

Robust Serum-Free Expansion of Human B Cells In Vitro with an Animal Component-Free Cell Culture Supplement

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INTRODUCTION

Achieving robust expansion of B cells in culture typically requires the addition of serum, feeder cells, or specialized culture plates. Addition of serum can improve performance, albeit with high lot-to-lot variability, but introduces the risk of contamination by adventitious agents. Here we report the development of a serum-free supplement that can be added to any base medium to achieve robust in vitro expansion of human B cells in bulk cultures. ImmunoCult™-ACF Human B Cell Expansion Supplement is an animal component-free (ACF) 50X supplement containing recombinant proteins and factors. This supplement can be added to various base media such as RPMI, DMEM, or ImmunoCult™-XF to prepare a complete medium suitable for expanding enriched pan-B, CD19⁺, naïve, and memory B cell populations. ImmunoCult™-ACF Human B Cell Expansion Supplement elicits robust expansion of human B cells in vitro for > 40 days. This supplement may facilitate studies using human B cells for antibody discovery and diverse basic and translational applications such as gene editing.

METHODS

Immunomagnetic Isolation of Human B Cells

Human pan-B cells were enriched from peripheral blood mononuclear cells (PBMCs; leukopaks) using EasySep™ Human Pan-B Cell Enrichment Kit (STEMCELL Technologies) according to the manufacturer's instructions. Human memory and naïve B cells were enriched from PBMCs using EasySep™ Human Memory B Cell Isolation Kit (STEMCELL Technologies) according to the manufacturer's instructions. This kit was used to isolate memory and naïve B cell populations from the same sample. Enriched cells were analyzed by flow cytometry using a CytoFLEX instrument (Beckman Coulter Life Sciences).

