EasySep™ Kits for Immunomagnetic Purification of CD138+ cells from Mouse and Rat Samples

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Introduction

CD138 is found on several B cell subsets but is highly expressed on antibody-secreting cells (plasma cells and plasmablasts). Because CD138+ antibody-secreting cells represent a small fraction of the total B cell population (0.4 ± 0.1% (n = 52), 1.6 ± 1.1% (n = 43), and 0.6 ± 0.5% (n = 18) when using naïve C57BL/6 mice, immunized BALB/c mice, or naïve rat (Sprague Dawley and Wistar) spleens, respectively), pre-enrichment of CD138+ cells can improve downstream assays and processes such as hybridoma generation and screening. Therefore, we have developed a facile immunomagnetic method to isolate CD138+ cells from mouse and rat samples.

The column-free immunomagnetic method allows for isolation of CD138+ cells in less than 25 minutes from mouse or rat spleen, bone marrow, and other tissues using fast and simple EasySep™ technology. Bispecific antibody complexes are used to cross-link CD138+ cells to dextran-coated magnetic particles and the labeled cells are separated from the unwanted cells using an EasySep™ magnet. Single-cell suspensions of splenocytes and bone marrow cells do not require red blood cell lysis prior to isolation.

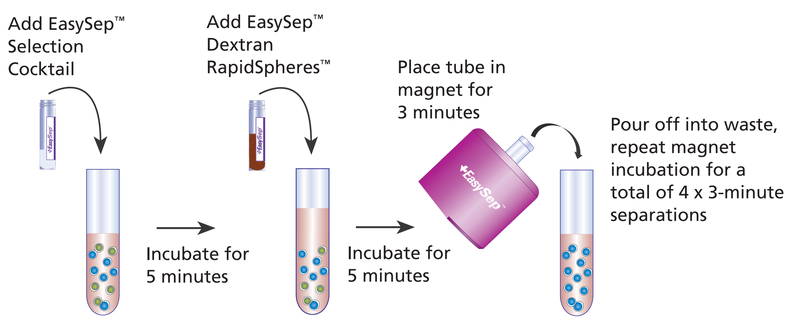
Using this method, the total viable CD138+ content of the isolated fraction is typically 75.0 ± 10.2% (n = 41), 77.9 ± 1.6% (n = 4), and 55.5 ± 10.3% (n = 52), when using naïve C57BL/6 mice, immunized BALB/c mice, or naïve rat spleens, respectively, while the high-expressing CD138+ antibody-secreting cell content of the isolated fraction is typically 49.5 ± 16.2% (n = 41), 71.6 ± 3.4% (n = 4), and 48.6 ± 10.8% (n = 52), respectively. The isolated CD138+ populations remain functional and are immediately ready for downstream applications, including antibody development projects.

Methods

Preparation of Starting Cell Suspension

To prepare a single-cell suspension of splenocytes, spleens from naïve C57BL/6 mice, immunized BALB/c mice, or naïve Sprague Dawley or Wistar rats were disrupted in phosphate-buffered saline (PBS) containing 2% fetal bovine serum (FBS) and 1 mM EDTA. To remove aggregates and debris, cells were passed through a 70 µM mesh nylon strainer and centrifuged at 120 x g for 10 minutes. A mononuclear cell suspension was prepared from rat bone marrow by flushing cells from the femur and tibia bones into PBS/2% FBS/1 mM EDTA using a 23-gauge needle. All cells were resuspended at 1 x 10^8 cells/mL in PBS/2% FBS/1 mM EDTA prior to EasySep™ separation.

Figure 1. EasySep™ protocol for isolating mouse or rat CD138+ cells from splenocytes or bone marrow suspensions



The procedure involves the addition of the EasySep™ Cocktail and EasySep™ Dextran RapidSpheres™ to the cells, followed by immunomagnetic separation.

Results

Table 1. Purities and average yields of CD138+ cells isolated from rat spleen or bone marrow cells by EasySep™

CD138 Total					
Sample	Start		Isolated		
	n	Purity (% ± SD)	*n*	Purity (% ± SD)	Target cells recovered per 1 x 10^8 start cells
Naïve Rat Spleen	17	0.9 ± 0.8	52	55.5 ± 10.3	0.39 x 10^6
Naïve Rat Bone Marrow	15	8.8 ± 5.1	87	62.7 ± 9.0	1.30 x 10^6
Plasma cells/Plasmablasts					
Sample	Start		Isolated		
	n	Purity (% ± SD)	*n*	Purity (% ± SD)	Target cells recovered per 1 x 10^8 start cells
Naïve Rat Spleen	18	0.6 ± 0.5	52	48.6 ± 10.8	0.28 x 10^6
Naïve Rat Bone Marrow	15	0.4 ± 0.3	86	9.2 ± 7.8	0.10 x 10^6

