Fast and easy immunomagnetic positive selection of PE- or biotin-conjugated antibody labeled cells with Releasable RapidSpheres™ results in functional, pure, and particle-free cells

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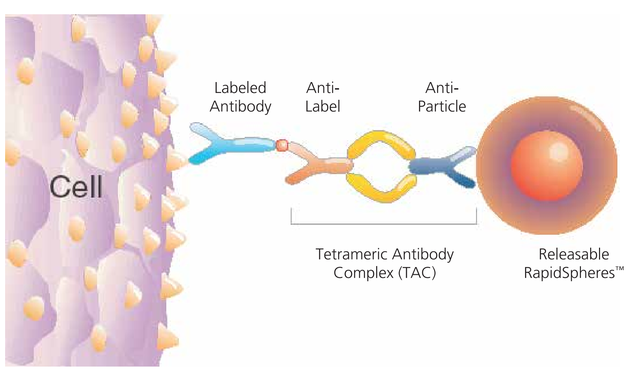
Abstract

Immunomagnetic cell isolation has long been recognized as a cost-effective and gentle method of obtaining purified cell populations. However, isolating novel or rare cell populations often relies on time consuming cell sorting techniques due to the lack of off-the-shelf immunomagnetic cell isolation kits. To bridge this gap, we developed the EasySep Release isolation kits for PE- or biotin-conjugated antibodies. EasySep™ Release incorporates our latest magnetic particles that have low non-specific binding characteristics, and are rapidly removed from isolated cells after positive selection using a simple reagent.

The system is highly adaptable, targeting cells through the use of any biotin or PE-conjugated primary antibody or ligand. We have thoroughly tested the EasySep™ Release kits with biotin and PE-conjugated to antibodies to human CD19 and human CD3, obtaining 93% to 98% purity and 35% to 60% recovery. Additionally, the technology has been used to isolate numerous other human immune cell subsets such as CD4, CD45RO, CD20, and HLA-DR expressing cells from leukopak samples and cryopreserved peripheral blood mononuclear cells. The EasySep™ Release Biotin and PE positive selection kits have also undergone preliminary testing on rat splenocytes, and various mouse tissues such as spleen, thymus, lung, and liver. EasySep™ Release technology is amenable to sequential isolations, which can enable the isolation of immune cell subsets with complex cell surface marker phenotypes. Ultimately, this will offer a gentle, and cost-effective, magnetic particle-free cell isolation method for the isolation of virtually any cell population.

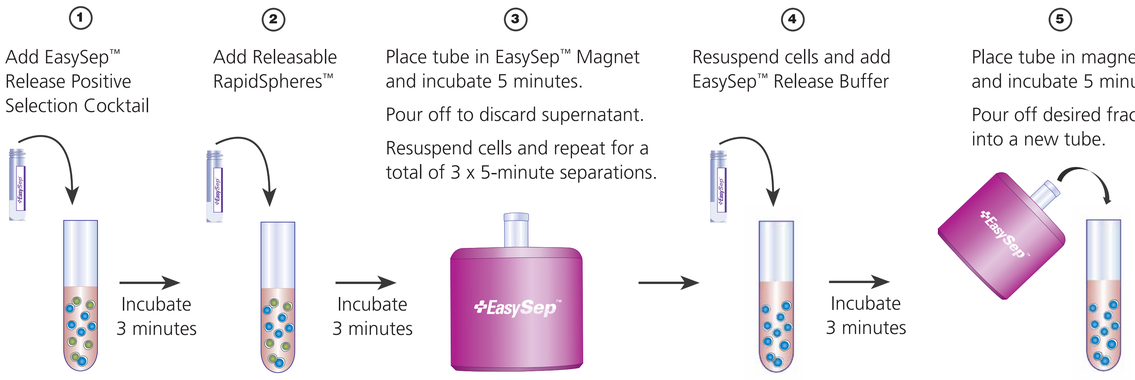
Methods

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-labeled antibody. The antibody complexes link targeted cells to the Releasable RapidSpheres™ magnetic particles. Labeled cells are then purified using a hand-held magnet and the particles are removed using a mild dissociation reagent.

