Lyophilized antibody cocktails offer long term shelf life and excellent reproducibility for longitudinal studies

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Abstract

Multi-color flow cytometric assays require laboratory personnel to mix from four up to 15 antibodies on a daily basis, which is time and labor intensive and can lead to manual errors due to missing antibodies, pipetting incorrect amounts of antibody, cross contamination or spillage during the workflow. In addition, liquid cocktails with tandems dyes such as PE/Cy7 and APC/Cy7 suffer from stability issues, leading users to prepare a fresh cocktail for every use. One solution to prevent these issues is to lyophilize antibody cocktails, allowing increased shelf-life and providing researchers with the ability to easily conduct long-term, multi-site studies to obtain reliable data. Using our unique lyophilization technique, we have successfully lyophilized a variety of multi-color immuno-phenotyping cocktails. Here we show that a lyophilized CD3 APC/Cy7, CD4 PE/Cy7, CD8a Alexa Fluor™ 700, CD14 APC, CD16 PE, CD19 Pacific Blue™, CD45 PerCP, CD56 PE, HLA-DR Alexa Fluor® 488, CD11c PE/Dazzle™ 594, and CD123 Brilliant Violet 510™ cocktail performs similar to (*i.e.*, % positive, MFI and compensation parameters) a freshly mixed cocktail. Stability studies have shown that these lyophilized cocktails are stable for up to 3 months under heat (37°C) stress storage conditions. These results suggest that the lyophilized cocktail will be stable for several years. Real-time stability studies are ongoing.

Introduction

Multi-color flow cytometry studies are tedious and time consuming due to a variety of reasons, such as the need to titrate antibodies when new lots are needed and instability of tandem dyes when cocktailed. Lyophilized cocktails help address the issue of tandem dye instability. Our unique lyophilization technique allows drying down of multi-color cocktails containing tandem dyes such PE/Cy5, PE/Cy7, PerCP/Cy5.5 and APC/Cy7 together. This increases the shelf life of such cocktails from a few months to potentially a few years.

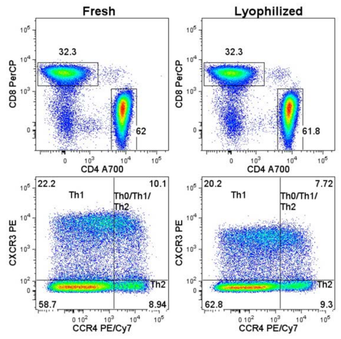
Here we show that lyophilized cocktails show performance similar to that of a freshly mixed cocktail, based on the percent positive of cells detected and signal-to-noise ratio for most markers tested. Some markers show a lower signal-to-noise ratio post-lyophilization, but still allow optimal resolution between positive and negative populations. Once lyophilized, these cocktails are stable, limiting inter and intra-assay variability. Differences between pipetting techniques between users mixing the single color reagents contribute to inter and intra assay variability, particularly in multi-center trials.

BioLegend offers custom packaging solutions, including single and multi-test sizes. Large lot sizes can also be made, allowing further reduction of assay variability.

Materials and Methods

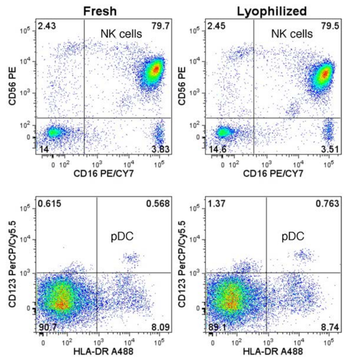
Antibodies were titrated to determine optimal concentration and then lyophilized in a single test format. On the day of usage the cocktail was reconstituted with distilled, deionized water and then cells were added to the antibody cocktail, incubated for 15-20 minutes, washed and then acquired on a flow cytometer equipped with appropriate lasers and detectors.