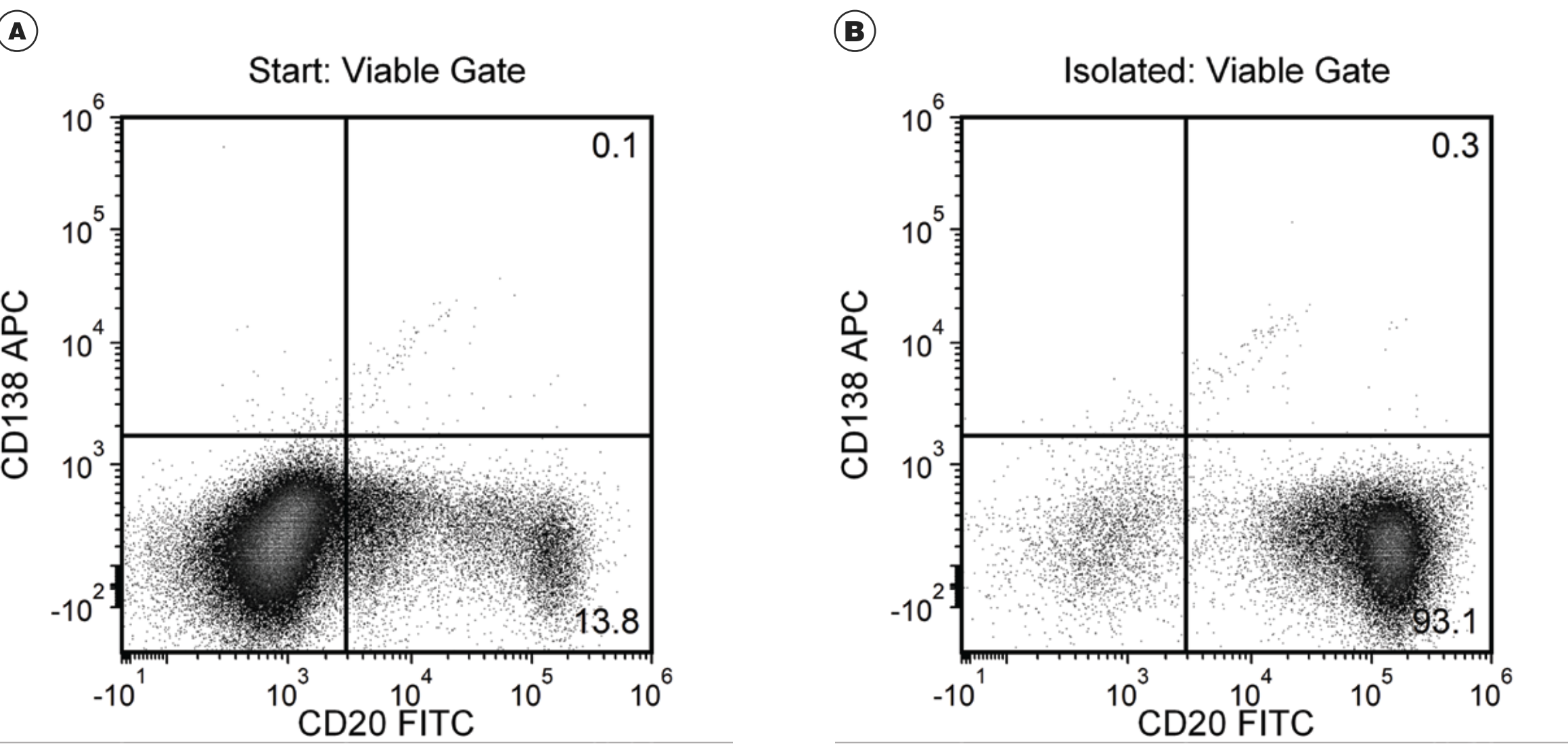


FIGURE 1. Enrichment of CD20⁺ Human Pan-B Cells Using EasySep™ Immunomagnetic Negative Selection

Representative flow cytometry plots gated on viable cells for **(A)** start cells (total leukocytes) and **(B)** isolated pan-B cells. The purity of the isolated CD20⁺ pan-B cells in this example was 93.4%, and typically ranged from 93 - 96% (n = 4).

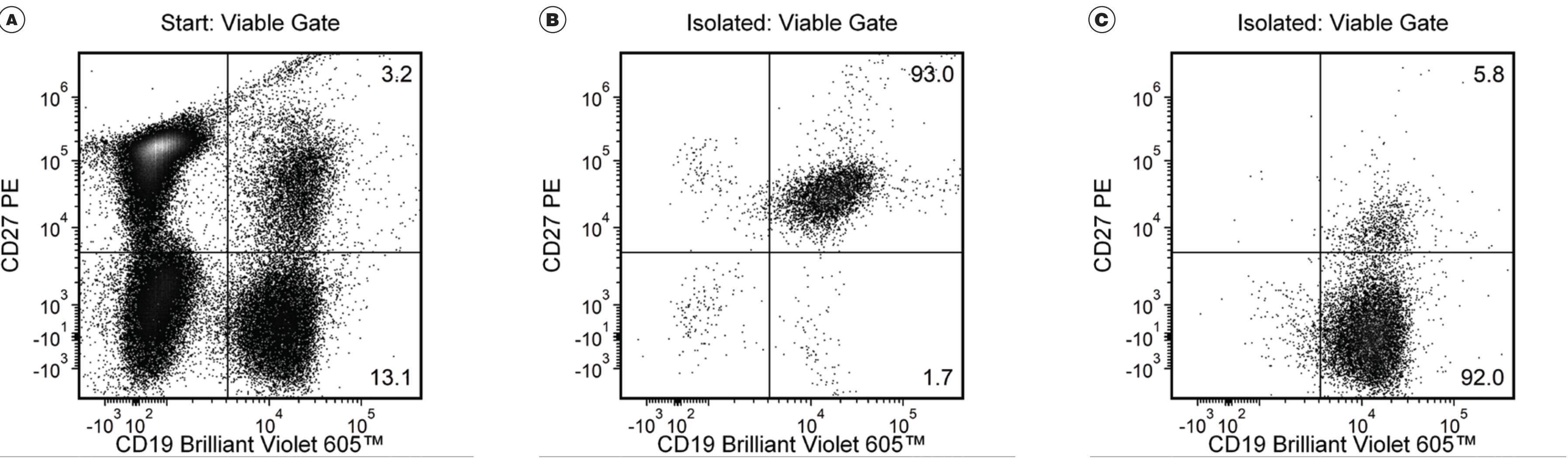


FIGURE 2. Enrichment of Human Memory and Naïve B Cells Using EasySep™ Immunomagnetic Negative Selection

Representative flow cytometry plots gated on viable cells for **(A)** start cells (total leukocytes), **(B)** isolated CD19⁺CD27⁺ memory B cells, and **(C)** isolated CD19⁺CD27⁻ naïve B cells. The purity of the isolated memory and naïve B cells in this example was 93.0% and 92.0%, and typically ranged from 95 - 99% and 88 - 98%, respectively (n = 22).

