Fast and Easy Immunomagnetic Positive Selection of PE- or Biotin-Conjugated Antibody Labeled Cells with Releasable RapidSpheres™ Results in Highly Purified, Functional and Particle-Free Cells

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Abstract

Immunomagnetic cell isolation is a cost-effective and gentle method of obtaining purified cell populations. However, isolating novel or rare cell populations often relies on relatively time consuming and expensive cell sorting techniques due to the lack of off-the-shelf immunomagnetic cell isolation kits. To bridge this gap, we have developed the EasySep™ Release isolation kits for PE- or biotin-conjugated antibodies. EasySep™ Release uses magnetic particles with low non-specific binding characteristics that can be rapidly removed from isolated cells after positive selection.

The system is highly customizable, targeting cells through the use of any biotin- or PE-conjugated primary antibody or ligand. Starting with a variety of sample materials, including single-cell suspensions from mouse spleen, lymph nodes, lungs, rat spleen, or human peripheral blood mononuclear cells (PBMCs), the EasySep™ Release kits have consistently demonstrated purities above 85%.

The EasySep™ Release technology can also be used to isolate subsets of cells when used in combination with our other cell separation products. For example, functional and particle-free mouse CD25$^+$ or CD304$^+$CD4$^+$ regulatory T cells were isolated by using our EasySep™ Release Mouse PE Positive Selection Kit and EasySep™ Mouse CD4$^+$ T Cell Isolation Kit. We have used EasySep™ Release Mouse kits with EasySep™ Dextran RapidSpheres™ to isolate CD4$^+$CD8$^+$ mouse thymocytes (double positive selection) and to isolate mouse lung epithelial cells by EpCAM positive selection, followed by the removal of contaminating leukocytes via CD45 depletion.

These kits offer a customizable and cost-effective magnetic isolation approach for the isolation of virtually any cell population.

Methods

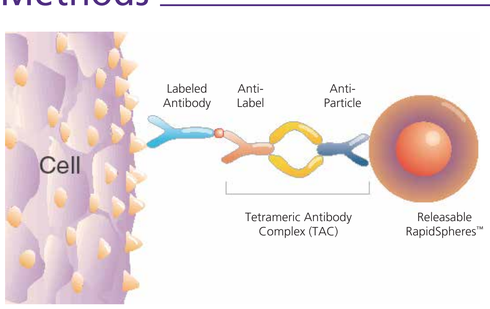
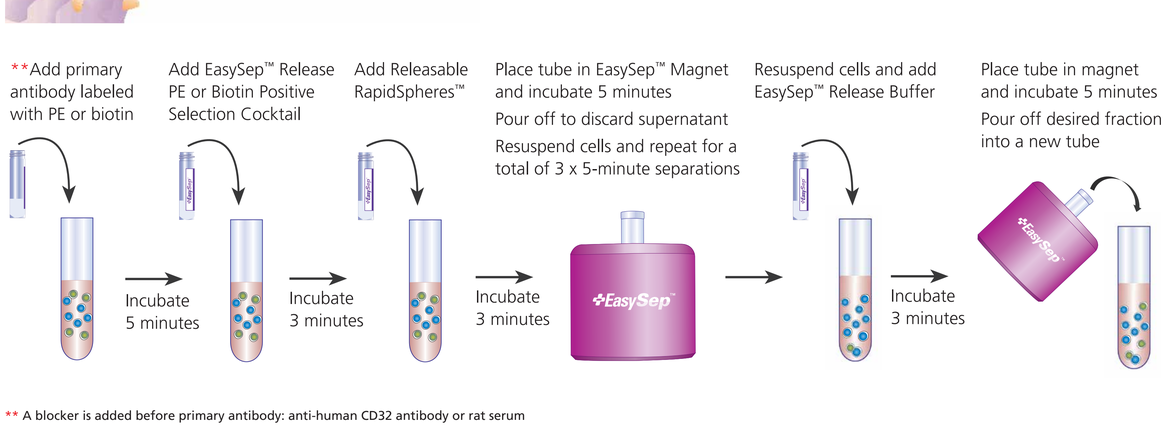


Figure 1. Schematic Overview of EasySep™ Release Technology. Cells of interest are targeted for selection using a cocktail of tetrameric antibody complexes directed to a PE- or biotin-conjugated antibody. The antibody complexes link targeted cells to the Releasable RapidSpheres™ magnetic particles. Labeled cells are then isolated using a hand-held magnet and the particles are removed using the EasySep™ Release Buffer.



** A blocker is added before primary antibody: anti-human CD32 antibody or rat serum

Figure 2. Typical EasySep™ Release protocol for isolation of particle-free cells in less than 30 minutes. Prior to separation, single-cell suspensions of PBMCs from leukapheresis samples, cell-strained mouse or rat splenocytes, or mouse lungs digested with Liberase™ and DNase I Solution (Catalog #07900) were prepared and resuspended at concentrations of 5 x 10^7 - 1 x 10^8 cells/mL in PBS containing 2% FBS and 1 mM EDTA. Cells were then labeled with biotin- or PE-conjugated primary antibodies for 5 minutes at room temperature, followed by the addition of the EasySep™ Release Positive Selection reagents.

