A SMARTER WAY TO ISOLATE CELLS

Cell Separation with EasySep™

Developed with Efficiency in Mind

Working efficiently is key to overcoming the demands of scientific research. That's why our scientists developed EasySep™—a smarter, more efficient way to isolate cells.

At STEMCELL Technologies we provide you with the necessary information to make the right decisions for your research. Here are the facts about EasySep™, backed by data, so you can see the EasySep™ advantages for yourself.

How EasySep™ Works

EasySep™ is a column-free immunomagnetic system for the fast and easy isolation of cells that are immediately ready for downstream applications. Cells of interest are targeted with antibody complexes and magnetic particles for negative or positive selection using an EasySep™ magnet (Figure 1). In as little as 8 minutes (Figure 2), you can obtain highly purified cells from a variety of sample sources, including peripheral blood mononuclear cells, whole blood, leukapheresis products, bone marrow, and cord blood.

> "[EasySep™] enables us to isolate B cells from the spleen really fast with good viability. That is what we need. You don't have to stand in front of a column and wait for it to drip. The viability of the cells that you recover tends to be higher just because it is so fast."
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> **Steevenson N., PhD**
> **National Institutes of Health**

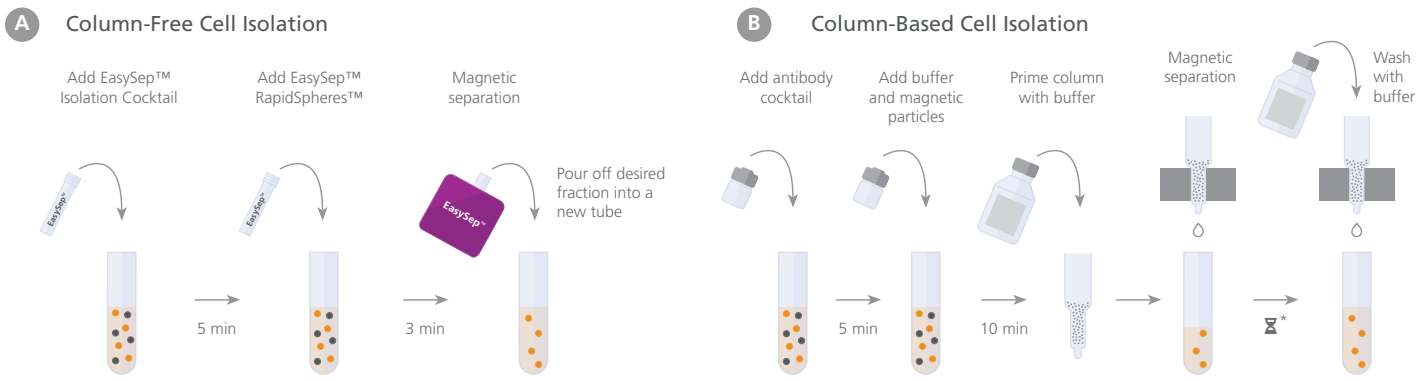

Figure 1. Comparison of Two Different Immunomagnetic Cell Isolation Protocols

Steps involved in the isolation of human T cells by negative selection using two different immunomagnetic cell isolation technologies are shown: (A) column-free EasySep™ technology (time taken may vary depending on the type of magnet used) and (B) a commercially available column-based technology (*elution time varies).

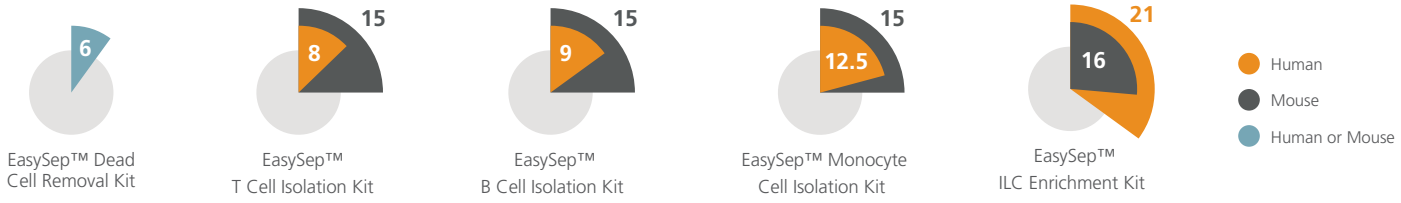

Figure 2. Cell Isolation Protocol Lengths

Time taken (in minutes) to isolate cells using select EasySep™ kits.

