Abstract #1064053

Fast, Easy and Column-Free Procedure for the Isolation of Human Memory B Cells

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Introduction

Human memory B cells are long-lived cells having the unique ability to rapidly proliferate and differentiate when re-exposed to the same antigen. They can be distinguished from naïve B cells by the presence of somatic hypermutations in their Ig-V region gene sequences. CD27 is widely used as a marker of memory B cells because its surface expression correlates with the presence of such mutations. CD27 also plays a key role in regulating B cell activation and immunoglobulin synthesis. Several CD27$^+$ memory subsets collectively make up 20-60% of peripheral B cells.

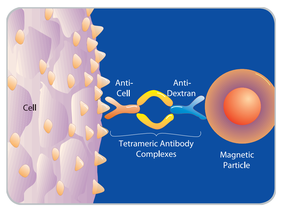
We describe a two-step EasySep™ method for the isolation of memory CD27$^+$ B cells from fresh or previously frozen peripheral blood nucleated cells. First, non-B cells are targeted for depletion with dextran-coated magnetic particles using a cocktail of tetrameric antibody complexes (TAC). Labeled cells are separated in an EasySep™ magnet without the use of columns and pre-enriched, unlabeled B cells are collected. Next, CD27$^+$ B cells are selected from the pre-enriched fraction using TAC recognizing CD27 and dextran-coated magnetic particles. Labeled cells are separated and remain in the tube in the magnet while unwanted cells are poured off. The unwanted cell fraction may be used to obtain naïve B cells. The selection steps can be fully automated using RoboSep™. Isolation of memory B cells from human samples is increasingly important for the investigation of B cell signaling pathways and regulation mechanisms central to a robust immune response.

Methods

Preparation of starting cell suspension

A single cell suspension of mononuclear cells (PBMC) was prepared from either fresh whole blood or buffy coat suspensions of peripheral blood using Ficoll-paque PLUS. Alternatively, peripheral blood apheresis (Leucopak) cells were used following red blood cell lysis and one or more washes to remove platelets. Either fresh or frozen cells were used for these experiments.

FIGURE 1: EasySep™ labeling of human cells



Cells are targeted for selection or depletion using monoclonal antibodies directed against specific cell surface markers. The labeled cells are then crosslinked to EasySep™ magnetic particles using tetrameric antibody complexes (TAC). Magnetically-labeled cells are then separated from unlabeled cells using the EasySep™ procedure.

Results

TABLE 1: Percentages of B cells and memory B cells in starting human PBMC or apheresis samples before and after B cell pre-enrichment (n=35)

% CD19$^+$ B cells start	% CD19$^+$ CD27$^+$ memory B cells start	% CD19$^+$ CD27$^+$ after B cell pre-enrichment
8.5 ± 4	3.2 ± 1.6	37.3 ± 14

Percentage of CD19$^+$CD27$^+$ memory B cells in the peripheral B cell population can affect purity. Purity is donor related, and typically samples from younger donors have fewer CD19$^+$CD27$^+$ memory B cells in the starting cell sample. This generally results in lower overall purity of the target population.

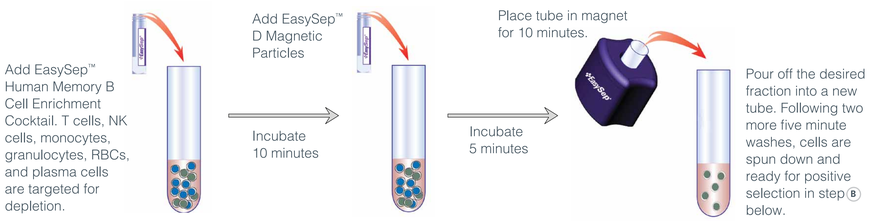
TABLE 2: Purity and recovery of memory B cells enriched from peripheral blood nucleated cells by manual EasySep™ or RoboSep™

method	n	% purity CD19$^+$CD27$^+$	average yield of memory B cells
EasySep™	14	93.5 ± 3	1.0 - 2.7 x 10^6 per 1 x 10^8 starting cells
RoboSep™	8	90.6 ± 5	

Purities determined by flow cytometry. All samples gated on viable (PI negative) cells.
Values are expressed as means ± 1 sd.

FIGURE 2: EasySep™ procedure for column-free selection of memory B cells from human PBMC or apheresis

(A) Depletion of non-B cells (pre-enriched memory B cells)



(B) Positive selection of CD27$^+$ cells from pre-enriched B cells

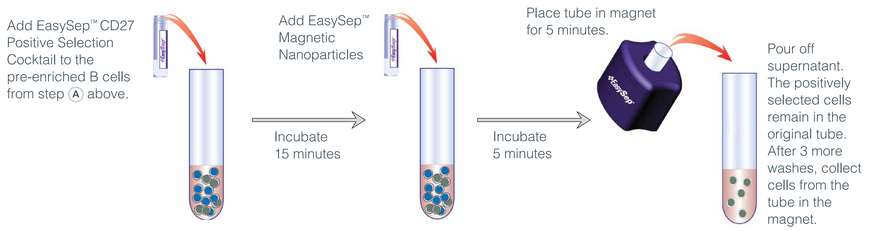
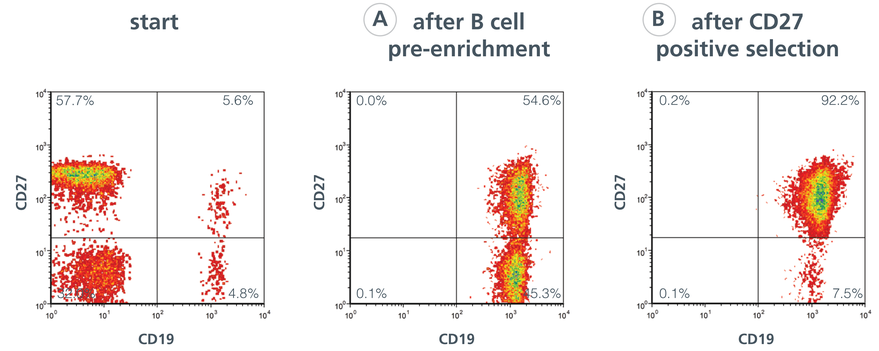


FIGURE 3: Typical FACS plots before and after enrichment of memory B cells



Plots show viable PI negative cells

TABLE 3: Comparison of protocols for isolation of memory B cells from human PBMC or apheresis

	STEMCELL EasySep™ 18164 Human Memory B Cell Kit	competitor column-based Human Memory B Cell Kit	STEMCELL RoboSep™ (automated)
(A) B cell pre-enrichment	45 min	60 min	54 min
(B) Memory B cell CD27$^+$	40 min	60 min	48 min
total time	85 min	120 min	102 min
number of columns	0	3	0

Conclusions

- Rapid and column-free: Memory B cells of up to 96% purity and 99% viability can be enriched from peripheral blood nucleated cell samples using EasySep™. The entire procedure takes 85 minutes.
- Automated: Memory B cell enrichment can be fully automated using RoboSep™.
- Naïve B cells can be isolated from the same cell sample.
- Memory B cells can be directly isolated from washed apheresis samples – no need to Ficoll.
- On average, approximately 1.0 - 2.7 x 10^6 memory B cells are recovered from 1 x 10^8 starting cells by the described method.
- The investigation of B cell signaling pathways and regulation mechanisms will be facilitated by this rapid and simple isolation method.