Results

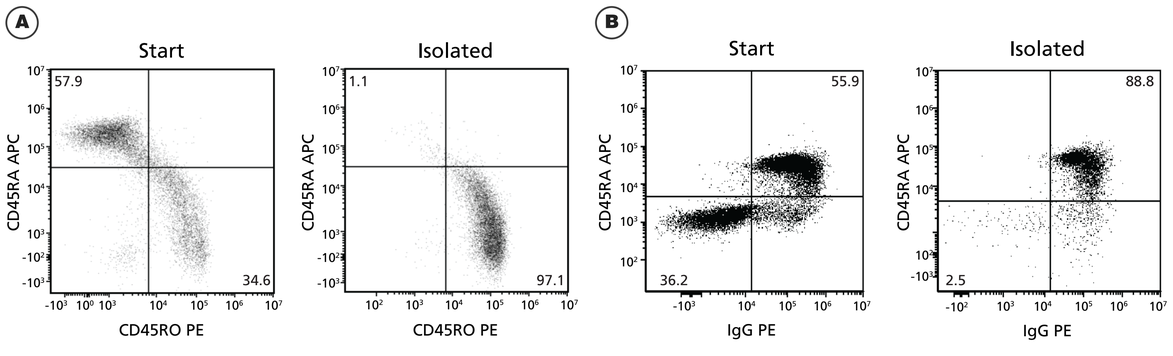


Figure 3. Single-step positive selection with EasySep™ Release. A) Human CD45RO$^+$ cells were isolated from PBMCs using a biotin-conjugated anti-human CD45RO antibody and EasySep™ Release Human Biotin Positive Selection Kit. The purities of the start and final isolated fractions were 34.6% and 97.1%, as assessed with PE-conjugated CD45RO and APC-conjugated anti-human CD45RA antibodies. The final recovery was 40%. **B)** Rat IgG$^+$ cells were isolated from spleen using 2 µg/mL of polyclonal PE-conjugated anti-rat IgG antibody and EasySep™ Release PE Positive Selection Kit. Cells were labeled with APC-conjugated anti-rat CD45RA antibody, a rat B cell marker. The purities of the start and final isolated fractions were 55.9% and 88.8%, respectively and the final recovery was 22%.