Cell Culture

A complete human B cell expansion medium (Complete Medium) was prepared by diluting the 50X ImmunoCult™-ACF Human B Cell Expansion Supplement 1:49 in ImmunoCult™-XF (STEMCELL Technologies). At day 0, pan-B cells were seeded at 1 x 10⁵ cells/mL in 24-well plates, and memory or naïve B cells were seeded at 2.5 x 10⁵ cells/mL in 96-well culture plates. Cultures were incubated at 37°C and 5% CO₂ in a humidified incubator. The cell density was adjusted to 1 x 10⁵ cells/mL (pan-B cells) or 2.5 x 10⁵ cells/mL (memory or naïve B cells) every 2 - 3 days by the addition of fresh Complete Medium. Cell counting and measurement of viability were performed using a NucleoCounter® NC-250™ instrument (ChemoMetec A/S) according to the manufacturer's instructions. The fold expansion of viable cells was calculated as the fold-change in cell number relative to the number of viable cells seeded at day 0. Flow cytometric analyses were performed with a CytoFLEX instrument to assess changes in the expression of cell surface markers characteristic of B cell activation and maturation (CD19, CD20, CD86, CD138). Cultures were imaged using an Olympus CKX53 inverted microscope.

RESULTS

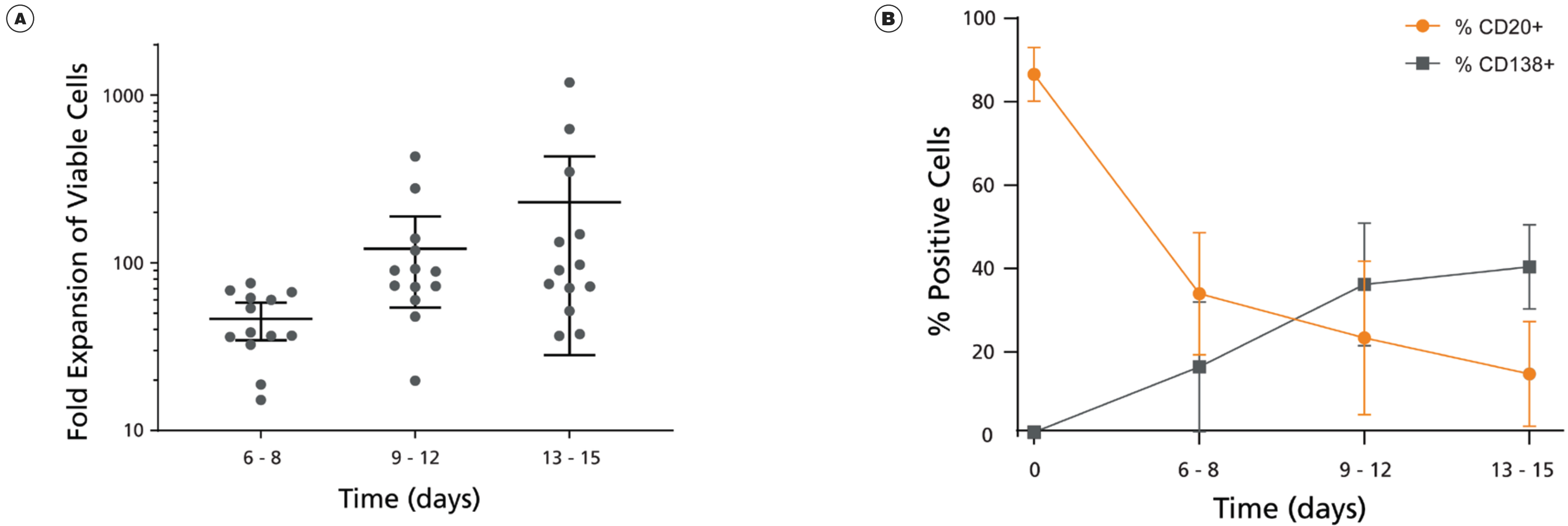


FIGURE 3. ImmunoCult™-ACF Human B Cell Expansion Supplement Promotes the Expansion and Maturation of Human Pan-B Cells

