

B Cells and B Cell Blasts Withstand Cryopreservation While Retaining Their Functionality for Producing Antibody

Philipp Fecher, Richard Caspell, Villian Naeem, Alexey Y. Karulin, Stefanie Kuerten and Paul V. Lehmann
Cellular Technology Limited • R&D Department • 20521 Chagrin Boulevard • Shaker Heights, OH USA

ABSTRACT: In individuals who have once developed humoral immunity to an infectious/foreign antigen, the antibodies present in their body can mediate instant protection when the antigen re-enters. Such antigen-specific antibodies can be readily detected in the serum. Long term humoral immunity is, however, also critically dependent on the ability of memory B cells to engage in a secondary antibody response upon re-exposure to the antigen. Antibody molecules in the body are short lived, having a half-life of weeks, while memory B cells have a life span of decades. Therefore, the presence of serum antibodies is not always a reliable indicator of B cell memory and comprehensive monitoring of humoral immunity requires that both serum antibodies and memory B cells be assessed. The prevailing view is that resting memory B cells and B cell blasts in PBMC cannot be cryopreserved without losing their antibody secreting function, and regulated high throughput immune monitoring of B cell immunity is therefore confined to – and largely limited by – the need to test freshly isolated PBMC. Using optimized protocols for freezing and thawing of PBMC, we show here that both resting memory B cells and B cell blasts retain their ability to secrete antibody after thawing, and thus demonstrate the feasibility of B cell immune monitoring using cryopreserved PBMC.

