, conventional DCs (cDCs), and plasmacytoid DCs (pDCs) were analyzed in parallel from unenriched or EasySep™-enriched samples by flow cytometry. In the unenriched sample, red blood cells were first lysed before performing flow cytometry. Alternatively, pan-DCs were first enriched using EasySep™ before analysis on the flow cytometer. Each sample was run on the flow cytometer for an equivalent amount of time with a consistent flow rate and the data was used to extrapolate the total theoretical time required to sort 1 million pan-DCs, cDCs, or pDCs using FACS. Data shown as mean ± SD; n = 2.

Results and Summary

Starting with a single-cell suspension of mouse splenocytes, pre-enrichment with EasySep™ Mouse Pan-DC Enrichment Kit II increased the pre-FACS purity of cDCs from 2.9% to 44.9% and pDCs from 0.8% to 7.1%, and resulted in high recoveries of both cell subsets for downstream FACS (Figures 1).

To evaluate the effect of pre-enrichment on the time required to obtain DC subsets using FACS, unenriched and EasySep™-enriched samples were run on the flow cytometer and the data was used to extrapolate the total theoretical time required to sort 1 million DCs and DC subsets using FACS. Pre-enrichment with EasySep™ Mouse Pan-DC Enrichment Kit II reduced the time to sort 1 million pan-DCs by 79.6%, cDCs by 80.2%, and pDCs by 87.1% (Figure 2). On average, the overall time to acquire cDCs was reduced from 3.5 hours to just 42 minutes, and pDCs from 11.5 hours to just 91 minutes, saving significant time.

Case Study: Improving the Isolation and Analysis of Rare ILC Subsets

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^{+} leukocytes in mouse and human peripheral blood.[4,5] The low frequency and lack of unique surface markers for ILCs create challenges when it comes to their identification, isolation, and characterization. ILCs are typically isolated using multicolor FACS; however, this method is time-consuming and expensive, and can result in low cell recovery. Here, we discuss a method to drastically reduce the time required to isolate rare ILC subsets (ILC1, ILC2, and ILC3) while maintaining the functionality of the isolated cells.
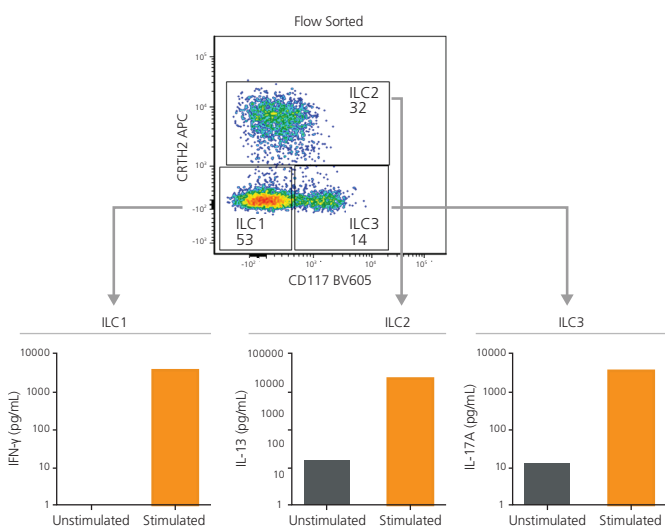
Methods

Single-cell suspensions were prepared by washing unprocessed leukapheresis samples twice with phosphate-buffered saline containing 2% fetal bovine serum. 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 EasySep™ Human Pan-ILC Enrichment Kit (Catalog #17975) prior to staining and FACS. ILCs were identified as CD45^{+}, lineage-negative (CD1a, CD3, CD11c, CD14, CD19, CD34, CD123, TCR$\alpha\beta$, TCR$\gamma\delta$, BDCA-2, FcϵR1α, CD94, CD4, CD16), and CD127^{+} by flow cytometry. ILC1s were further defined as CD117^{-} CRTH2^{-}, ILC2s as CD117^{+} CRTH2^{+}, and ILC3s as CD117^{+} CRTH2^{-}. Final purities, cytokine production, and protocol times were compared between the two isolation methods.
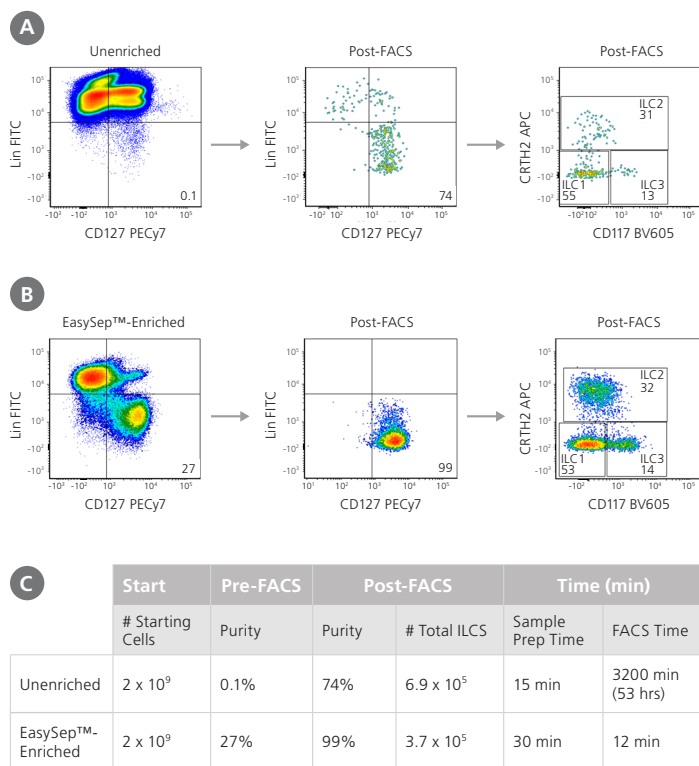
Figure 3. Pre-Enrichment with EasySep™ Reduces the Time Required to Obtain Pure ILCs

Starting with a fresh leukapheresis sample, ILCs (Lin⁻ CD127⁺) 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.

