

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

Nina M. Maeshima<sup>1</sup>, Vida K. Jovanovic<sup>1</sup>, Catherine L. Ewen<sup>1</sup>, Samuel J. Clarke<sup>1</sup>, Andy I. Kokaji<sup>1</sup>, Steven M. Woodside<sup>1</sup>, Terry E. Thomas<sup>1</sup> and Allen C. Eaves<sup>1,2</sup>  
<sup>1</sup>STEMCELL Technologies Inc., Vancouver, BC, Canada <sup>2</sup>Terry Fox Laboratory, BC Cancer Agency, Vancouver, BC, Canada

## Abstract

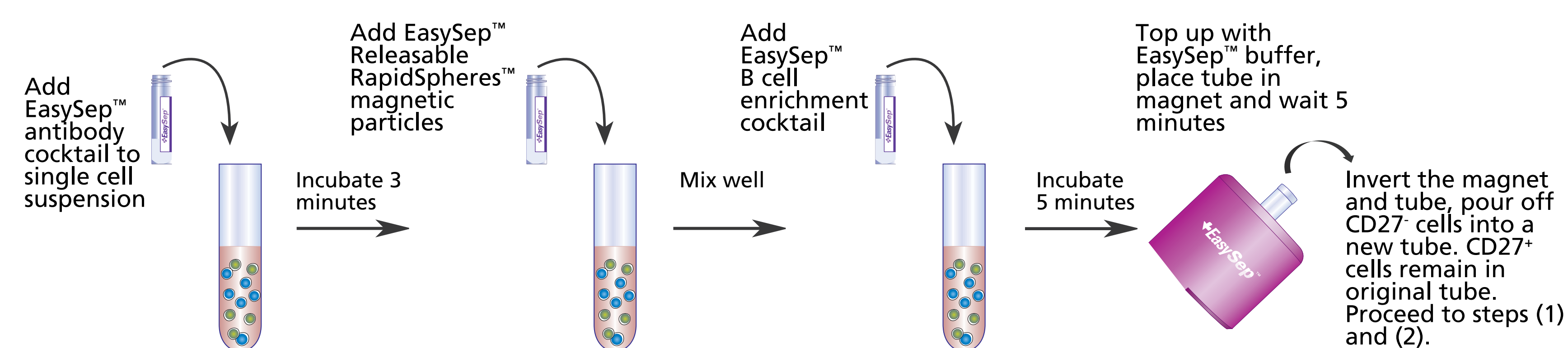
Over the course of a lifetime, memory B cells are key to maintaining antibody-mediated protection from viral and bacterial pathogens and they are one outcome of an effective vaccine. Since memory B cells are typically found at frequencies of less than 5% of normal human peripheral blood mononuclear cells (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<sup>+</sup>) human memory B cells from fresh PBMCs.

First, CD27<sup>+</sup> 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<sup>+</sup> fraction are targeted for depletion using a second antibody cocktail and EasySep™ Dextran RapidSpheres™. Following an additional magnetic separation step, the particle-free, CD27<sup>+</sup> memory B cells are poured off into a new tube and ready for use. With one additional step, CD27<sup>-</sup> 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.

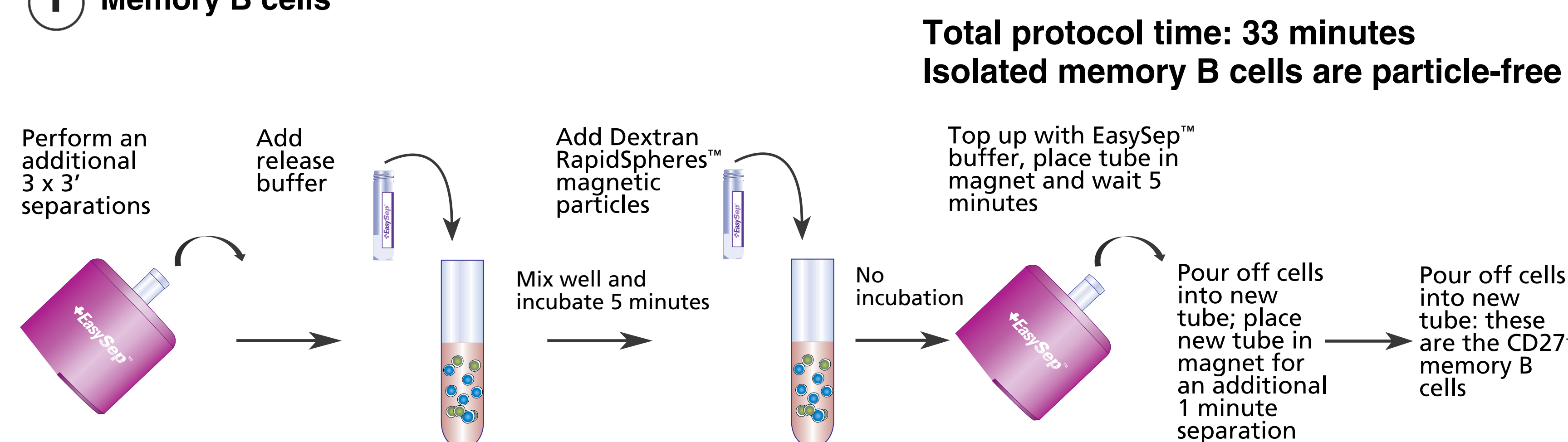
Using this method, CD19<sup>+</sup>CD27<sup>+</sup> memory B cells can be isolated to 97 ± 2% purity and 37 ± 14% recovery (mean ± SD; n = 19). The CD19<sup>+</sup>CD27<sup>-</sup> naïve B cells can be isolated to 93 ± 5% purity and 28 ± 11% recovery (mean ± SD; n = 9). When stimulated with CpG and IL-15,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