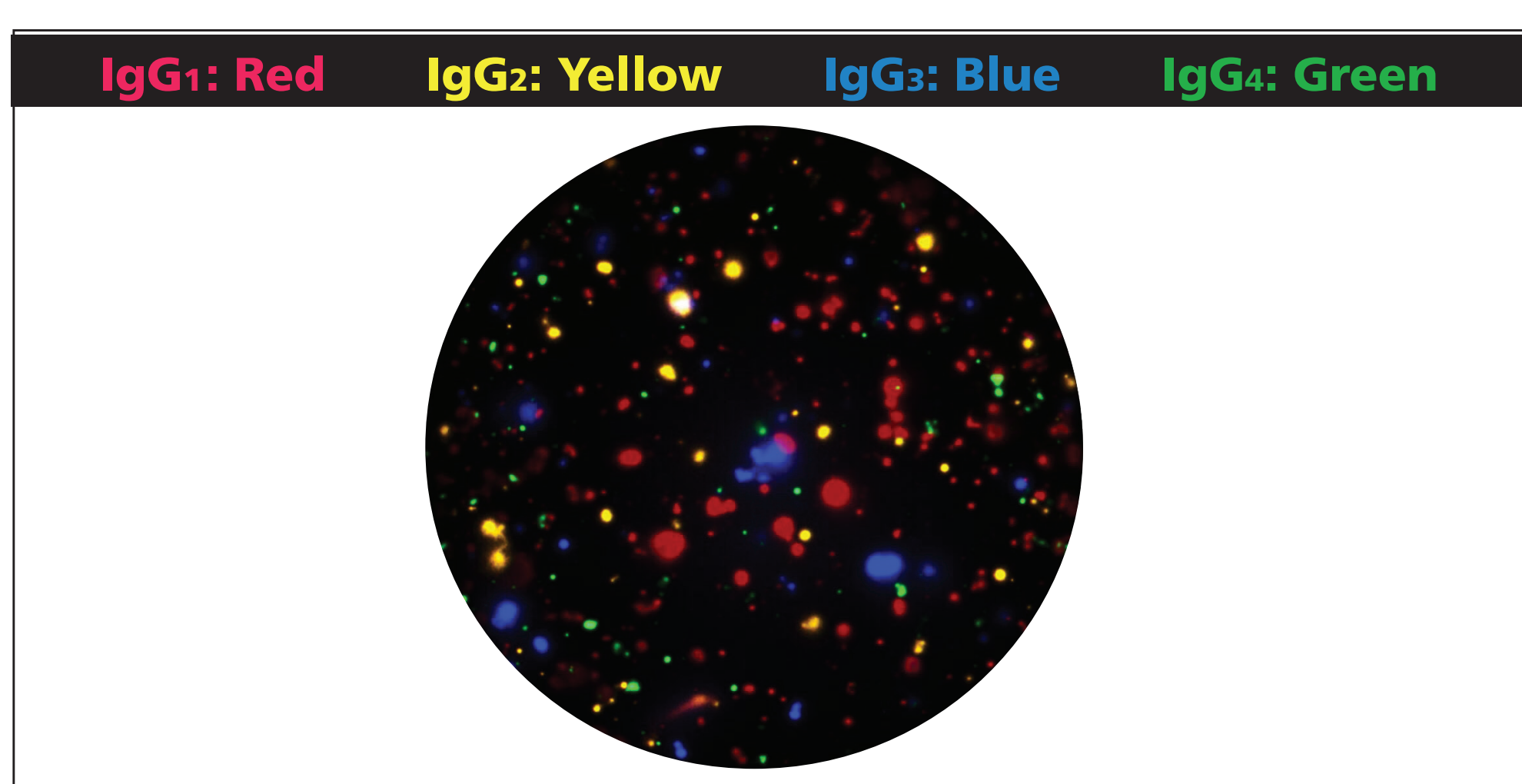


Figure 2. Representative four-color well. The membrane was coated with anti- κ/λ capture antibody and polyclonally activated PBMC were plated at 50,000 cells/well. Detection reagents for each of the four IgG subclasses were added to visualize the membrane-bound IgG molecules. Images were captured for each individual fluorescent channel (see Fig. 1) and superimposed using the following artificially assigned colors for each color plane: IgG1: Red; IgG2: Yellow; IgG3: Blue, and IgG4: Green.