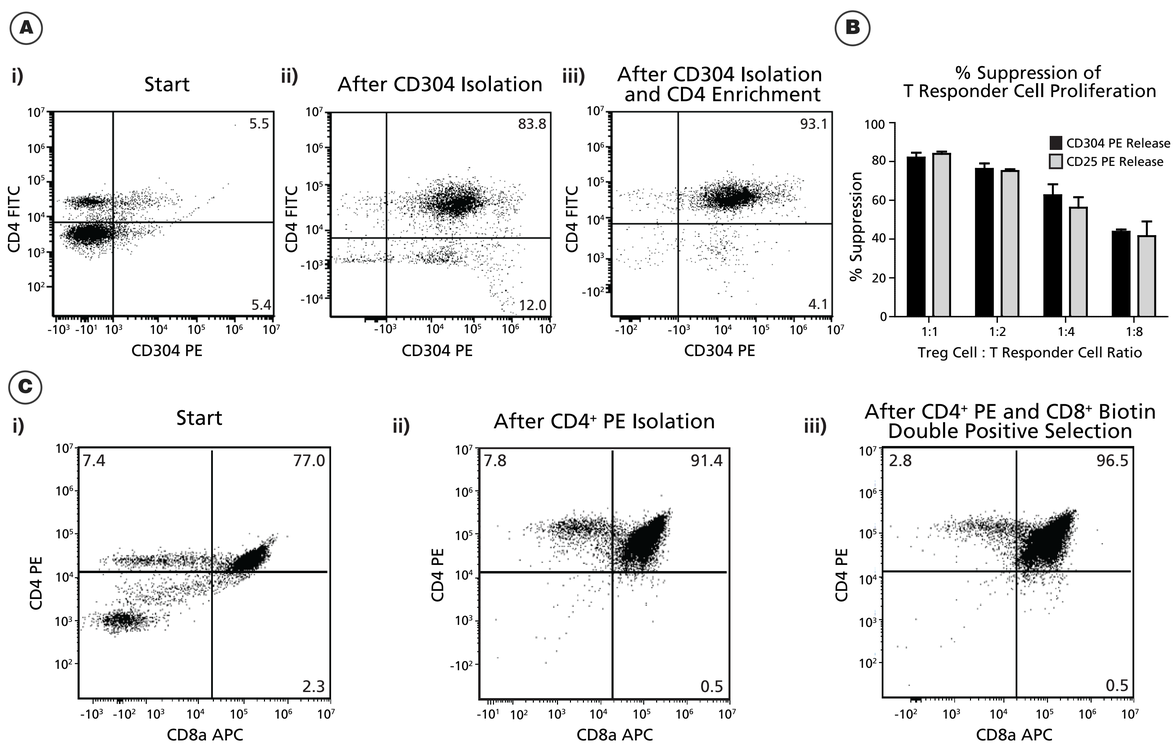


Figure 4. Sequential isolation strategies to isolate functional subsets of T cells A) An example of a sequential selection with EasySep™ Release Mouse PE Positive Selection Kit, followed by a negative enrichment step to isolate highly pure CD4$^+$CD304$^+$ regulatory T cells (Treg). i) Start cells were labeled with PE-conjugated anti-mouse CD304 antibody and ii) isolated using the EasySep™ Release Mouse PE Positive Selection Kit. iii) Highly pure CD4$^+$ cells were then isolated using the EasySep™ Mouse CD4$^+$ T Cell Isolation Kit (Catalog #19852). Purity was assessed using FITC-conjugated anti-mouse CD4 antibody. The purities of the start and final isolated fractions were 5.5% and 93.1%, respectively. The final recovery was 29%. **B)** Isolated CD4$^+$CD25$^+$ and CD4$^+$CD304$^+$ Tregs suppressed proliferative responses of CD4$^+$ responder T cells (CD4$^+$CD25$^-$), when cultured for 4 days in the presence of anti-mouse CD3 and anti-mouse CD28 stimulating antibodies. Responder T cells were monitored for their proliferative responses using a flow cytometry-based assay. Percent suppression was determined by assessing the level of proliferation relative to responder T cells cultured without regulatory T cells (N = 4; + SD). **C)** Sequential double positive selection with EasySep™ Release Mouse PE Positive Selection Kit, followed by selection with an anti-biotin isolation cocktail and EasySep™ Dextran RapidSpheres™. i) Start cells from mouse thymi were labeled with PE-conjugated anti-mouse CD4 antibody and ii) isolated using the EasySep™ Release Mouse PE Positive Selection Kit. iii) Highly pure CD4$^+$CD8$^+$ cells were then isolated using a biotin-conjugated anti-mouse CD8a antibody, an anti-biotin selection cocktail, and EasySep™ Dextran RapidSpheres™. Purity was assessed using PE-conjugated anti-mouse CD4 and APC-conjugated anti-mouse CD8a antibodies. The purities of the start and final isolated fractions were 77.0% and 96.5%, respectively. The final recovery was 30%.

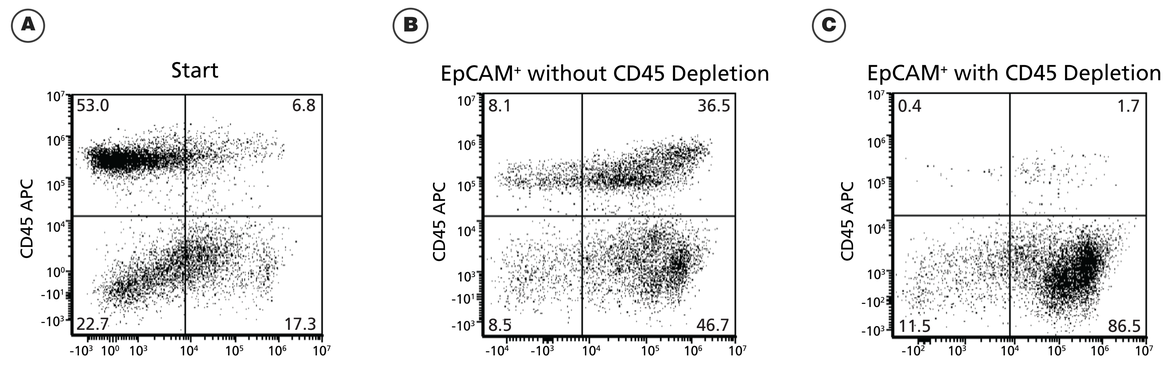


Figure 5. Isolation of epithelial cells from mouse lungs using EasySep™ Release Mouse PE positive selection. A) Single-cell suspensions from digested mouse lungs were labeled with PE-conjugated anti-mouse CD326 (EpCAM) antibody. **B)** EpCAM$^+$ cells were isolated with the EasySep™ Release Mouse PE Positive Selection Kit, but contained many contaminating leukocytes. **C)** Contaminating leukocytes were depleted using a biotin-conjugated anti-mouse CD45 antibody, an anti-biotin depletion cocktail, and EasySep™ Dextran RapidSpheres™. Purity was assessed by labeling with APC-conjugated anti-mouse CD45, PE-conjugated anti-mouse CD326 (EpCAM), and a viability dye 7AAD. The purities of the start and final isolated fractions were 17.3% and 86.5%, respectively. The final recovery was 15%.

Summary

- EasySep™ Release is a fast and easy cell isolation method for particle-free positive selection using the novel Releasable RapidSpheres™ magnetic particle technology
- The EasySep™ Release procedure is compatible with a wide range of processing volumes and magnets
- EasySep™ Release PE and Biotin kits offer the flexibility to isolate cells using any PE or biotin-conjugated primary antibodies
- The method provides flexibility to isolate almost any cell type from various tissues and species and accommodates a wide-range of sample volumes
- EasySep™ Release PE and Biotin kits can be used in sequential strategies for the isolation of purified and functional unique immune cell subsets