## Methods

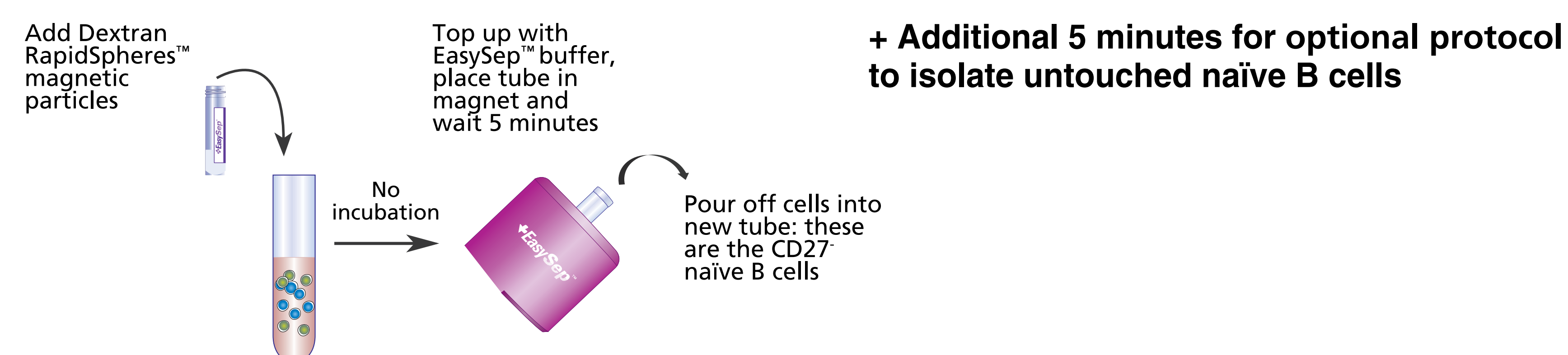
### Isolates memory B cells in as little as 33 minutes