Figure 2. Typical EasySep™ Release protocol for isolation of particle-free cells in less than 30 minutes



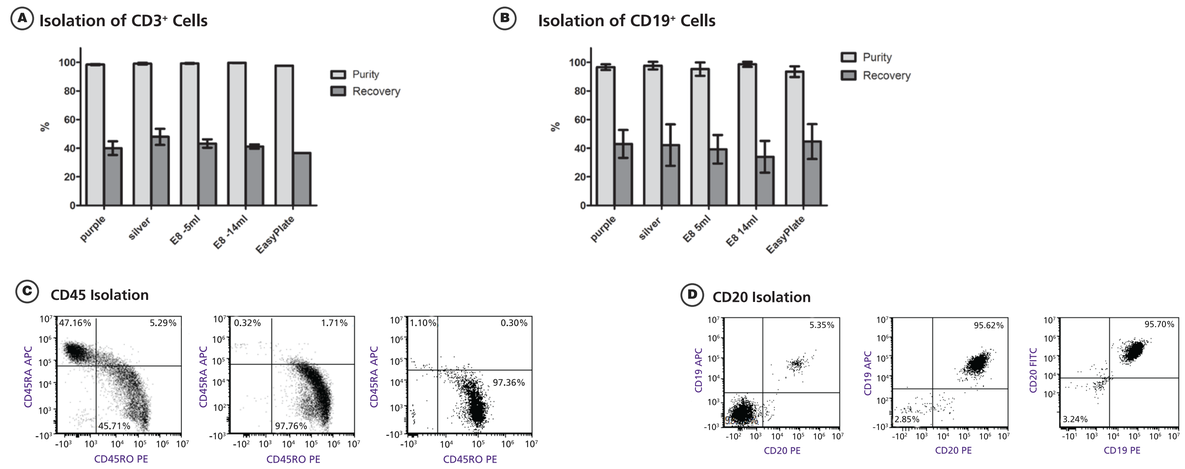
Prior to separation, single-cell suspensions of human peripheral blood mononuclear cells (PBMCs) from Leukopak samples, cell-strained mouse or rat splenocytes, or mouse lungs digested with Liberase™ and DNase I Solution (Catalog #07900) were prepared and resuspended at concentrations of 5 x 10^7 - 1 x 10^8 cells/mL in PBS containing 2% FBS and 1 mM EDTA. Cells were then labeled with the indicated concentrations of biotinylated or PE-conjugated primary antibodies for 5 minutes at room temperature, followed by the addition of the EasySep™ Positive Selection Cocktails.

Figure 3. Overview of EasySep Release™ magnet compatibility

The EasySep™ Release procedure is compatible with a wide-range of processing volumes using the (A) EasySep™ Magnet (Catalog #18000), (B) "The Big Easy" EasySep™ Magnet (Catalog #18001), (C) Easy 50 EasySep™ Magnet (Catalog #18002), (D) EasyPlate EasySep™ Magnet (Catalog #18102) and the (E) EasyEights™ EasySep™ Magnet (Catalog #18103).