Benefits of an Efficient Workflow

Ensure that your research has a strong foundation by adopting an efficient cell isolation technology like EasySep™ as the first step in your experimental workflow.

- Fast protocols preserve cell quality and allow you to get to your downstream applications sooner.
- Robust and optimized protocols minimize variability between samples and allow you to isolate a large number of samples in a short period of time.

Maintain High Cell Viability

Column-free EasySep™ technology allows you to get to your downstream experiments quickly, without extended manipulation of your sample. With the simple, stress-free EasySep™ protocol, cells remain highly viable after separation (Figure 3).

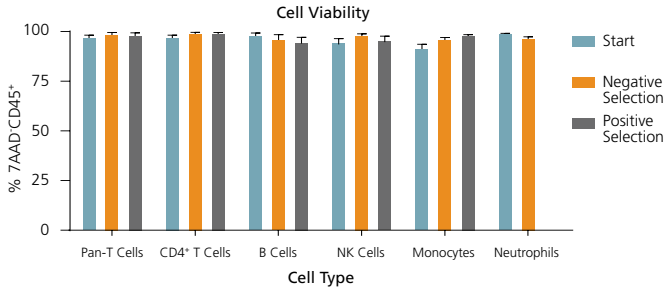

Figure 3. Cells Isolated Using EasySep™ Show Comparable Viability to Starting Samples

Immune cells were isolated from processed leukapheresis or peripheral blood samples using EasySep™ positive selection or negative selection kits. Pre- and post-isolation samples were stained with the cell viability dye 7-AAD and appropriate cell surface markers, and were assessed by flow cytometry. Cells isolated using EasySep™ showed no significant decrease in viability compared to the starting samples. Data shown as mean ± SEM; n = 3 - 7.

Minimize Protocol Optimization

Our scientists have carefully optimized EasySep™ protocols and reagents so you don't have to.

- Cocktails are titrated to ensure proper cell labeling and to avoid epitope blocking (Figure 4).
- Magnetic particles are titrated to minimize non-specific binding.
- Protocols are designed to ensure robust, optimal performance across samples.

> "EasySep™ allows me to pre-enrich my samples for FACS much faster and with less handling. Monocytes are so plastic and sensitive to external factors, and speed is essential when working with these cells. EasySep™ is the fastest separation kit out there."
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> **Sara M., PhD**
> **University of Queensland**

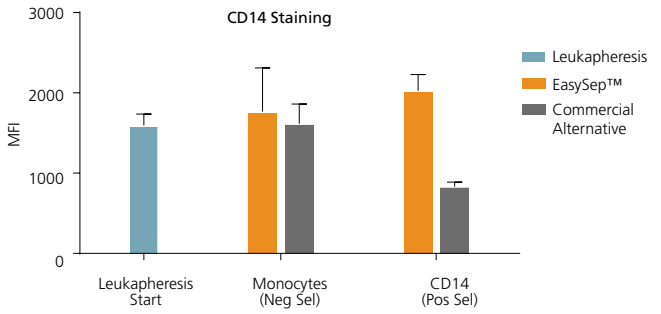
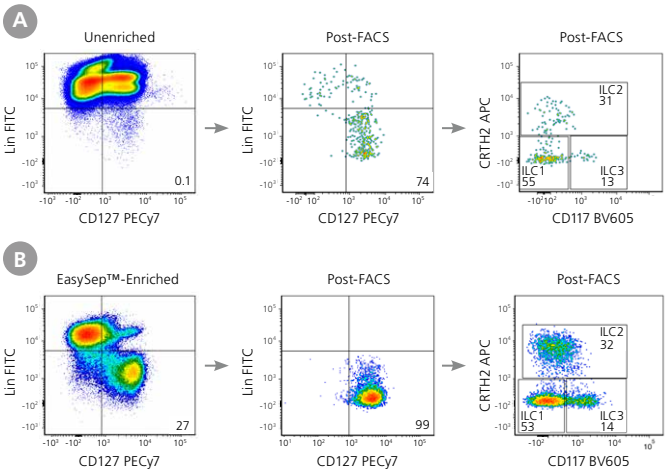


