Releasable RapidSpheres™ enable immunomagnetic purification of highly viable and functional immune cells from complex tissues in less than 30 minutes

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Abstract

Recent advances in cellular therapy and diagnostics promise dramatic improvements in disease prevention and treatment. This research is driven by scientists studying how cells of the immune system communicate and relies on the success of cell based assays. However, it remains challenging and time consuming to purify large numbers of high quality cells from complex tissues. EasySep™ Release is a fast and easy cell isolation method which utilizes the Releasable RapidSpheres™. This novel magnetic particle technology improves cell purities by reducing contaminants and allows for gentle particle removal to mitigate potential interference in downstream assays. The 29 minute protocol involves first incubating target cells with antibody complexes followed by the Releasable RapidSpheres™. Next, labeled cells are purified using a handheld magnet. Particle-free purified cells are obtained by applying a mild dissociation reagent and a final magnetic separation. We validated EasySep™ Release by purifying T, B and NK cells from peripheral blood mononuclear cell samples containing 5 to 800 million cells. Cell purities were 98.6 ± 0.9% (n = 67) for CD3+ T cells, 96.3 ± 4.1% (n = 67) for CD4+ cells, 95.1 ± 4.8% (n = 67) for CD8+ T cells, 96.2 ± 2.7% (n = 51) for CD19+ cells and 94.1 ± 2.8% (n = 51) for CD56+ cells. Isolated cells were confirmed particle free, viable and functional. We obtained similar high performance using unprocessed leukapheresis samples of up to 5 billion cells, demonstrating excellent scalability and compatibility with more complex samples. Finally, we show how EasySep™ Release can be paired with commercially available antibodies or sequential separations to isolate almost any cell type, including those with a complex phenotype.

Methods

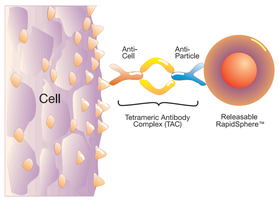

Figure 1: Schematic overview of EasySep™ Release technology. Cells of interest are targeted for selection using a cocktail of tetrameric antibody complexes directed to specific cell surface antigens. The antibody complexes link targeted cells to the Releasable RapidSpheres™ magnetic particles. Labeled cells are then purified using a hand-held magnet and the particles are removed using a mild dissociation reagent.

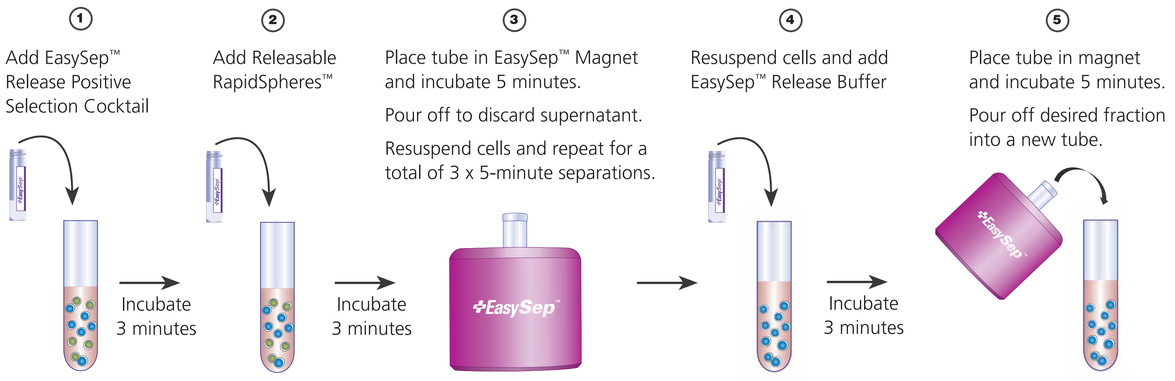

Figure 2: Typical EasySep™ Release protocol for isolation of particle-free cells in less than 30 minutes. Prior to separation, a single-cell suspension of peripheral blood mononuclear cells (PBMCs) from whole blood was prepared by centrifugation over Lymphoprep™ density gradient medium (Cat. #07801). Previously-frozen PBMCs were thawed and incubated with DNase I Solution (Cat. #07900) at a concentration of 100 µg/mL at room temperature for 15 minutes and filtered through a 40 µm cell strainer (Cat. #27305). After preparation, cells were resuspended at 1 x 10^8 cells/mL in PBS containing 2% FBS and 1 mM EDTA.

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 (Cat. #18000), **B)** "The Big Easy" EasySep™ Magnet (Cat. #18001), **C)** Easy 50 EasySep™ Magnet (Cat. #18002), **D)** EasyPlate EasySep™ Magnet (Cat. #18102) and the **E)** EasyEights™ EasySep™ Magnet (Cat. #18103).