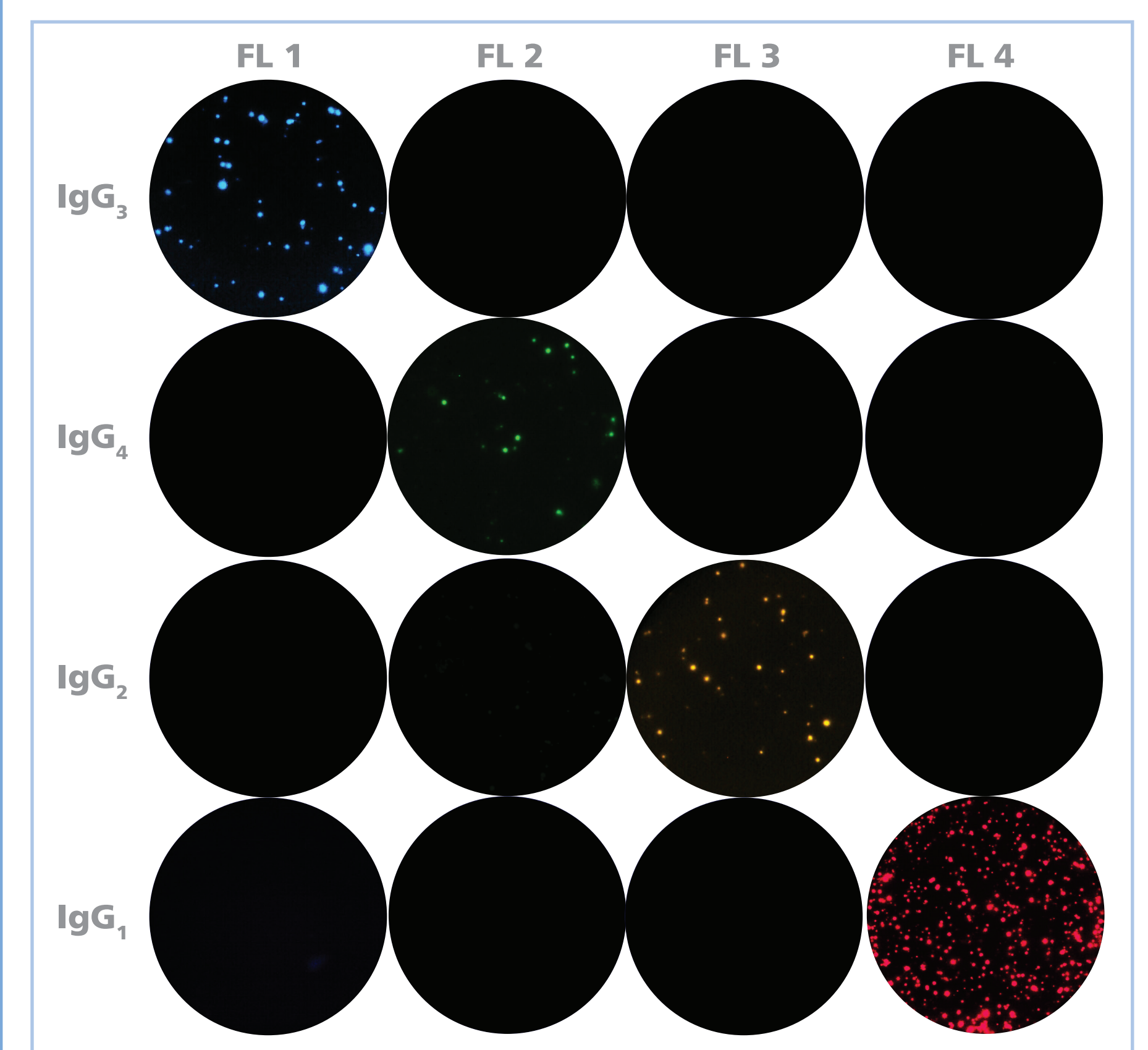


Figure 1. Unambiguous detection of the four fluorescent tags identifying the individual IgG subclasses. In each row, a single-color B cell ELISPOT assay was performed using the respective IgG subclass-specific detection reagent, as specified in the figure. Each assay was analyzed with filter settings optimized for the individual colors. Across three independent experiments (of which representative scans are shown), individual fluorescent channels (FL1 to FL4), readily detected corresponding fluorescent tags. Importantly, no cross-bleeding of colors between channels occurred.