Figure 4. CD14 Epitopes Are Not Blocked Following Cell Isolation Using EasySep™

Cells were isolated by negative selection (Neg Sel) or positive selection (Pos Sel) methods using EasySep™ or a commercially available column-based technology. Isolated cells were stained using an anti-CD14 antibody (clone M5E2), and assessed by flow cytometry. EasySep™-isolated cells showed similar levels of CD14 staining (MFI) compared to unprocessed CD14+ cells (Leukapheresis Start). Data shown as mean ± SEM; n = 4 - 5.

Reduce or Eliminate Cell Sorting Time

By using EasySep™ as a pre-enrichment step when isolating rare or complex cell types, you can significantly reduce or eliminate the time you spend performing fluorescence-activated cell sorting (FACS; Figure 5) while maintaining the original subset ratios.



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	Start	Pre-FACS	Post-FACS		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 h)*
EasySep™-Enriched	2 x 10^9	27%	99%	3.7 x 10^5	30 min	12 min

*Time was extrapolated

Figure 5. Pre-Enrichment with EasySep™ Significantly Reduces the Time Required to Obtain Purified ILCs by FACS

Starting with a fresh leukapheresis sample, human innate lymphoid cells (ILCs) were isolated by fluorescence-activated cell sorting (FACS) 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.

Compatible with Gene Expression Profiling Assays

Pre-enriching your samples with EasySep™ can make your next-generation sequencing workflow more efficient by improving the sequencing coverage in your cells of interest, saving you time and money.

Cells isolated using EasySep™ kits are fully compatible with next-generation sequencing workflows including library preparation, amplification, and sequencing, resulting in high-quality reads (Table 1). Gene expression of CD4⁺ T cells isolated using EasySep™ are similar to the PBMC control (Figure 6), indicating that EasySep™ cell isolation protocols do not introduce artifacts that affect gene expression.

Table 1. Using the 10x Genomics Chromium™ Platform to Compare Single-Cell Gene Expression of EasySep™-Isolated CD4⁺ Cells to PBMC Controls

	PBMC Control	Positive Selection[a]	Negative Selection[b]	Positive Selection with Particle Release[c]
Reads Mapped to Genome (%)	88.70%	90.60%	88.40%	91.10%
Valid Barcodes (%)	96.90%	97.00%	96.90%	96.90%
Q30 Bases in Barcode (%)	96.70%	96.80%	96.70%	96.80%

Human CD4⁺ cells were isolated by negative selection or positive selection using a variety of EasySep™ kits containing different types of magnetic particles:
[a] EasySep™ Dextran RapidSpheres™
[b] EasySep™ D Magnetic Particles
[c] EasySep™ Releasable RapidSpheres™