Pan-B cells isolated from human PBMCs were seeded at 1 x 10⁵ cells/well in 24-well tissue culture plates with ImmunoCult™-ACF Human B Cell Expansion Supplement. ImmunoCult™-XF was used as a base medium in these experiments. The cells were passaged every 3 - 4 days, with data shown from day 7 to 14 (± 1 day). **(A)** Fold expansion of viable cells is shown for 13 donors, with bars representing the mean ± 95% confidence interval (range 37- to 1,190-fold at day 14 ± 1). **(B)** Expression of CD138 and CD20 were determined by flow cytometry at each timepoint (data shown are the mean % positive viable cells ± 1 SD). Upregulation of CD86 and some downregulation of CD19 were also observed (data not shown). These changes in phenotype are consistent with maturation of B cells to plasma cells/blasts.

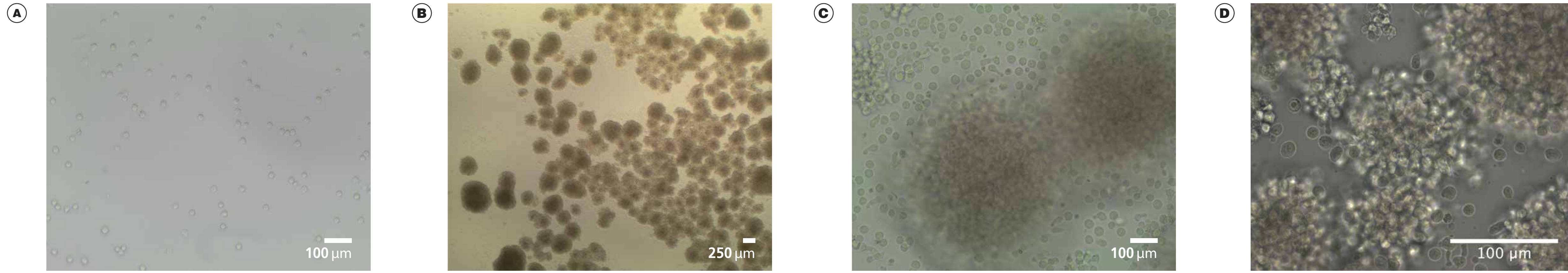


FIGURE 4. Robust Growth of Human Pan-B Cells Cultured with ImmunoCult™-ACF Human B Cell Expansion Supplement

Pan-B cells isolated from human PBMCs (leukopaks) were seeded at 1 x 10⁵ cells/well in a 24-well tissue culture plate with ImmunoCult™-ACF Human B Cell Expansion Supplement. ImmunoCult™-XF was used as a base medium. The cells were passaged on day 4 after seeding and imaged at **(A,C)** 20X, **(B)** 2X, or **(D)** 40X magnification, 6 days after seeding.