Figure 1. Lyophilized cocktails show similar T cell staining profile as that observed with a liquid cocktail



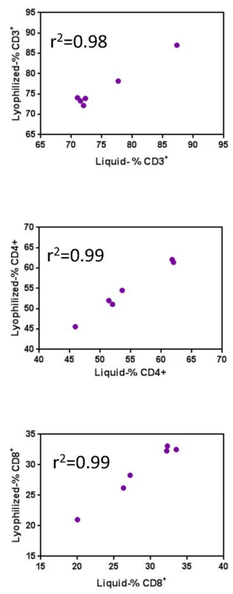
An 8 color cocktail containing CD3 APC/Cy7, CD4 Alexa Fluor ®700, CD8 PerCP, CXCR5 Brilliant Violet 421™, CXCR3 PE, CCR6 APC, CCR4 PE/Cy7 and HLA-DR Alexa Fluor ® 488 was lyophilized and then tested on a PBMC preparation in comparison to a freshly mixed liquid cocktail. Left panel shows staining with fresh liquid cocktail and right panel shows staining with a lyophilized cocktail. Similar frequencies of $CD4^+$, $CD8^+$, Th0/Th1/Th2, Th1 and Th2 cells were detected.

Figure 2. Lyophilized cocktails show similar T cell staining profile as that observed with a liquid cocktail



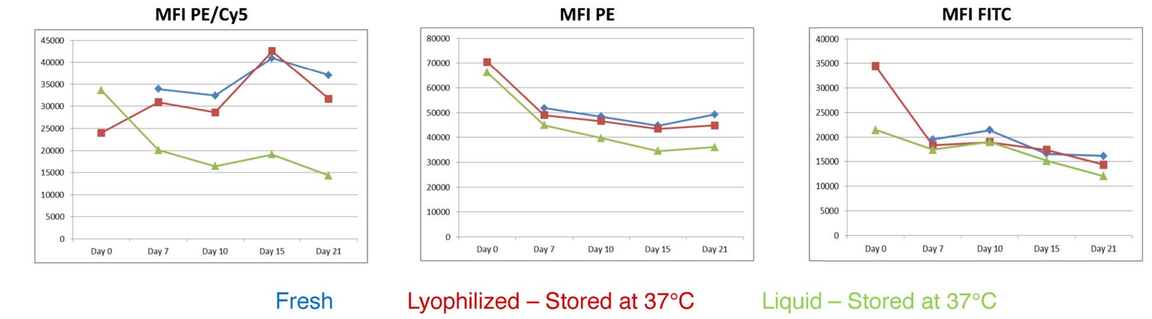
A 12-color cocktail consisting of CD3 Brilliant Violet 510™, CD4 Alexa Fluor ®700, CD11b PE/Dazzle 594™, CD11c APC, CD14 Pacific Blue™, CD16 PE/Cy7, CD19 Brilliant Violet 510™, CD20 Brilliant Violet 510™, CD34 APC/Cy7, CD56 PE, HLA-DR Alexa Fluor ® 488 , CD123 PerCP/Cy5.5, was lyophilized and then tested on a PBMC preparation in comparison to a freshly mixed liquid cocktail. Left panel shows staining with fresh liquid cocktail and right panel shows staining with a lyophilized cocktail. Similar frequencies of NK cells subsets ($CD56^+CD16^-$, $CD56^+CD16^+$ and $CD56^-CD16^+$) and pDCs ($CD123^+HLA\text{-}DR^+$) were detected.

Figure 3. Lyophilized and liquid cocktails show good correlation of percent positive cells



