Next-Level Efficiency: StraightFrom Spleen Isolation Kits. Standardizing Isolation of Untouched B cells with Minimal Hands-On Time.

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Introduction

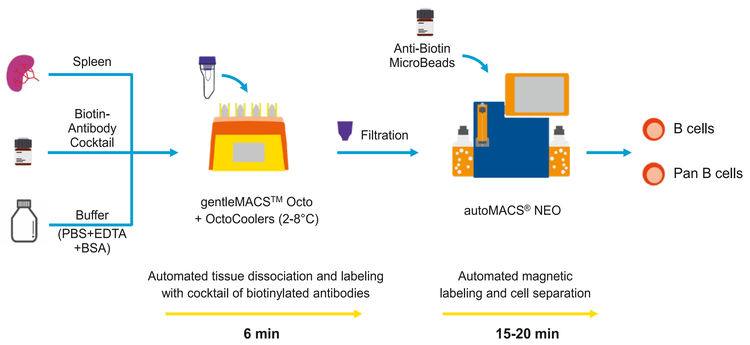
The study of mouse B cells is essential for advancing our understanding of the immune system, developing new vaccines and progressing in the field of immunotherapy. B cells in mice are present at high frequencies in the spleen, one of the major lymphoid organs. The standard workflow for their isolation relies on manual mechanical disruption of the mouse spleen and subsequent magnetic purification. The combination of these two processes is rather time-consuming and operator dependent, with important implication for reproducibility and major impact on downstream applications. To overcome these challenges, we at Miltenyi developed the StraightFrom Spleen kit, an all-in-one solution for isolation of highly pure B cells from mouse spleen. Our results demonstrate that this innovative kit enables the isolation of highly viable and pure B cells, which successfully proliferate *in vitro*, differentiate into CD138+ antibody-secreting cells, and undergo class switching. In conclusion, this new solution represents a simple, fast and reliable cell isolation method that significantly accelerated the isolation of functional cells. It presents an attractive alternative to traditional multi-step protocols commonly used in academic and industrial laboratories, potentially setting a new standard for B cell isolation.

Methods

Simultaneous spleen dissociation and labeling of unwanted cells with StraightFrom Spleen B Cell and Pan B Cell Biotin-Antibody Cocktails was performed using the GentleMACS™ Octo Dissociator with Heaters. The optimal temperature for labeling the cells was maintained by the gentleMACS Octo Coolers. Subsequent automated magnetic labeling of cells with Anti-Biotin MicroBeads and isolation of untouched cells was carried out using the autoMACS® NEO Separator. Target cell purity, yield, proliferation capacity, IgG class switching (following stimulation with αCD40 and IL-4), and differentiation into antibody-secreting cells (after stimulation with 25 µg/mL LPS) were assessed using *in vitro* assays. Cells were stained with fluorophore-conjugated antibodies, including CD19 PE (clone REA749), CD1d FITC (clone 1B1), CD23 PE-Vio770 (clone REA1068), CD5 APC-Vio770 (clone REA521), CD43 PE-Vio615 (clone REA840), CD138 APC (clone REA104), IgG FITC (clone REA1017), and IgM APC-Vio770 (REA979). Dead cell exclusion was performed using 7-AAD, PI or DAPI staining. All reagents were purchased from Miltenyi Biotec unless otherwise noted. The Proliferation Tracer was purchased from ThermoFisher Scientific. LPS was purchased from Sigma-Aldrich. Competitive Kits A–E were either purchased from other suppliers or obtained from Miltenyi Biotec. Samples were acquired with the MACSQuant® Analyzer 10, MACSQuant X or MACSQuant Analyzer 16. Flow cytometric data were analyzed with the MACSQuantify™ or FlowJo™ Software. Data quantification and statistical analysis was performed using GraphPad Prism Software.