Results

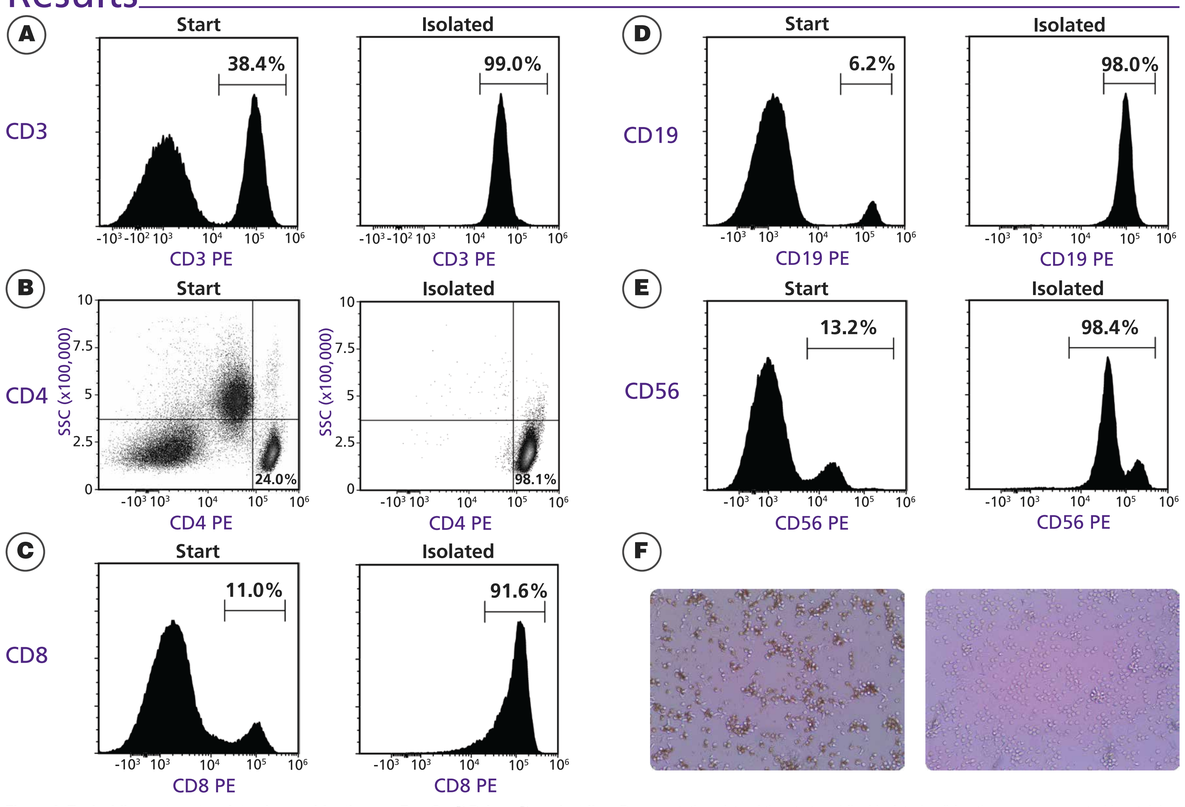

Figure 4: Typical flow-cytometry plots observed for the new EasySep™ Release™ product line. Representative plots of the start and final isolated cell fractions were generated by staining samples with non-blocking fluorochromes, flow-cytometry analysis, and a gating strategy based on viable cells and the cell marker of interest. Results are shown for the **A)** EasySep™ Release Human CD3 Positive Selection Kit (Cat. #17751), **B)** EasySep™ Release Human CD4 Positive Selection Kit (Cat. #17752), **C)** EasySep™ Release Human CD8 Positive Selection Kit (Cat. #17753), **D)** EasySep™ Release Human CD19 Positive Selection Kit (Cat. #17754), and **E)** EasySep™ Release Human CD56 Positive Selection Kit (Cat. #17755). **F)** Optical microscopy images showing purified CD8+ T cells targeted with the Releasable RapidSpheres™ (left) and particle-free cells obtained by gentle removal of the magnetic particles (right) during the EasySep™ Release protocol.