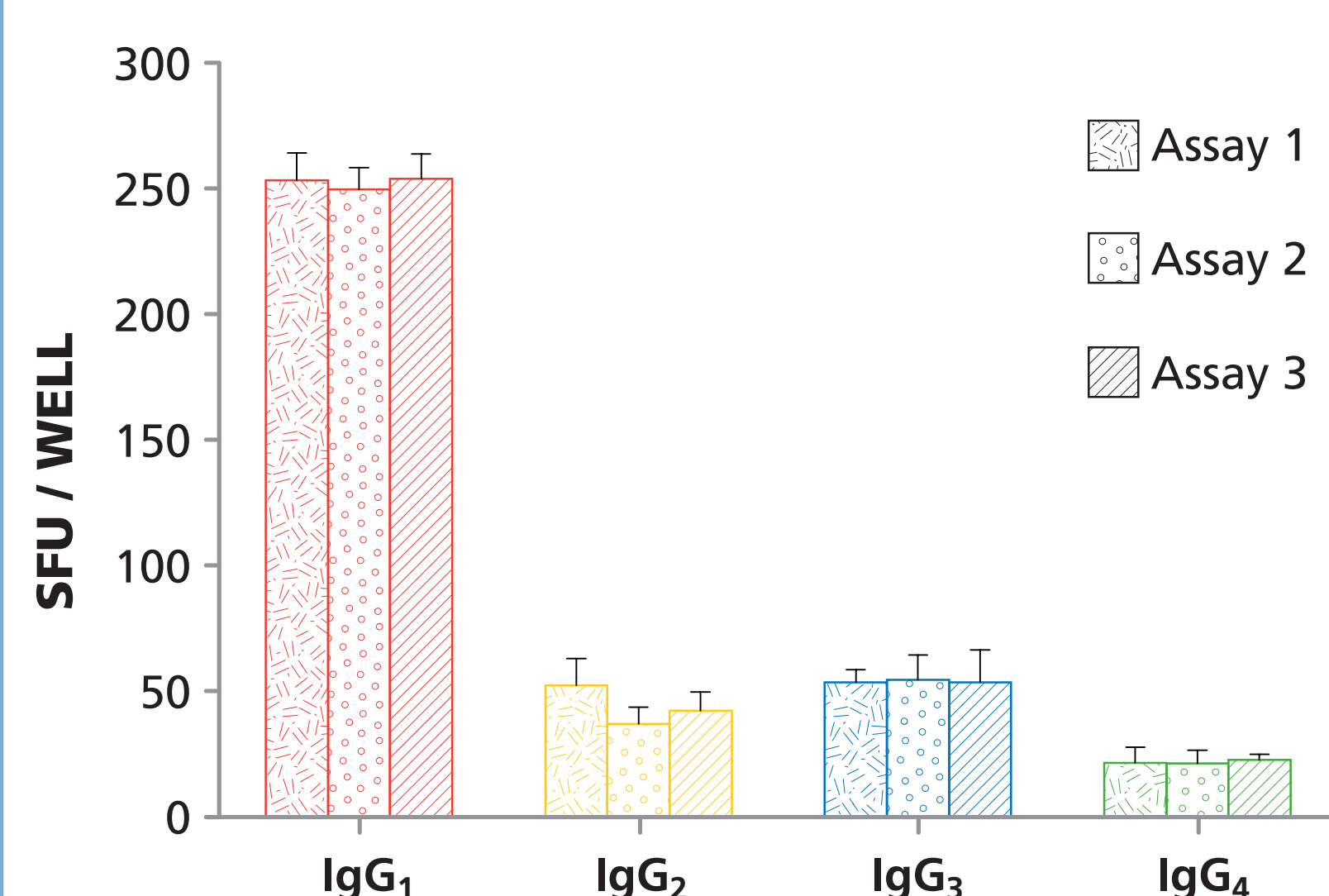


Figure 3. Reproducibility of the 4 color B cell assay. To assess inter-assay variation in spot numbers, PBMC were stimulated polyclonally, and frozen in aliquots on day four. Three independent assays were performed after thawing an aliquot, measuring the four IgG subclasses on anti- κ/λ capture antibody-coated membranes. Each bar represents the mean spot count + SD for the tests done in four replicate wells.