1 Rapid and standardized isolation of untouched B cells

Figure 1

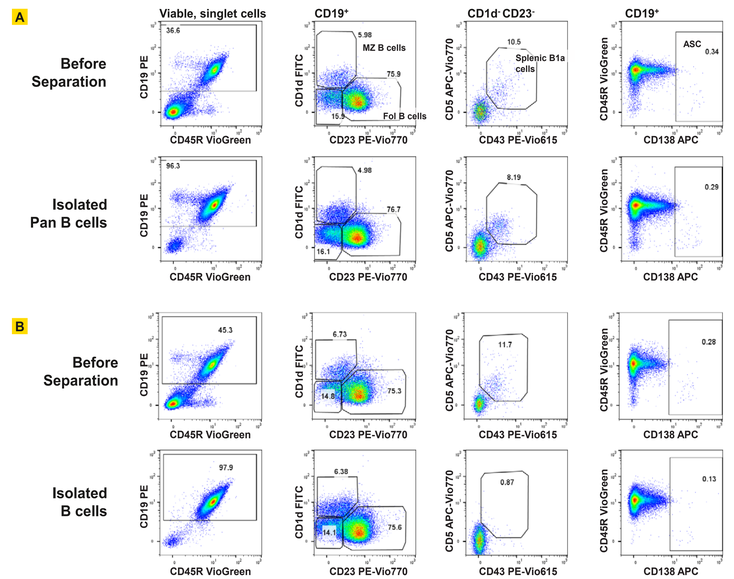


A graphical depiction of the StraightFrom Spleen workflow for rapid and standardized isolation of splenic B cells and Pan B cells. In the first step, the spleen, Biotin-Antibody Cocktail and Buffer (PBS, 5mM EDTA, 0.5% BSA) are placed in gentleMACS™ C Tube. Spleen is gently mechanically dissociated using the gentleMACS Octo Dissociator with Heaters. During the dissociation step, non-target cells are indirectly labeled with a cocktail of biotin-conjugated monoclonal antibodies as the primary labeling reagent while the gentleMACS Octo Coolers provide an optimal temperature for labeling the cells. Afterwards, anti-biotin monoclonal antibodies conjugated to MicroBeads are used as secondary labeling reagent. In between the two labeling steps no washing steps are required. After dissociation and magnetic labeling, the sample is passed through a 70 µm filter to remove larger particles from the singlecell suspension. Then, the cell suspension is loaded onto MACS Column placed in the magnetic field of a MACS Separator. The magnetically labeled non-target cells are depleted by retaining them within a MACS Column in the magnetic field of a MACS Separator, while the unlabeled B cells or Pan B cells run through the column.

Results

1 Isolation of untouched B cells and Pan B cells with StraightFrom Spleen workflow

Figure 2



To test whether all B cell subsets were maintained with our newly developed kit, we compared the frequencies of major spleen subsets before isolation and after using both the Pan B cell and B cell isolation kits. Using a combination of different markers, we identified the most frequent B cell subsets present in naïve SPF mice. From the B cell gate (CD19+CD45R+), we identified FO B cells as CD23+ CD1d-, and Marginal Zone B cells. In naïve mice, a small but detectable population of innate-like B cells, namely splenic B-1a, was present, defined by the coexpression of CD43 and CD5. Additionally, a small population of cells secreting natural antibodies (ASCs) was detected as CD138+ with dim to low expression of CD45R. By using the same gating strategy, we demonstrated that after isolation with both the Pan B cell and B cell isolation kits, all relevant subsets were identified at frequencies identical to those in the unseparated samples. The representative plots were acquired with a MACSQuant device, and samples were stained with a collection of conjugated REA antibodies (Miltenyi Biotec).