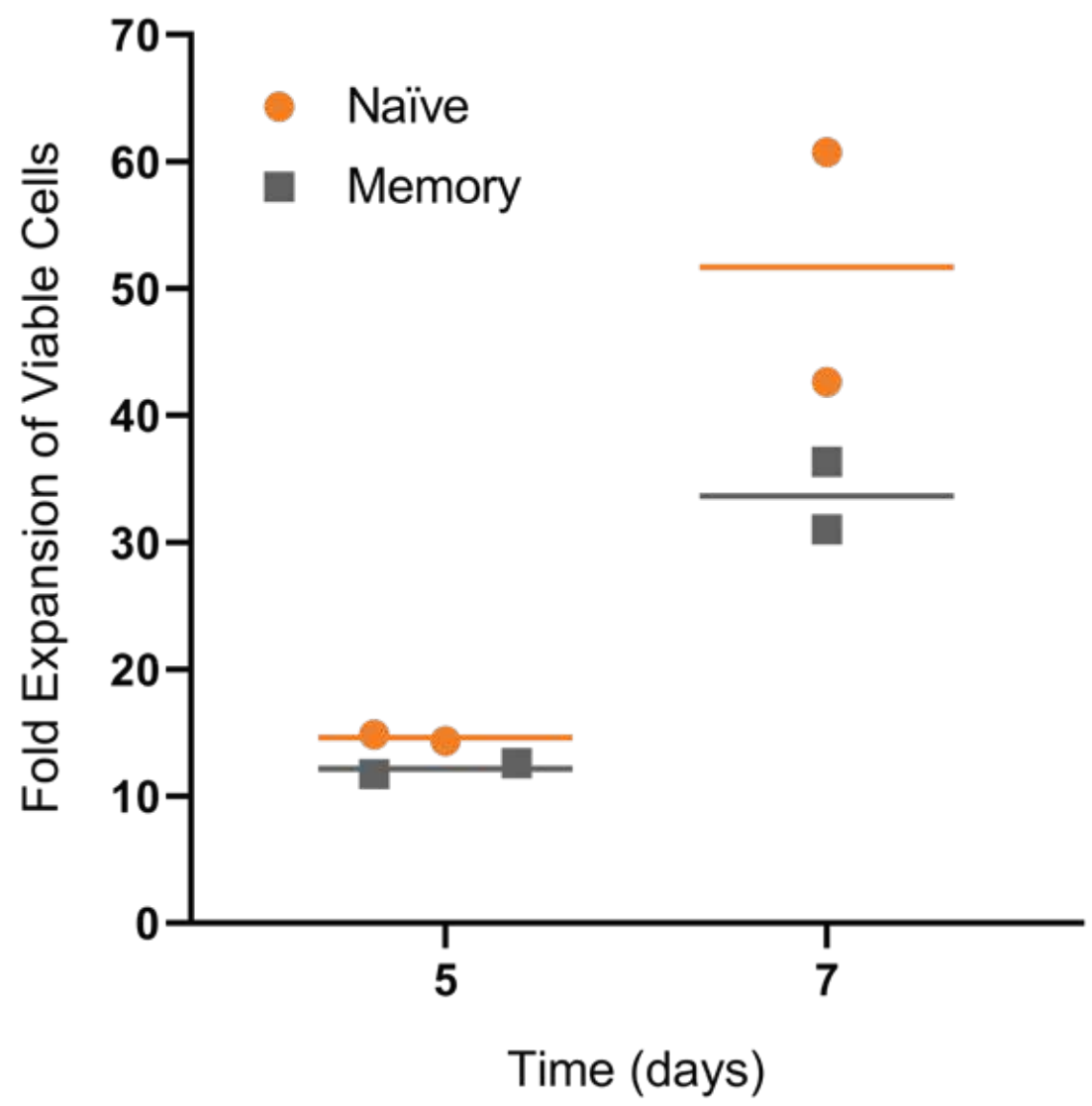


FIGURE 5. ImmunoCult™-ACF Human B Cell Expansion Supplement Promotes the Expansion of Memory and Naïve B Cells

Human memory and naïve B cells isolated from PBMCs (leukopaks) using EasySep™ Human Memory B Cell Isolation Kit were seeded at 2.5 x 10⁵ cells/mL in 96-well tissue culture plates with ImmunoCult™-ACF Human B Cell Expansion Supplement. ImmunoCult™-XF was used as a base medium. The cells were passaged on day 5 after seeding. Fold expansion of viable cells is shown for two independent experiments performed using the same human donor cell sample.

Summary

- An animal component-free (ACF) ImmunoCult™-ACF Human B Cell Expansion Supplement has been developed that facilitates robust in vitro expansion of bulk cultures of human primary B cells, including enriched pan-B, naïve, and memory B cell populations. Enriched CD19⁺ cells exhibited ~50-fold expansion after 10 - 11 days (n = 1; data not shown).
- Formulated as a 50X concentrate, the Supplement can be added to an appropriate base culture medium to prepare a complete medium suitable for activation and expansion of human B cells.
- This Supplement may facilitate studies employing human B cells for antibody discovery and diverse basic and translational applications such as gene editing.