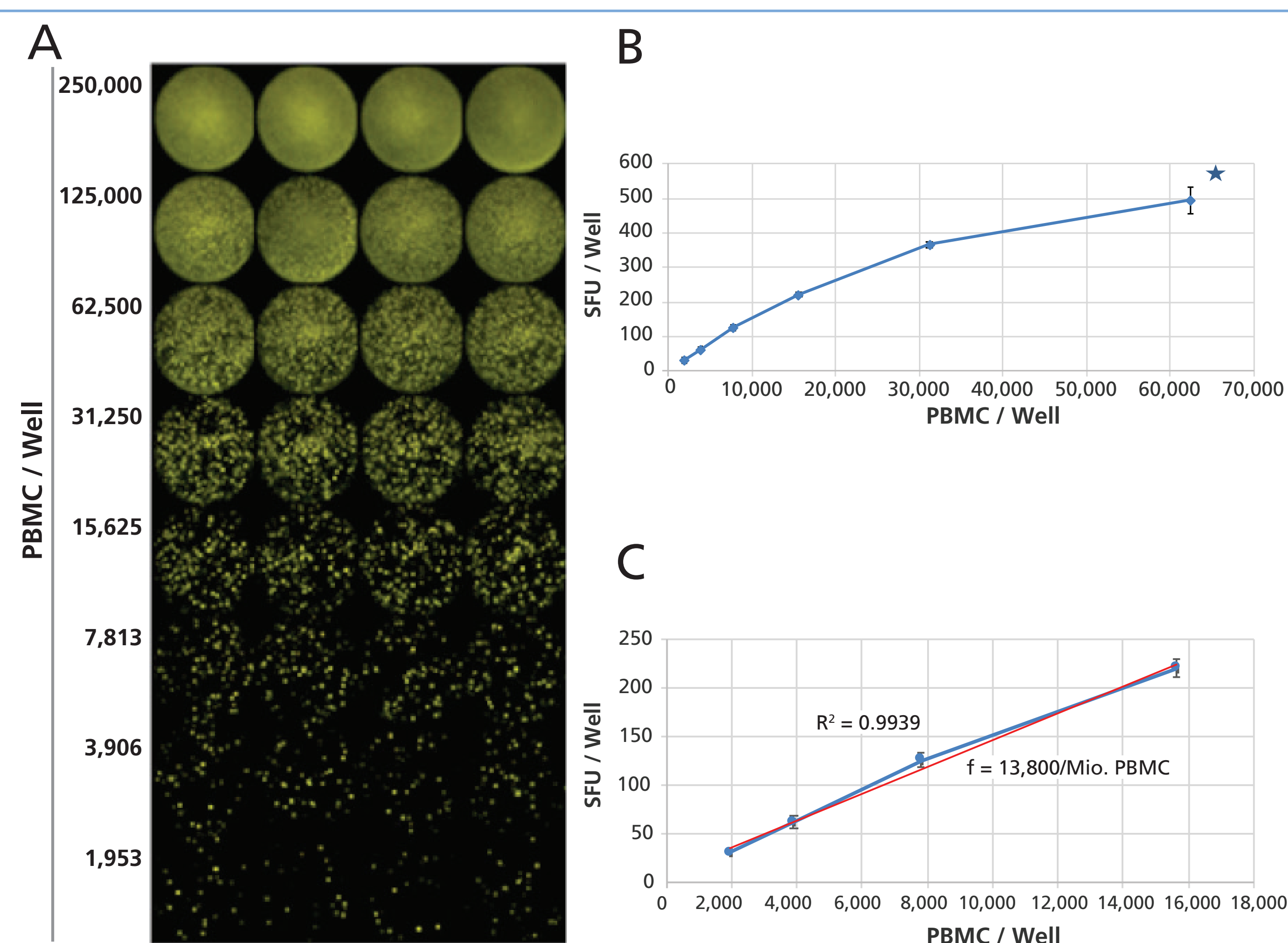


Figure 4. Calculating frequencies for pan IgG-SFU/million PBMC illustrated. Polyclonally stimulated PBMC were plated in the specified cell numbers in quadruplicate wells, and a Three Color ImmunoSpot® assay was performed for Ig classes pan-IgG, M and A. (A) Raw images for the IgG color plane are shown with the cell numbers plated per well specified. (B) The mean spot count for each cell concentration, with the SD for the quadruplicate wells tested is plotted vs. the cell numbers plated. As the spots at high ASC densities start crowding (plus when the number of ASC is high, there is an “ELISA” effect with analyte captured from the supernatant causing a high background carpet staining), for high cell numbers SFU become no longer precisely countable: “too numerous to count” is shown by a star. (C) While at high numbers spot counts tend to deviate from linearity due to crowding, in lower numbers they followed a linear relationship between cell numbers plated and SFU counted, in this case with an R2 value of 0.9939. Based on the regression line shown in red, the pan-IgG SFU frequency has been calculated to be, in the example shown here, 13,800 pan-IgG SFU per one million PBMC.