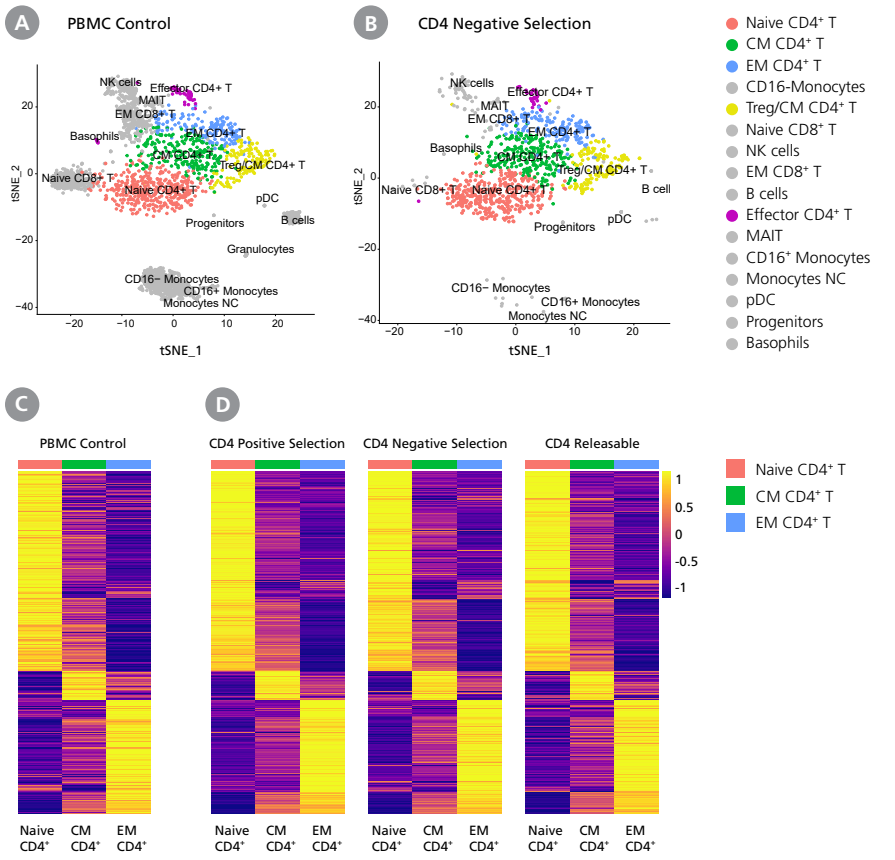

Figure 6. Gene Expression Profiles of EasySep™-Isolated CD4⁺ T Cells Are Similar to PBMC Control

(A,B) tSNE plots were generated using data from (A) PBMC control or (B) cells isolated using the EasySep™ Human CD4⁺ T Cell Enrichment Kit. CD4⁺ T cell clusters are colored as indicated in the legend. (C,D) 500 genes were selected from a previously published list of CD4⁺ T cell signature markers (Zhang et al., 2018). Expression heatmaps were generated for CD4⁺ cells from (C) PBMC control and (D) cells isolated using the EasySep™ Human CD4 Positive Selection Kit II (Catalog #17852), EasySep™ Human CD4⁺ T Cell Enrichment Kit (Catalog #19052), or the EasySep™ Release Human CD4 Positive Selection Kit (Catalog #17752). The average expression was calculated within each sample for three CD4⁺ T cell clusters identified by Seurat (naïve, central memory, and effector memory CD4⁺ T cells).

Efficiency Without Compromising Functionality

EasySep™ kits are designed with cell functionality in mind. During product development, our scientists characterize isolated cells using a variety of relevant functional assays to ensure that isolated cells are representative of their original state.

Isolated Cells Are Responsive to Stimuli

Cells isolated using EasySep™ are functional and respond appropriately to a variety of stimuli (Figures 7 and 8).

- Isolated cells remain unactivated in the absence of stimulation.
- Stimulated cells show an activated phenotype and produce appropriate cytokines.

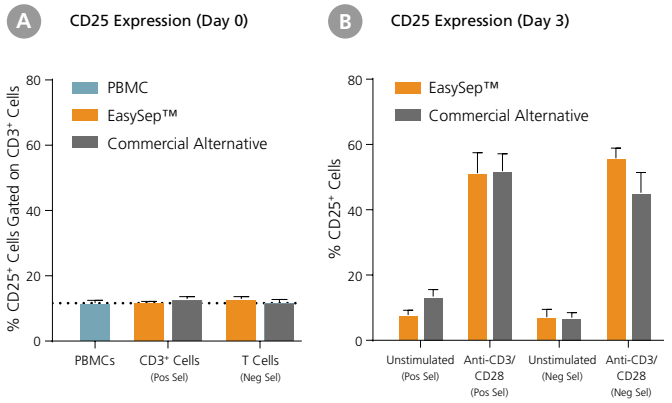

Figure 7. T Cells Isolated Using EasySep™ Show an Appropriate Activation Phenotype