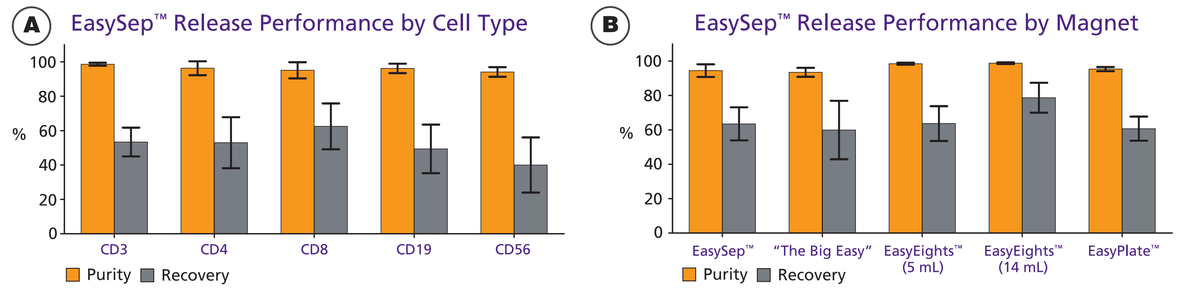

Figure 5: EasySep™ Release cell separation performance data. A) Overall performance data demonstrating the high cell purity (P) and recovery (R) obtained using EasySep™ Release to purify CD3+ T cells (P = 98.6 ± 0.9%, R = 53.4 ± 8.4%, n = 67), CD4+ T cells (P = 96.3 ± 4.1%, R = 53.0 ± 14.8%, n = 67), CD8+ T cells (P = 95.1 ± 4.8%, R = 62.5 ± 13.3%, n = 67), CD19+ B cells (P = 96.2 ± 2.7%, R = 49.4 ± 14.2%, n = 51) and CD56+ NK cells (P = 94.1 ± 2.8%, R = 40.0 ± 16.1%, n = 51). **B)** Similar high performance data was obtained across a wide range of processing volumes. Results for CD8+ T cells are shown for the EasySep™ Magnet (P = 94.5 ± 3.7%, R = 63.5 ± 9.6%, n = 14), "The Big Easy" EasySep™ Magnet (P = 93.5 ± 2.6%, R = 59.9 ± 17.0%, n = 5), EasyEights™ EasySep™ Magnet with a 5 mL tube (P = 98.5 ± 0.6%, R = 63.7 ± 10.1%, n = 9) and a 14 mL tube (P = 98.8 ± 0.5%, R = 78.7 ± 8.7%, n = 9) and the EasyPlate EasySep™ Magnet (P = 95.4 ± 1.2%, R = 60.7 ± 7.0%, n = 24). All experiments were performed across numerous donors.

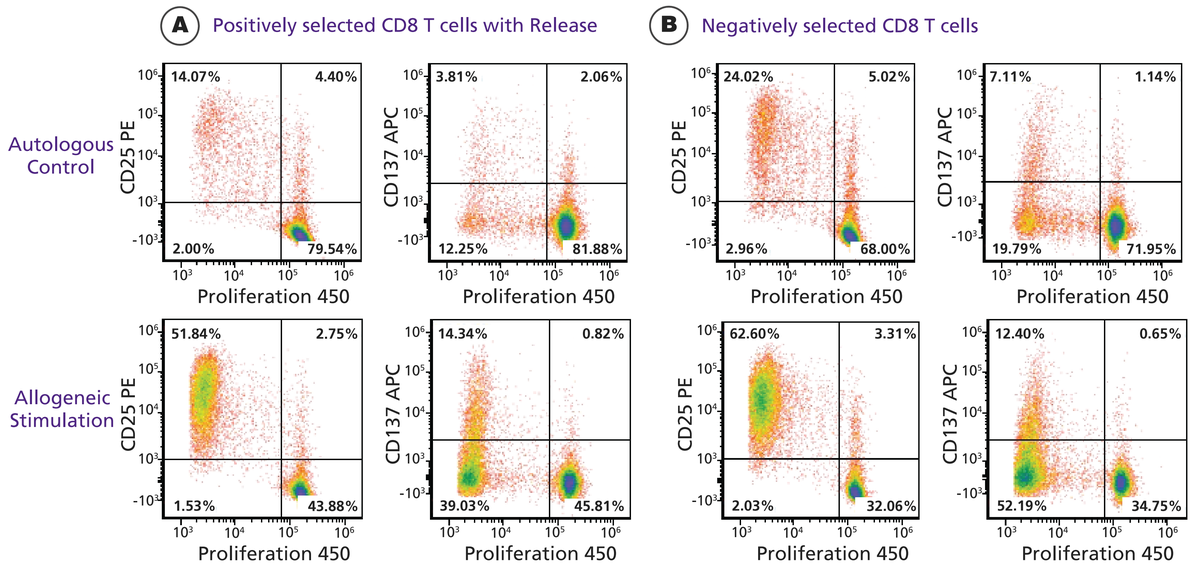

Figure 6: Cells isolated with EasySep™ Release are responsive to allogeneic stimulation. A) Cells were isolated with EasySep™ Release Human CD8 Positive Selection Kit (Cat. #17753) or **B)** using negative selection with EasySep™ Human CD8+ T Cell Isolation (Cat. #17953) and stimulated with mitomycin-C treated autologous control or allogeneic PBMCs for 5 days. Proliferation was measured using violet cell proliferation dye eFluor® 450 and activation of T cells were assessed with stains for human CD137 and human CD25. Cells were gated on viable CD8+CD3+ T cells. Cells stimulated with control PBMCs demonstrated small levels of spontaneous activation and division (<25%), while allogeneic-stimulated CD8+ cells underwent extensive proliferation and up-regulation of activation markers CD25 and CD137 (>50%).