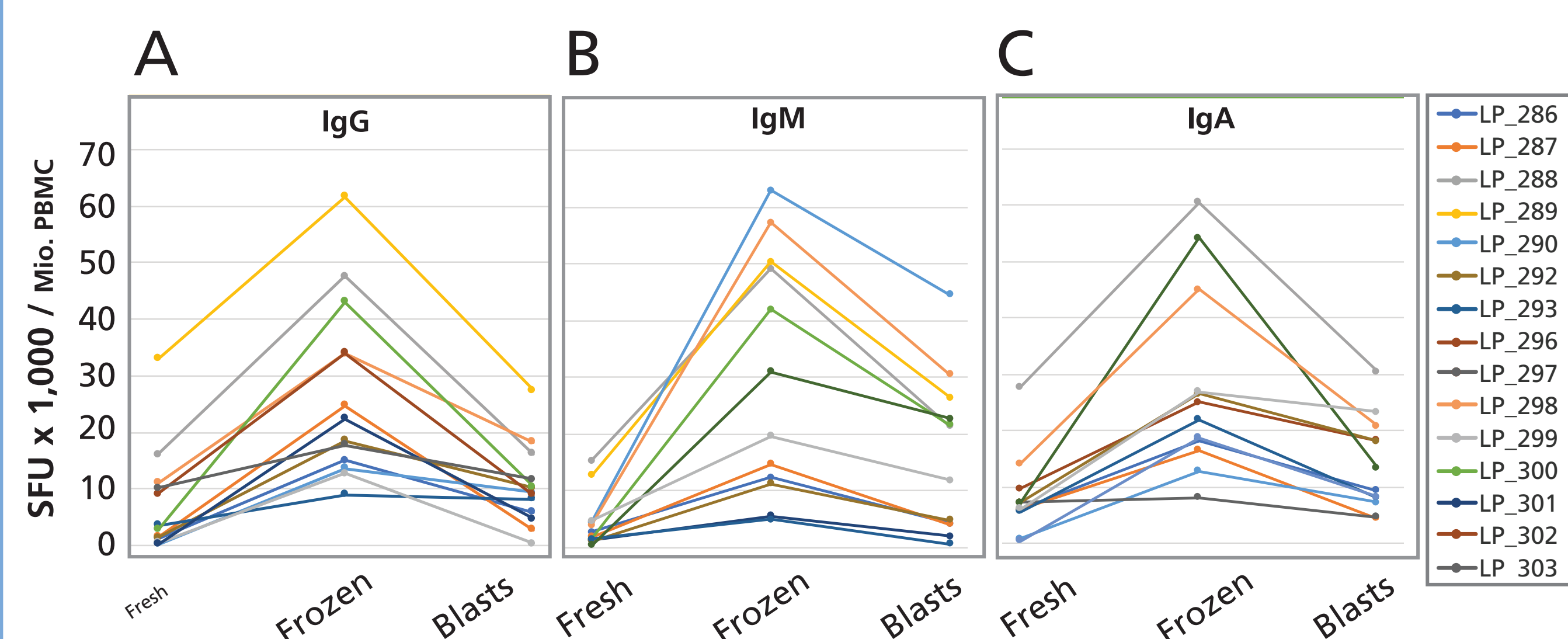


Figure 5. Effect of cryopreservation on Ig class production by B cells. Freshly isolated PBMC were polyclonally stimulated for 4 days and then tested in a 3 color B cell ImmunoSpot® assay, with serial dilution of the cells in 4 replicate wells, (“Fresh”), as illustrated in Figure 4. The same PBMC were cryopreserved, thawed, and then polyclonally stimulated, seeded and tested as above (“Frozen”). The latter cells were cryopreserved at the end of the 4 day polyclonal stimulation culture, when B cell blasts have been engaged, then thawed, and seeded and tested as above (“Blasts”). The relationship of Fresh/Frozen and Blast cells is graphically illustrated in Figure S1. The results are shown here for pan-IgG (A), IgM (B), and IgA (C). The SFU counts per million PBMC have been established as specified in Figure 4. For each donor, as defined by color in the insert, the mean spot counts are connected by the corresponding color coded lines.

