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

Catherine L. Ewen, Andy J. Kokai, Samuel J. Clarke, Drew W. Kellerman, Martha N. Chambers, Mary Chan, Steven M. Woodside, Terry E. Thomas, Allen C. Eaves

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 nonspecific 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 95% to 98% purity and 95% to 99% 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 also have 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 purged using a handheld 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-stained 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 10⁶ - 1 x 10⁷ 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 Cocktail.

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 #18001), (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).

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

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

(A) Rat CD3⁺ cells were isolated from spleen using 2 µg/mL PE-labeled anti-rat CD3 (clone OKT3) antibody and EasySep™ Release PE Positive Selection Kit. Cells were co-stained with FITC-labeled anti-rat CD4. Following isolation, cells were 86% for CD3 and 80% pure for CD4⁻/CD3⁺ cells. Recovery of CD3⁺ cells was 29%. (B) Rat IgG₁ cells were isolated from spleen using 1 µg/mL PE-labeled anti-rat IgG₁ antibody and EasySep™ Release PE Positive Selection Kit. Cells were co-stained with APC-labeled anti-rat CD45RA, a B cell marker. Recovery of IgG₁/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™ Deraitin RapidSphere™. Isolated cells were 96% pure with a recovery of 13%. All viable cells were assessed using the viability dye 7AAD. (B) Mouse CD4⁺CD304⁺ cells were isolated from mouse spleen using 5 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 RapidSphere™ (Cat #18582). 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.