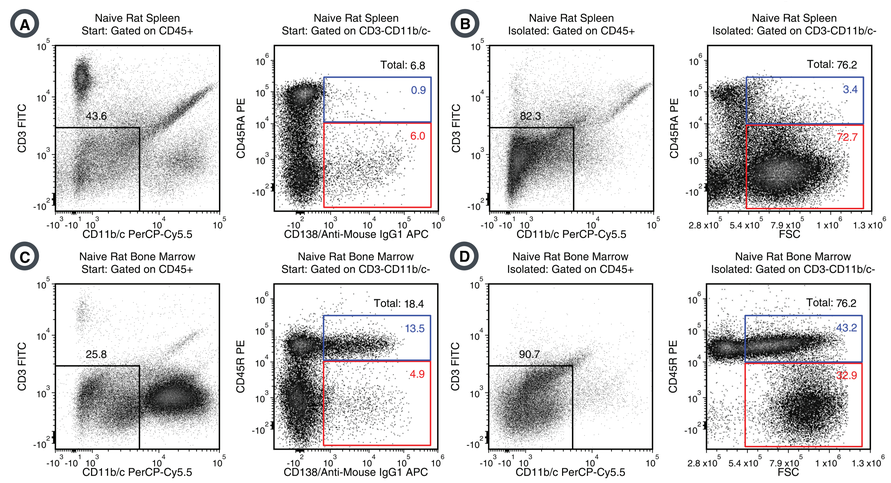
Purities and average cell yields of isolated rat CD138+ cells are based on the percentage of viable CD45+CD3-CD11b/c- cells. Purity values are expressed as mean ± SD. Average cell yield is presented as the number of cells recovered per 1 x 10^8 start cells. Results include data for EasySep™ Magnet, "The Big Easy" Magnet, and EasyEights™ Magnet.

Table 2. Purities and average cell yields of mouse CD138+ cells isolated by EasySep™

CD138 Total					
Sample	Start		Isolated		
	n	Purity (% ± SD)	*n*	Purity (% ± SD)	Target cells recovered per 1 x 10^8 start cells
Naïve Mouse Spleen	52	6.0 ± 2.1	41	75.0 ± 10.2	0.36 x 10^6
Immunized Mouse Spleen	43	5.9 ± 2.3	4	77.9 ± 1.6	2.48 x 10^6
Plasma cells/Plasmablasts					
Sample	Start		Isolated		
	n	Purity (% ± SD)	*n*	Purity (% ± SD)	Target cells recovered per 1 x 10^8 start cells
Naïve Mouse Spleen	52	0.4 ± 0.1	41	49.5 ± 16.2	0.24 x 10^6
Immunized Mouse Spleen	43	1.6 ± 1.1	4	71.6 ± 3.4	1.47 x 10^6

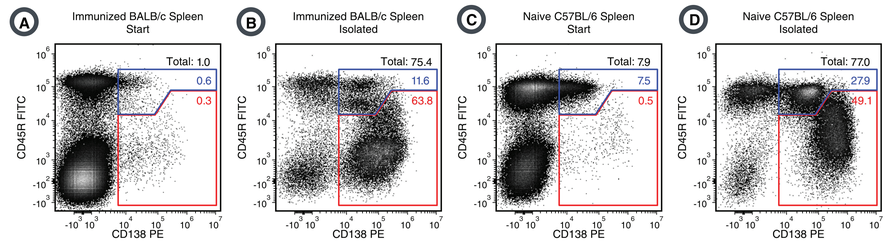
Purities and average cell yields of isolated mouse CD138+ cells is based on percentage of viable cells. Average cell yield is indicated as the number of cells recovered per 1 x 10^8 start cells from naïve C57BL/6 or immunized BALB/c mice spleens (purity values are expressed as mean ± SD). Results include data for EasySep™ Magnet, "The Big Easy" Magnet, and EasyEights™ Magnet.

Figure 2. Representative flow cytometry data before and after EasySep™ isolation of CD138+ cells from naïve rat spleen or bone marrow samples



The viable CD138+ content (gated on CD45+CD3-CD11b/c- cells) is shown for start **(A)** and isolated **(B)** splenocytes, and for start **(C)** and isolated **(D)** bone marrow cells from naïve Sprague Dawley rats. Cells were assessed by flow cytometry with antibodies against CD45, CD3, CD11b/c, and either CD45RA (splenocytes) or CD45R (bone marrow cells) in the presence of DAPI. Start CD138+ cells were identified with an anti-rat CD138 antibody, followed by a rat anti-mouse IgG1 antibody, APC. Isolated plasma cells and blasts are identified as large FSC/CD45RA$^{Low/-}$ (spleen) or large FSC/CD45R$^{Low/-}$ (bone marrow).

Figure 3. Representative flow cytometry data before and after EasySep™ isolation of CD138+ cells from the spleens of immunized or naïve mice



The viable CD138+ content is shown for start **(A)** and isolated **(B)** cells from immunized BALB/c mice, and for start **(C)** and isolated **(D)** cells from naïve C57BL/6 mice. Samples were assessed by flow cytometry using antibodies against CD138 and CD45R in the presence of DAPI. Plasma cells/plasmablasts are identified as CD138^{High} and CD45R$^{Low/-}$.

Summary

- Fast, easy-to-use, and column-free isolation of CD138+ cells directly from mouse or rat tissues in as little as 25 minutes
- No red blood cell lysis required prior to isolation
- Isolated CD138+ populations remain functional and are immediately ready for downstream assays and processes such as hybridoma generation and screening