Human T cells were isolated from peripheral blood mononuclear cells (PBMCs) by negative selection (Neg Sel) or positive selection (Pos Sel) using EasySep™ or a commercially available column-based system. Isolated T cells were assessed for CD25 expression immediately after isolation (Day 0) and after 3 days in culture with or without anti-CD3/CD28 stimulation. (A) At Day 0, isolated T cells expressed similar levels of CD25 compared to CD3+ cells in unmanipulated PBMCs. (B) At Day 3, cells remained unactivated in the absence of stimulation, while stimulated cells expressed the activation marker CD25. Data shown as mean ± SEM; n = 3.

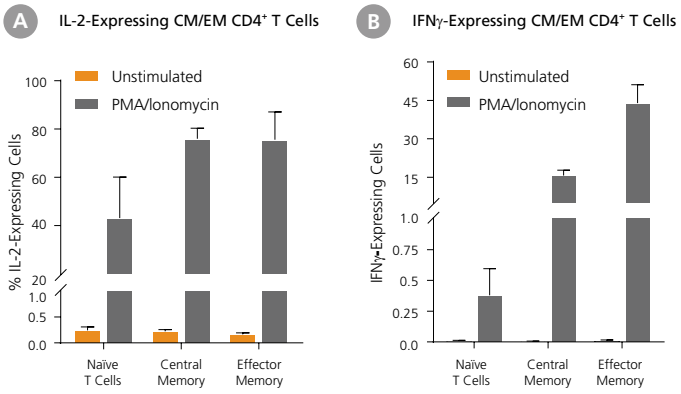

Figure 8. Central Memory and Effector Memory CD4+ T Cells Isolated Using EasySep™ Produce Cytokines When Stimulated

T cell subsets were isolated using the EasySep™ Human Central and Effector Memory CD4+ T Cell Isolation Kit. Isolated cells were cultured in medium with or without PMA and ionomycin stimulation for 4 hours. T cell subsets were assessed for the expression of (A) IL-2 and (B) IFNγ by intracellular flow cytometry. In the absence of stimulation, T cell subsets produced low levels of IL-2 and IFNγ. (A) When stimulated, central memory (CM) and effector memory (EM) CD4+ T cell subsets showed significantly higher IL-2 expression compared to naïve T cells. (B) EM CD4+ T cells expressed higher levels of IFNγ than CM CD4+ T cells when stimulated. Both CM and EM CD4+ T cell subsets showed higher IFNγ expression than naïve T cells. Data shown as mean ± SEM; n = 3.

Isolated Cells Differentiate and Mature As Expected

EasySep™-isolated cells express appropriate surface markers following differentiation and maturation (Figure 9).