Results and Summary

Starting with 7.5 x 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 FACS time. A second round of FACS 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 FACS time to process 2 x 10^9 cells (Figure 3). Conversely, starting with 2 x 10^9 total cells from the same donor, pre-enrichment using EasySep™ increased the pre-FACS ILC frequency from 0.1% to 27%. FACS time for this EasySep™-enriched sample was reduced to 12 minutes, with a final Lin⁻CD127⁺ cell purity of 99% (Figure 3). Sorted ILC1, ILC2, and ILC3 subsets from EasySep™-enriched samples remained functional as assessed by cytokine secretion (Figure 4).

Conclusion

Isolating complex immune cell subsets or rare cells by FACS can be time-consuming and can negatively impact the purity of your isolated cells, slowing you down in reaching your research goals. In the case studies above, we show how EasySep™ can be used to quickly deplete unwanted cells to enrich for rare, untouched, and functional DC subsets and ILCs that are ready for downstream analysis or FACS. Overall, adding EasySep™ upstream of FACS can result in drastically reduced FACS times while improving the quality of your isolated cells.

Figure 4. 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.

Products for Cell Pre-Enrichment Prior to FACS

EasySep™ is a column-free immunomagnetic cell separation technology that enables rapid isolation of highly purified and functional cells for downstream applications. Pre-enrichment of cell populations using EasySep™ reduces FACS time and enables isolation of complex immune cell subsets and rare cell types.

Table 1. Examples of Reagents for Human Cell Enrichment and Downstream Subset Sorting

Cell Type of Interest	EasySep™ Kits Commonly Used for Pre-Enrichment	Examples of Cell Subsets That Can Be Obtained by FACS
T Cell Subsets[6-9]	EasySep™ Human T Cell Isolation Kit (17951) EasySep™ Human CD4+ T Cell Isolation Kit (17952) EasySep™ Human CD8+ T Cell Isolation Kit (17953)	Th1 Cells Th2 Cells Th17 Cells Th9 Cells Naïve T Cells Central Memory T Cells (Tcm) Effector Memory T Cells (Tem) T Follicular Helper Cells (Tfh) CD161+ Regulatory T Cells (Treg) Naïve Tregs Invariant Natural Killer T Cells (iNKT) Exhausted T Cells (PD1high) Effector T Cells
B Cell Subsets[7]	EasySep™ Human Pan-B Cell Enrichment Kit (19554)	Memory B Cells Regulatory B Cells
Dendritic Cell (DC) Subsets	EasySep™ Human Pan-DC Pre-Enrichment Kit (19251)	Conventional DCs (cDC1 and cDC2) Plastmacytoid DCs (pDC)
Innate Lymphoid Cell (ILC) Subsets	EasySep™ Human Pan-ILC Enrichment Kit (17975) EasySep™ Human ILC2 Isolation Kit (17782)	ILC1 ILC2 ILC3

Table 2. Examples of Reagents for Mouse Cell Enrichment and Downstream Subset Sorting

Cell Type of Interest	EasySep™ Kits Commonly Used for Pre-Enrichment	Examples of Cell Subsets That Can Be Obtained by FACS
T Cell Subsets[10-11]	EasySep™ Mouse T Cell Isolation Kit (19851) EasySep™ Mouse CD4+ T Cell Isolation Kit (19852) EasySep™ Mouse CD8+ T Cell Isolation Kit (19853)	Effector T Cells (Teff) Memory T Cells (Tmem) Regulatory T Cells (Treg) Gamma-Delta T Cells Natural Killer T Cells (NKT) Antigen-specific T Cells Naïve T Cells (CD45RBhigh)
B Cell Subsets	EasySep™ Mouse Pan-B Cell Isolation Kit (19844) EasySep™ Mouse CD138 Positive Selection Kit (18957)	Memory B Cells Plasma Cells
Dendritic Cell (DC) Subsets	EasySep™ Mouse Pan-DC Enrichment Kit II (19863)	Conventional DCs (cDC) Plasmacytoid DCs (pDC)
Innate Lymphoid Cell (ILC) Subsets	EasySep™ Mouse Pan-ILC Enrichment Kit (19875) EasySep™ Mouse ILC2 Enrichment Kit (19842)	ILC1 ILC2 ILC3
Myeloid-Derived Suppressor Cells (MDSCs)[12]	EasySep™ Mouse MDSC (CD11b+Gr1+) Isolation Kit (19867)	Monocytic MDSCs (M-MDSC) Polymorphonuclear MDSCs (PMN-MDSC)
Hematopoietic Stem Cells[13]	EasySep™ Mouse SCA1 Positive Selection Kit (18756) EasySep™ Mouse CD117 (cKIT) Positive Selection Kit (18757) EasySep™ Mouse Hematopoietic Progenitor Cell Isolation Kit (19856)	Lin−Sca-1+c-Kit+ (LSK) Cells Long-Term Hematopoietic Stem Cells (LT-HSC) Short-Term Hematopoietic Stem Cells (ST-HSC)

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