2 StraightFrom Spleen workflow provides a rapid method for isolation of functional B cells

Figure 3

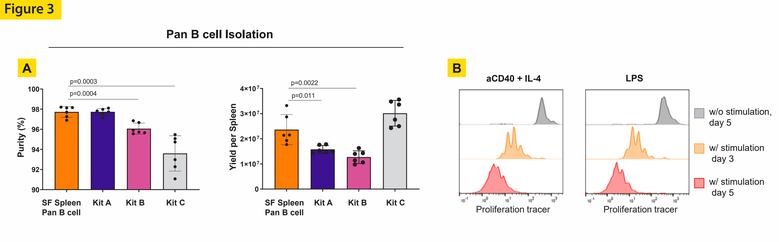
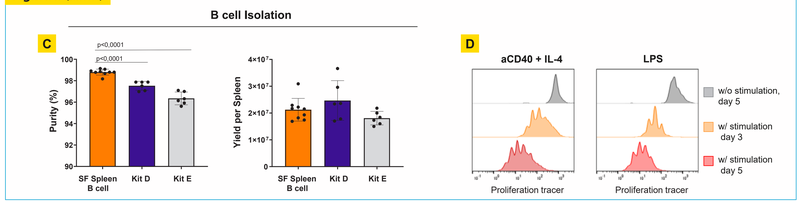


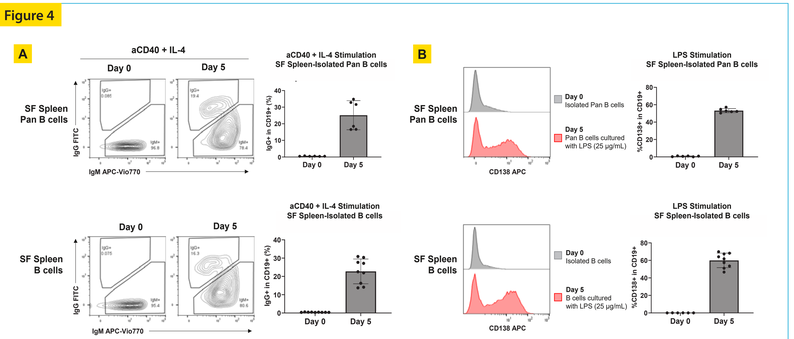
Figure 3 (cont.)



To examine whether the performance of our innovative workflow provides a good separation outcome despite a significant reduction in sample preparation time, we compared it to competitive protocols offered by various vendors. In terms of the purity of isolated B cells, both the Pan B cell isolation kit and the B cell isolation kit displayed significantly better performance compared to other commercially available kits. Regarding the number of B cells recovered, our new Pan B cell kit showed a significantly higher recovery compared to at least two of the competitors, whereas the recoveries for the B cell isolation kit were comparable (Figure A, C). Isolated B cells were assessed for their capability to proliferate after being stimulated with T-dependent and T-independent stimuli (Figures B and D). Briefly, after isolation, cells were labeled with a cell tracer to measure cellular division. In both conditions, B cells isolated with either the Pan B cell or B cell isolation kit were able to proliferate after 3 days under stimulation, and by day 5, all the cells had divided at least twice, as measured by the count of the cell tracer peaks.

3 StraightFrom Spleen Workflow Enables B Cell IgG Switching and Antibody Secreting Cell Differentiation

Figure 4



Isolated B cells from the spleen of naïve mice were placed in culture and activated *in vitro* with T-dependent and T-independent stimuli to test functionality. In panel A, B cells isolated with either the Pan B cell isolation kit (upper panel) or the B cell isolation kit (lower panel) were activated by crosslinking CD40 on the surface with anti-CD40 and adding IL-4 to mimic T-cell help and promote class switch recombination from IgM to IgG. On day 5, with cells isolated using both kits, we detected a substantial switch occurring in the B cells, as measured by flow cytometry. Additionally, we activated B cells with LPS, a potent T-independent stimulus that, by interacting with TLR4, pushes B cell differentiation to plasmablasts and plasma cells. In both situations, we observed successful differentiation of B cells on day 5, confirming their functionality.

Conclusions

- We developed a rapid, efficient workflow for untouched B and Pan B cell isolation with minimal hands-on time. Our optimized protocols yield highly pure target cells, significantly accelerating downstream applications.
- StraightFrom Spleen protocols enable functional B and Pan B cell isolation, supporting proliferation, IgG class switching (αCD40 + IL-4 stimulation), and differentiation into antibody-secreting cells (LPS stimulation).
- This streamlined approach offers a robust alternative to conventional multi-step methods in academic and industrial settings.
- The integration of the autoMACS® NEO Separator enhances workflow efficiency by automating cell isolation, reducing hands-on time, and supporting a broad range of human and mouse cell isolation applications.