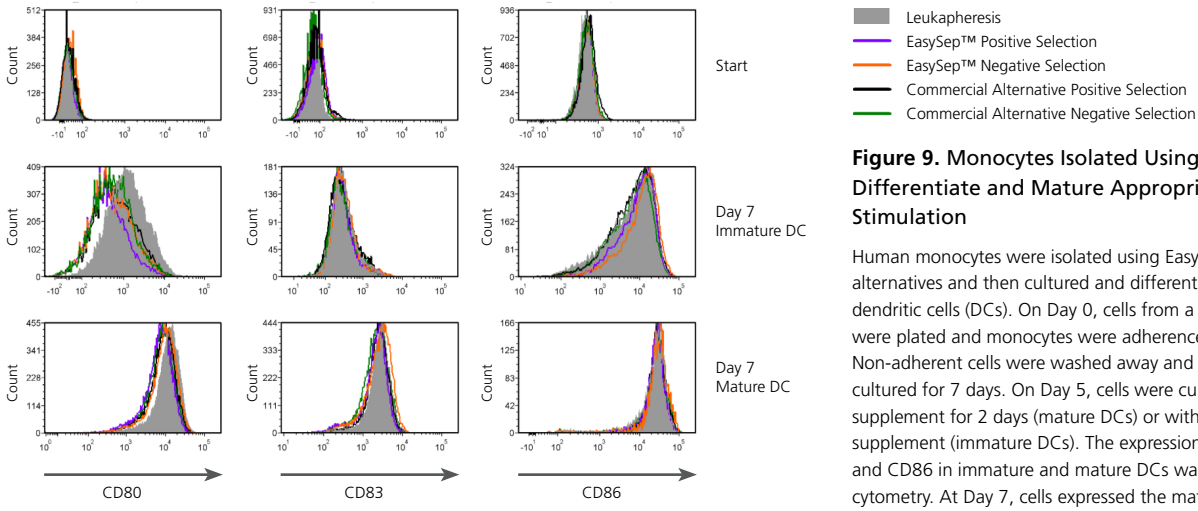

Figure 9. Monocytes Isolated Using EasySep™ Differentiate and Mature Appropriately Upon Stimulation

Human monocytes were isolated using EasySep™ or commercial alternatives and then cultured and differentiated into mature dendritic cells (DCs). On Day 0, cells from a leukapheresis sample were plated and monocytes were adherence-selected for 2 hours. Non-adherent cells were washed away and adherent cells were cultured for 7 days. On Day 5, cells were cultured with maturation supplement for 2 days (mature DCs) or without maturation supplement (immature DCs). The expression of CD80, CD83, and CD86 in immature and mature DCs was determined by flow cytometry. At Day 7, cells expressed the mature DC markers CD80, CD83, and CD86.

Efficiency Without Compromising Performance

High Purity and Recovery

Optimized protocols allow for the efficient isolation of both human (Figure 10) and mouse (Figure 11) target cells with high purity and recovery.

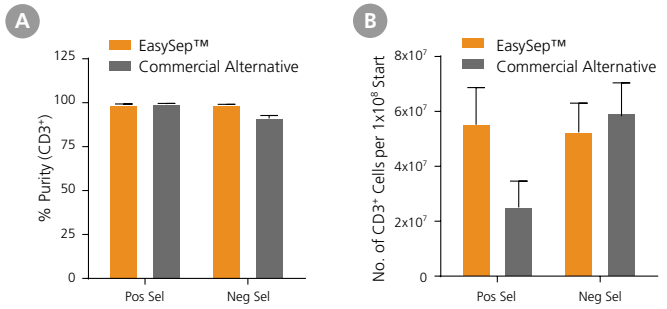

Figure 10. EasySep™ Yields Equivalent or Better Purity and Recovery of Human T Cells Compared to a Column-Based Technology

T cells were isolated by negative selection (Neg Sel) or positive selection (Pos Sel) using EasySep™ or a commercially available column-based technology. EasySep™ isolation yielded comparable or better (A) purity and (B) recovery to the other commercially available system. Data shown as mean ± SEM; n = 3.

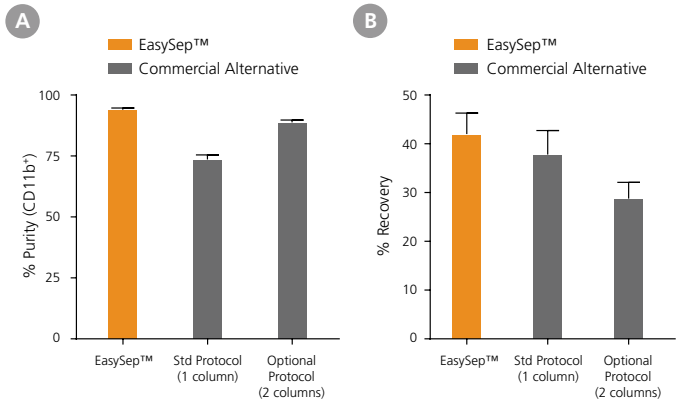

Figure 11. EasySep™ Yields Equivalent or Better Purities and Recoveries of Mouse CD11b$^+$ Cells Compared to a Column-Based Technology

CD11b$^+$ cells were isolated from mouse spleen by positive selection using EasySep™ or a commercially available column-based kit. The commercial alternative's protocol was followed using their standard protocol or their optional "high purity" protocol, which recommended an additional column. EasySep™ isolation yielded comparable or better (A) purity and (B) recovery. Data shown as mean ± SEM; n = 4 - 6.

