Fast and Easy Isolation of CD27-Positive Human Memory B Cells Using EasySep™ Releasable RapidSpheres™

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Abstract

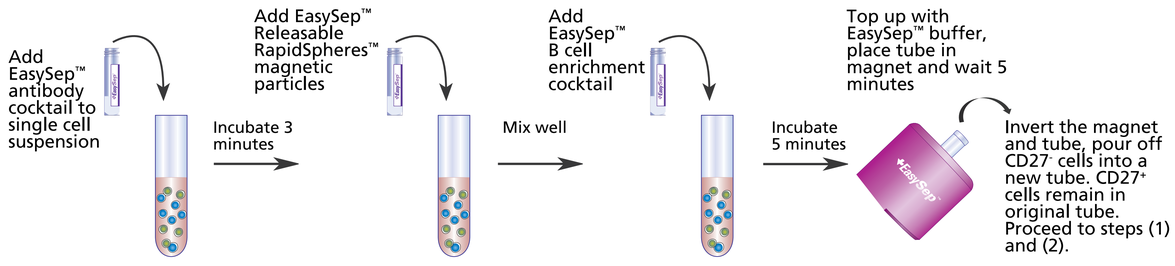
Over the course of a lifetime, memory B cells are key to maintaining antibody-mediated protection from viral and bacterial pathogens and they are one outcome of an effective vaccine. Since memory B cells are typically found at frequencies of less than 5% of normal human peripheral blood mononuclear cells (PBMC), research into their function would be aided by a fast and easy isolation method. To meet this need, we have developed an improved EasySep™ kit for the isolation of CD27-positive (CD27$^+$) human memory B cells from fresh PBMCs.

First, CD27$^+$ cells are labeled using an antibody cocktail and EasySep™ Releasable RapidSpheres™ and are positively selected using a hand-held EasySep™ magnet. Next, magnetic particles are released from the positively selected cells, and then non-B cells within the CD27$^+$ fraction are targeted for depletion using a second antibody cocktail and EasySep™ Dextran RapidSpheres™. Following an additional magnetic separation step, the particle-free, CD27$^+$ memory B cells are poured off into a new tube and ready for use. With one additional step, CD27$^-$ naïve B cells can be isolated from the same starting sample. The entire isolation protocol is completed in under 40 minutes without any centrifugation steps.

Using this method, CD19$^+$CD27$^+$ memory B cells can be isolated to 97 ± 2% purity and 37 ± 14% recovery (mean ± SD; n = 19). The CD19$^+$CD27$^-$ naïve B cells can be isolated to 93 ± 5% purity and 28 ± 11% recovery (mean ± SD; n = 9). When stimulated with CpG and IL-15, the isolated memory and naïve B cells are functional, as assessed by both proliferative responses and the secretion of IgG.