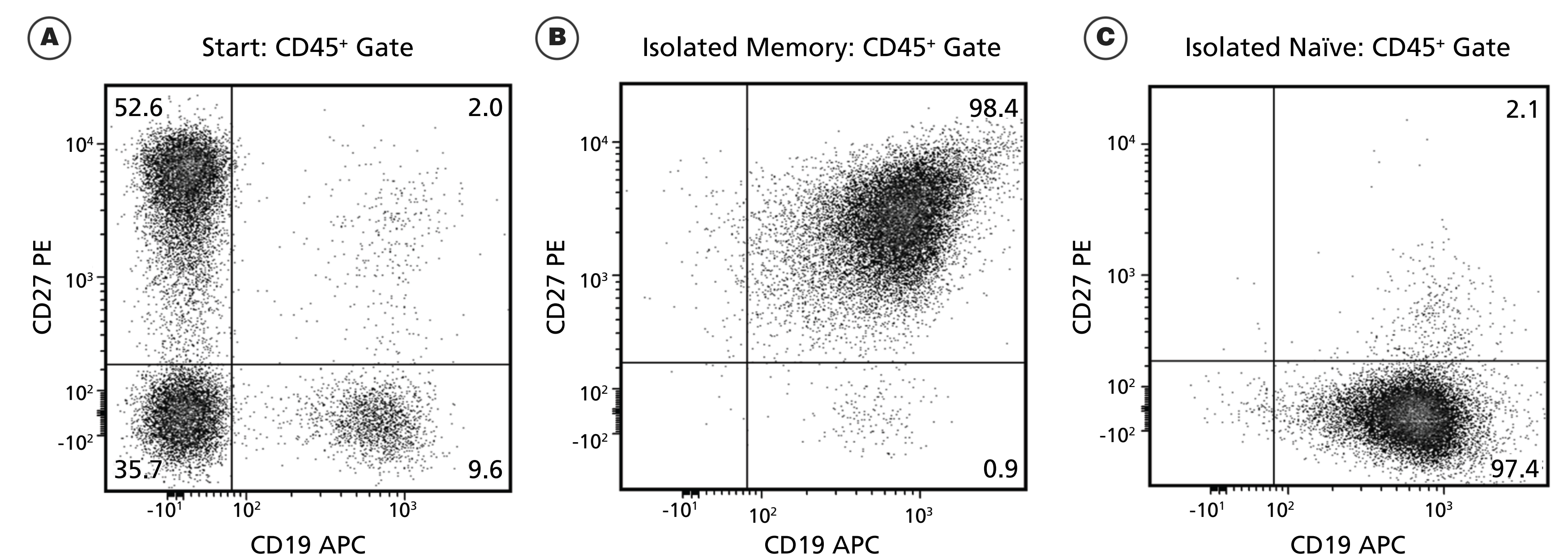
#### 1 Memory B cells



#### 2 Optional protocol to isolate naïve B cells



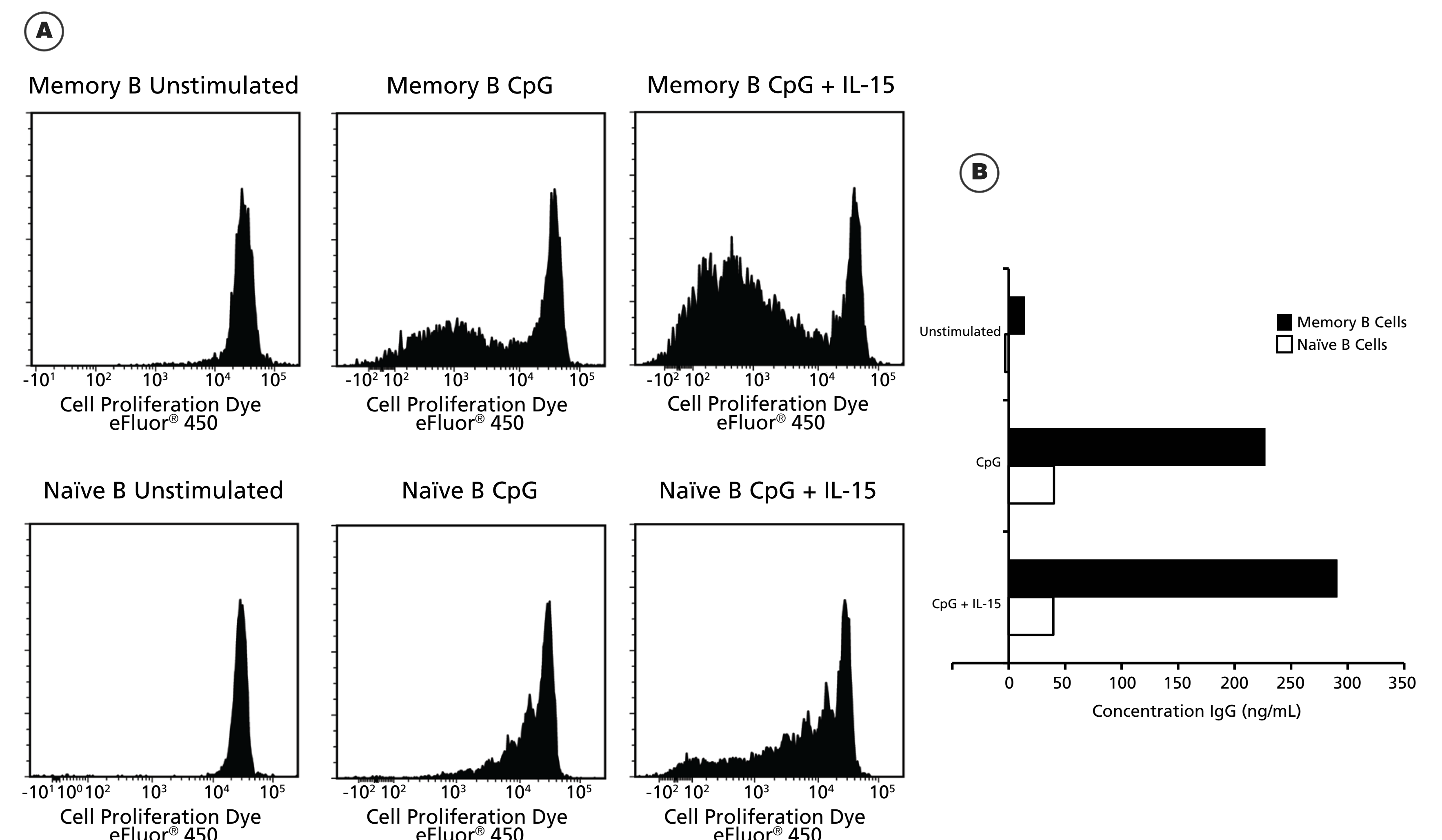
## Results



**Figure 1. Typical EasySep™ human memory B cell isolation kit purities.** (A) Start populations in PBMCs and final isolated cell populations for (B) CD19<sup>+</sup>CD27<sup>+</sup> memory B cells and (C) CD19<sup>+</sup>CD27<sup>-</sup> 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 <sup>+</sup> Memory B	95.4 ± 4.4	29.1 ± 11.4	97.2 ± 1.8	36.6 ± 14.0
CD27 <sup>-</sup> 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 × 10<sup>8</sup> 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<sup>®</sup> 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