"EasySep™ has allowed us to enrich for rare populations of cells ...[and] allowed us to accurately and efficiently phenotype our cells of interest. EasySep™ helped our lab analyze rare polyclonal antigen-specific CD4$^+$ T and B cell responses in an accurate and cost-effective manner."

Jessica Y., PhD Candidate
University of Minnesota

"We can quickly isolate T cells with high purity and great viability in order to culture them in vitro or do further analysis, like chromatin IP [immunoprecipitation] or RNA sequencing. We have great purities, so EasySep™ works great for these isolations and analyses."

Kyle B., PhD Candidate
University of British Columbia

A Smarter Way to Isolate Cells

Frequently Asked Questions

What cells can I isolate with EasySep™?

We have over 260 EasySep™ cell separation kits and protocols to isolate more than 80 different cell types from a variety of species and sample sources. Some EasySep™ kits even allow you to use your own antibody so you can isolate virtually any cell type you want. We also offer custom EasySep™ kits and will work with you to isolate your cell type of interest.

Is EasySep™ a well accepted method to isolate cells?

Absolutely! Since its launch in 2002, EasySep™ has been used by scientists all over the world to publish over 9,000 peer-reviewed articles.

Will it be easy to switch to EasySep™?

Like any new technology, you would want to test it in your own lab first. This may seem like extra work initially, but switching to more efficient technologies will benefit you and your entire lab for years to come. Contact your local sales representative or visit **www.EasySep.com** to request a sample.

What about cost?

With fewer consumables you will likely pay less than what you're paying right now. Depending on your current technology, EasySep™ may also reduce wasted materials and environmental cost.

New and Popular EasySep™ Kits

Kit	Catalog #	Automatable
EasySep™ Direct Human Total Lymphocyte Isolation Kit	19655	✓
EasySep™ Human CD4+CD127lowCD25+ Regulatory T Cell Isolation Kit	18063	✓
EasySep™ Human Monocyte Isolation Kit	19359	✓
EasySep™ Human NK Cell Isolation Kit	17955	✓
EasySep™ Human Pan-Extracellular Vesicle Positive Selection Kit	17891	-
EasySep™ Human Whole Blood and Bone Marrow CD138 Positive Selection Kit II	17887	✓
EasySep™ Mouse CD45 Positive Selection Kit	18945	-
EasySep™ Mouse CD4+ T Cell Isolation Kit	19852	✓
EasySep™ Mouse T Cell Isolation Kit	19851	✓

Did you know you can automate your EasySep™ cell isolations? RoboSep™ integrates the speed and simplicity of EasySep™ in a fully automated cell isolation system, allowing you to perform sequential or simultaneous cell isolations from up to 16 samples at once. Learn more at **www.RoboSep.com**

Why Use EasySep™ to Isolate Cells?

FAST AND EASY. Isolate cells in as little as 8 minutes with a simple pour.

HIGH PURITY. Achieve up to 99% cell purity with high recoveries.

GENTLE. Obtain viable, functional cells without the need for columns and washes.

VERSATILE. Isolate cells from various sample sources, including whole blood and leukapheresis samples.

PROVEN. Gain confidence with a tested and proven method used in over 9,000 peer-reviewed publications.

EasySep™ Direct

Isolate highly purified cells straight from whole blood in as little as 20 minutes without density gradient centrifugation, sedimentation, RBC lysis or other pre-processing steps that can alter cellular function.

EasySep™ Release

Positively select immune cells and then release bound magnetic particles to obtain highly purified and particle-free cells.

Our goal is to help you drive science forward, which is why we are committed to developing high-quality, efficient products for your research. Isolate your cells in a smarter, more efficient way with EasySep™. Try it in your own lab and see the advantage yourself. **www.EasySep.com**

References

1. Zhang L et al. (2018) Lineage tracking reveals dynamic relationships Of T cells in colorectal cancer. Nature 564(7735): 268–272.

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