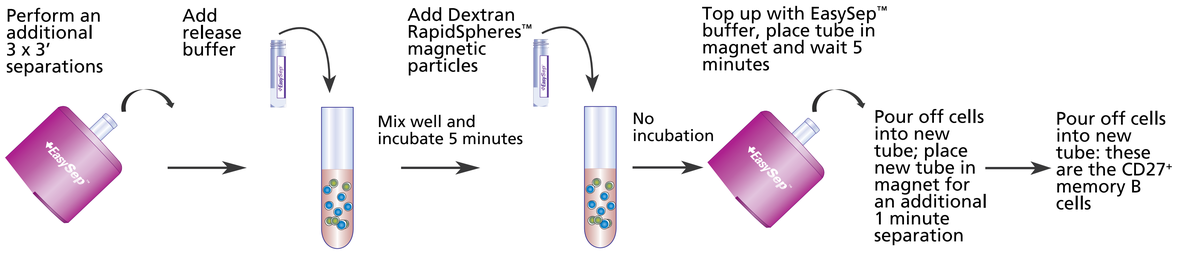
Methods

Isolates memory B cells in as little as 33 minutes

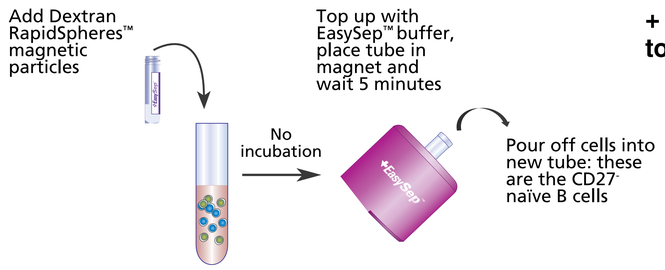


1 Memory B cells

Total protocol time: 33 minutes
Isolated memory B cells are particle-free



2 Optional protocol to isolate naïve B cells



+ Additional 5 minutes for optional protocol to isolate untouched naïve B cells

Results

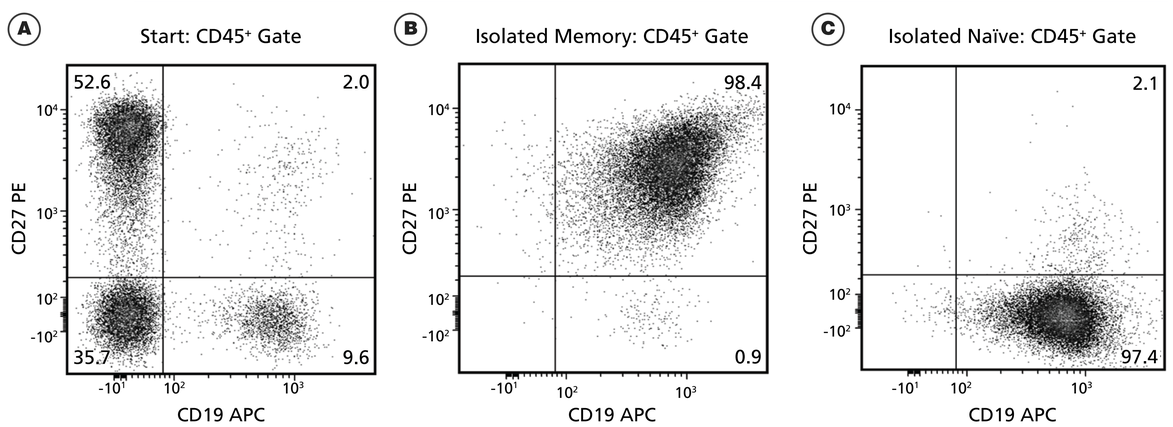


Figure 1. Typical EasySep™ human memory B cell isolation kit purities. (A) Start populations in PBMCs and final isolated cell populations for **(B)** CD19$^+$CD27$^+$ memory B cells and **(C)** CD19$^+$CD27$^-$ naïve B cells.

	Purple EasySep™ Magnet (n = 7 memory, n = 3 naïve)		"The Big Easy" EasySep™ Magnet (n = 19 memory, n = 9 naïve)	
	Purity (%)	Recovery (%)	Purity (%)	Recovery (%)
CD27$^+$ Memory B	95.4 ± 4.4	29.1 ± 11.4	97.2 ± 1.8	36.6 ± 14.0
CD27$^-$ Naïve B	90.6 ± 9.0	41.0 ± 17.4	92.9 ± 4.6	28.3 ± 11.0

Table 1. Performance summary in EasySep™ magnets. Starting with 1 x 10^8 cells, average purity and recovery (mean ± SD) of isolated memory and naïve B cells using the new EasySep™ Human Memory B Cell Isolation Kit and either the purple or "The Big Easy" EasySep™ magnets.

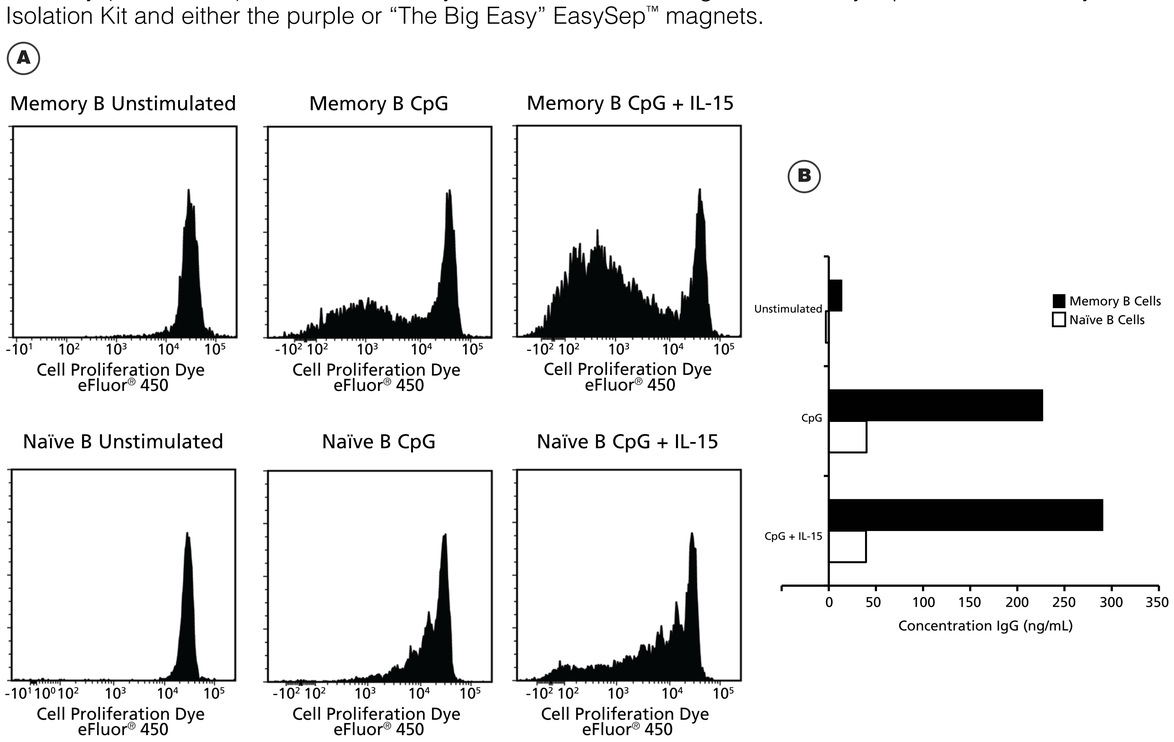


Figure 2. Isolated memory and naïve B cells are functional. Isolated human memory and naïve B cells were labeled with 5 µM Cell Proliferation Dye eFluor® 450 and then stimulated with CpG ODN2006 (2.5 µg/mL), with or without recombinant human IL-15 (10 ng/mL) for 5 days. **(A)** Proliferation was assessed by flow cytometry. **(B)** IgG in cell culture supernatants was analyzed by ELISA. One representative experiment is shown.

Summary

The new EasySep™ Human Memory B Cell Isolation Kit

- Facilitates the rapid isolation of both memory and naïve B cell populations from the same sample
- Compatible for use with EasySep™ (Catalog #18000) and "The Big Easy" EasySep™ (Catalog #18001) magnets
- Modified protocol is available for use with EasyEights™ EasySep™ (Catalog #18103) and Easy 50 EasySep™ (Catalog #18002) magnets
- Isolated cells are functional and ready for use in downstream assays