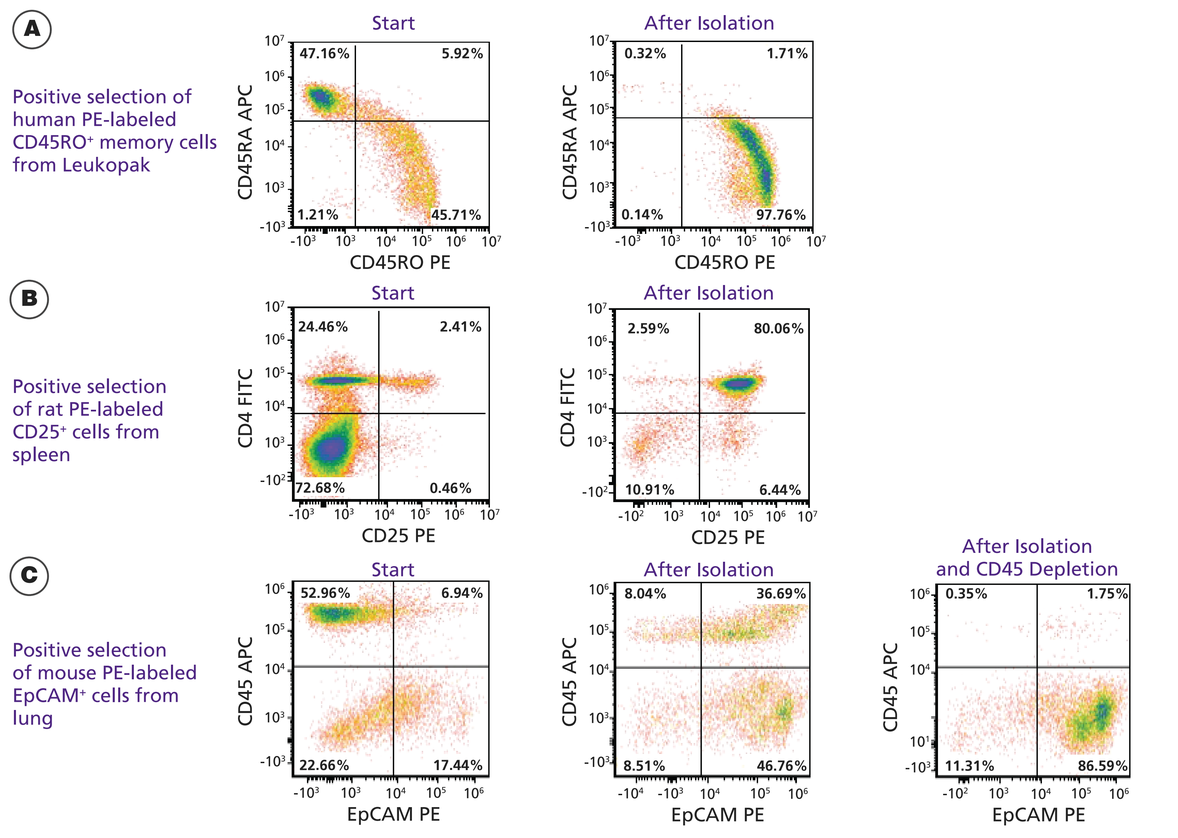

Figure 7: Flexibility of EasySep™ Release to cell type, tissue and species. A) Human CD45RO+ Memory Cells were isolated from processed leukapheresis samples using PE-labeled anti-human CD45RO antibody and EasySep™ Release PE Positive Selection Kit (Cat. #17654). Isolated cells were co-stained with CD45RA APC and demonstrated a purity of 97% CD45RO+CD45RA-. **B)** Rat CD25+ cells were isolated from spleen using PE-labeled anti-rat CD25 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. **C)** Mouse epithelial cells were isolated from mouse lungs using 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 then positively selected using EasySep™ Release, and subsequently, CD45+ contaminating leukocytes were depleted using an anti-biotin depletion cocktail. Isolated cells had a purity of 85%. All viable cells were assessed using the viability dye 7AAD.

Conclusions

- EasySep™ Release is a fast and easy cell isolation method which utilizes the novel Releasable RapidSpheres™ magnetic particle technology
- High purity target cells are obtained in under 30 minutes, remain viable and fully-functional and are confirmed particle-free
- The method also provides flexibility to isolate almost any cell type from numerous tissues and species, and accommodates a wide-range of sample volumes

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