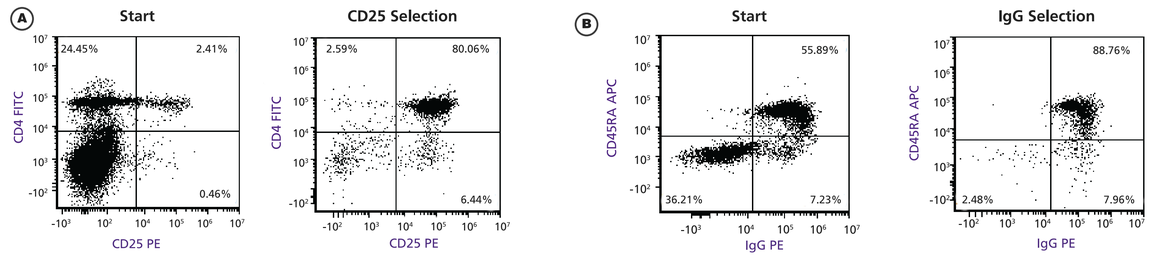
Results

Figure 4. Performance of Human PE and Biotin Positive Selection Kits



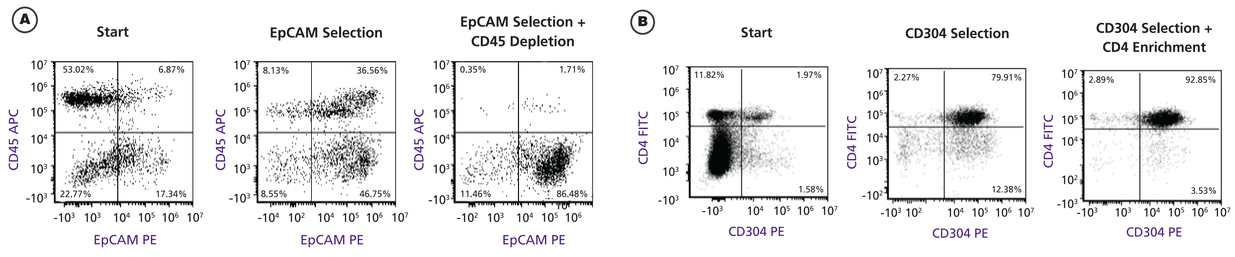
Human PE Positive Selection Kit was tested over 5 magnet platforms, using either PE-labeled anti-human CD3 (A) or anti-human CD19 antibodies (B). Purity was consistently over 90%, and recovery averaged between 30% - 60% for both cell types. (C) Human $CD45^+$ memory cells were isolated from PBMCs by labeling with 1 µg/ml of PE- labeled anti-human CD45RO (clone UCHL1) and 50 µL/mL of Human PE Selection Cocktail (middle panel), or 0.3 µg/mL of biotin-labeled anti-human CD45RO and 100 µL/mL of Human Biotin Selection Cocktail (Right Panel). Cells were subsequently stained for expression of CD45RA (APC) and CD45RO (PE). Recovery of PE-labeled $CD45RO^+$ cells was 31%, while biotin-labeled $CD45RO^+$ cells was 40%. (D) Human $CD20^+$ expressing B cells were isolated from PBMCs by labeling with 1 µg/mL PE-labeled anti-human CD20 (clone 2H7) and 25 µL/mL of Human PE Selection Cocktail (middle panel), or 0.3 µg/mL of biotin-labeled anti-human CD20 and 100 µL/mL of Human Biotin Selection Cocktail (right panel). Cells were subsequently stained for the expression of CD20 (FITC or PE) and CD19 (PE or APC). Recovery of PE-labeled CD20 cells was 30%, while biotin-labeled cells was 40%.

Figure 5. Isolation of Rat lymphocytes with EasySep™ Release PE Positive Selection



(A) Rat $CD25^+$ cells were isolated from spleen using 2 µg/mL PE-labeled anti-rat CD25 (clone OX-39) antibody and EasySep™ Release PE Positive Selection Kit. Cells were co-stained with FITC labeled anti-rat CD4. Following isolation, cells were 86% for CD25 and 80% pure for $CD4^+CD25^+$ cells. Recovery of $CD25^+$ cells was 25%. B) Rat IgG^+ cells were isolated from spleen using 2µg/mL of polyclonal PE-labeled anti-rat IgG antibody and EasySep™ Release PE Positive Selection Kit. Cells were co-stained with APC labeled anti-rat CD45RA, a rat B cell marker. Recovery of $IgG^+CD45RA^+$ cells was 22%, and purity was 88%.

Figure 6. Isolation of Mouse Cells with EasySep™ Release PE Positive Selection and Sequential Selection Strategies



Mouse epithelial cells were isolated from mouse lungs using 2 µg/mL PE-labeled anti-mouse CD326 (EpCAM) and EasySep™ Release PE Positive Selection Kit. Cells were firstly labeled with PE-labeled anti-mouse CD326 and biotin-labeled CD45 antibodies. $EpCAM^+$ cells were positively selected using EasySep™ Release PE Positive Selection Cocktail, and subsequently, $CD45^+$ contaminating leukocytes were depleted using an EasySep™ Biotin Depletion Cocktail and EasySep™ Dextran Rapidspheres™. Isolated cells were 86% pure, with a recovery of 12%. All viable cells were assessed using the viability dye 7AAD. B) Mouse $CD4^+CD304^+$ cells were isolated from mouse spleen using 3 µg/mL PE-labeled CD304 (Neuropilin-1) and EasySep™ Release PE Positive Selection Kit followed by EasySep™ Mouse $CD4^+$ T cell enrichment using EasySep™ Streptavidin RapidSpheres™ (Cat #19852). Isolated cells were 92% pure, with a recovery of 29%.

Conclusions

- EasySep™ Release is a fast and easy cell isolation method which utilizes the novel Releasable RapidSpheres™ magnetic particle technology
- PE and Biotin EasySep™ Release offers the flexibility to isolate cells using any biotinylated or PE-conjugated primary antibodies
- The method also provides flexibility to isolate almost any cell type from various tissues and species, and accommodates a wide-range of sample volumes
- PE and Biotin EasySep™ Release can be used in sequential strategies, resulting in pure isolations of unique immune cell subsets

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