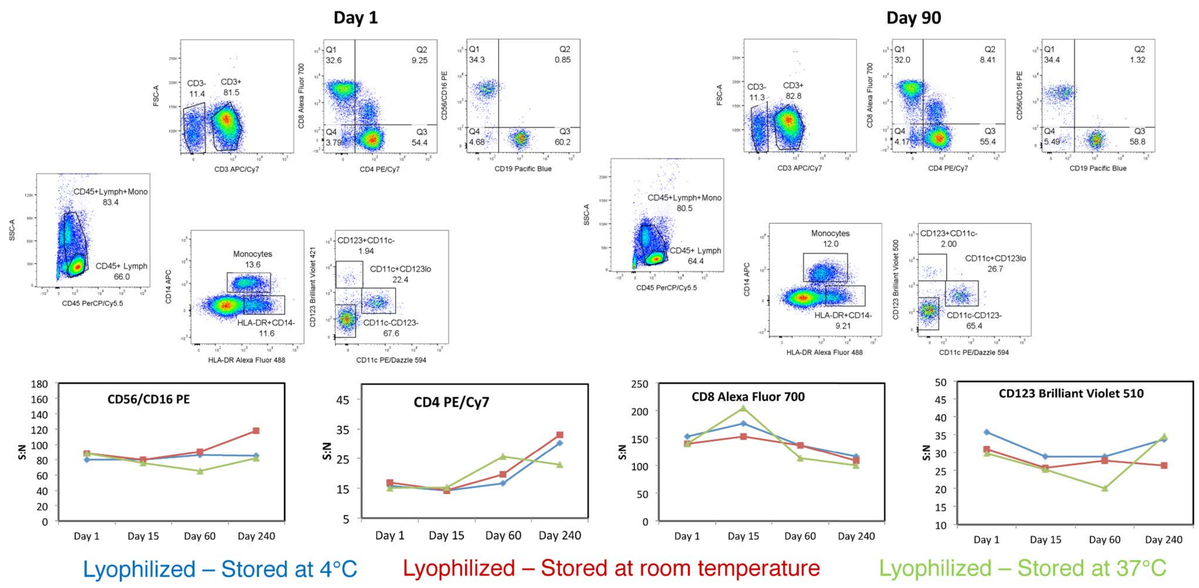
Multiple different lots of a lyophilized cocktail consisting of CD3 PE/Cy5, CD4 PE and CD8 FITC were used to stain human whole blood samples using a lyse-wash assay. Correlation of $CD3^+$ (top panel), $CD4^+$ (middle panel) and $CD8^+$ (bottom panel) T cells is depicted.

Figure 4. 3-color lyophilized cocktails containing PE/Cy5 show better stability compared to liquid cocktails, allowing long term storage at ambient temperature



A 3-color cocktail consisting of CD3 PE/Cy5, CD4 PE and CD8 FITC was prepared and lyophilized. The cocktail vials were tested immediately post lyophilization in comparison to a freshly mixed cocktail and a liquid pre-mixed cocktail and then at days 7, 10, 15 and 21. Mean Fluorescence Intensity (MFI) of FITC, PE and PE/Cy5 were recorded. Change in MFI at different times is attributed to donor variability and is not related to the cocktail reagents. MFI of FITC and PE remained stable over time, whereas PE/Cy5 signal dropped significantly in the pre-mixed cocktail stored at 37°C. In the lyophilized cocktail stored at 37°C, PE/Cy5 MFI stayed stable in comparison to the freshly mixed cocktail over a period of 21 days, demonstrating that lyophilized cocktails are more stable compared to liquid pre-mixed cocktails, thus allowing storage of lyophilized reagents at ambient temperatures, which cannot be done with liquid pre-mixed cocktails.

Figure 5. Accelerated stability testing demonstrates excellent long term stability of a 13-color lyophilized antibody cocktail



A 13-color cocktail consisting of CD3 APC/Cy7, CD4 PE/Cy7, CD8 Alexa Fluor® 700, CD11c PE/Dazzle 594™, CD14 APC, CD45 PerCP/Cy5.5, CD56/CD16 PE, CD123 Brilliant Violet 510™, HLA-DR Alexa Fluor ® 488, was prepared and lyophilized. The cocktail vials were tested post lyophilization in comparison to a freshly mixed cocktail and a liquid pre-mixed cocktail. Mean Fluorescence Intensity (MFI) and percent positive of all T cells, B cells, NK cells, monocytes, mDC and pDC were recorded at each time point. Veri-Cells™ PBMC were used as the target cell population for testing the cocktail to avoid donor-to-donor variability.

Conclusions

1. Antibody fluorophore conjugates can be lyophilized and stabilized.
2. All dyes including FITC, PE, APC and tandem dyes such as PE/Cy7, PE/Dazzle 594™, and APC/Cy7 can be stabilized without significant loss in signal.
3. Accelerated stability studies indicate that lyophilized reagents maintain their signal-to-noise ratio in warm (37°C) and cold (4°C) temperatures.

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