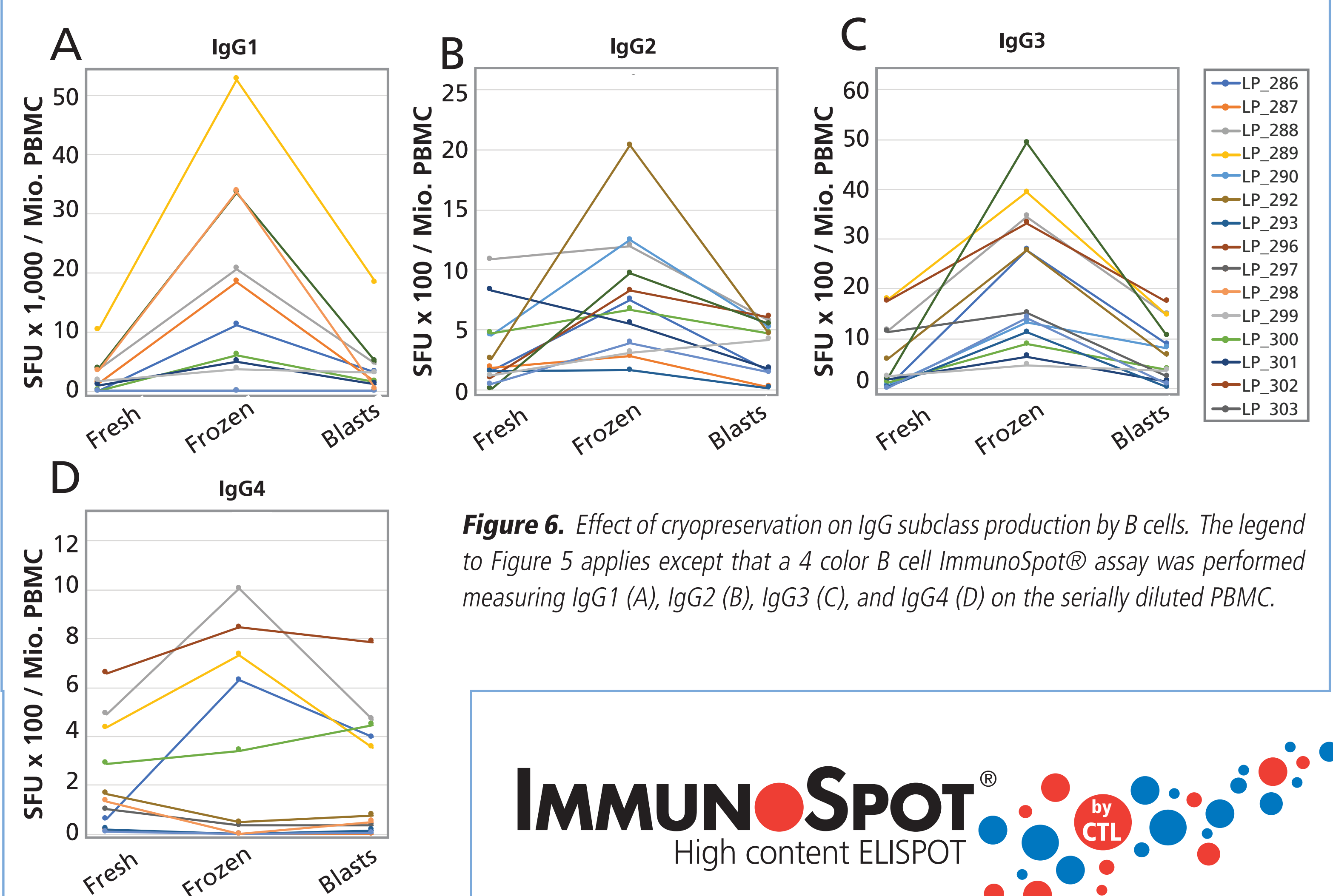


Figure 6. Effect of cryopreservation on IgG subclass production by B cells. The legend to Figure 5 applies except that a 4 color B cell ImmunoSpot® assay was performed measuring IgG1 (A), IgG2 (B), IgG3 (C), and IgG4 (D) on the serially diluted PBMC.