Development of a Dried-down, Multicolor Reagent Solution for Enhanced Flow-cytometric Applications

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Abstract
Multicolor flow cytometry provides a powerful tool to identify, analyze and enumerate multiple cell populations phenotypically, thereby making it a critical tool for hematological testing and diagnosis of hematological malignancies, as well as immune monitoring. As part of its custom reagents program, BD offers panel design tools and manufacturing of dried-down reagent cocktails for a diverse range of flow cytometry research applications. The dried-down cocktails afford enhanced reagent stability, simply the assay workflow and enable assay standardization across instruments, operators and testing sites.

The emergence of high-performing BD Horizon Brilliant™ dyes has resulted in significant demand to utilize these dyes in multicolor reagent panels. Conjugates made with BD Horizon Brilliant™ dyes are bright and provide excellent performance across multiple assays and applications. However, cocktails multiple BD Horizon Brilliant™ magnets may result in unwanted dye-to-dye interactions potentially impacting the stability of the cocktails. To address this issue, BD has developed a technology that enables the delivery of multiple BD Horizon Brilliant™ reagents in a single-use, dried-down format. The development of this technology enables BD to manufacture dried-down reagent cocktails containing up to five BD Horizon Brilliant™ magnets.

To demonstrate feasibility of this technology, we designed 2 panels (5-color and 7-color) comprising CD3, CD4, CD8, CD127, CD69 and CD161 for identification of regulatory T cells (Treg) and characterizing the different Treg subsets (naïve, effector and transitional), as well as the IL-17-producing Treg (CD161+) and the potentially immunosuppressive CD15s+. As part of assessing the feasibility of the reagent drying technology, we compared the performance of the 7-color Treg panel in a dried-down state and as a liquid cocktail using the BD FACSCalibur™-cell analyzer. Our results show that the performance of the dried-down cocktail is free of unwanted dye-to-dye interactions and is equivalent to the cell analyzer setup. In terms of resolution of the different functional Treg subsets, specifically, the percent of positive cells measured for the different Treg subsets (in a given sample) are comparable between the dried-down and the liquid cocktail. This data demonstrates the feasibility of the reagent drying technology.

Technology Overview
BD custom multicolor panels - BD Horizon™ On Chroma multicolor cocktails offer:

- Greater workflow efficiency
  - Proprietary technology to dry down up to 5 BD Horizon Brilliant™ dyes in one tube reduces the need for manual pipetting steps in your laboratory
  - Improves ease-of-use by providing up to 14 parameters in a single tube format
  - Reduces time to results with a streamlined workflow: simply resuspend the dried reagents, then add your sample, with no need for pipetting

- Greater standardization
  - An all-in-one tube format reduces operator errors due to liquid pipetting and co-staining
  - Batch matching of dried panels provides ideal format for inter- and intra-laboratory assays, enabling better data reproducibility
  - Reduces day-to-day variability, resulting in more consistent results

- Greater stability
  - 2-year shelf life when stored at room temperature (4-25°C)
  - Pouched in atright, resealable, light-resistant big bag
  - Resuspension of dried reagents with BD Horizon™ Brilliant Stain Buffer or BD Horizon Buffet™ Brilliant Stain Buffer Plus ensures minimal dye-to-dye interaction during staining

- Greater flexibility
  - Evaluation tubes included with every order ensure panel performance before scale-up
  - Dedicated BD Custom Technology Team available to optimize panel design for research use only (RUC) applications
  - Custom packaging options available to meet your lab’s specialized needs

Results 1. Single Color Analysis for CD4+

Results 2. Evaluation of the 5-color Treg Panel

Results 3. Evaluation of the 7-color Treg Panel

Conclusions
- The technology for BD Horizon™ On Chroma reagents provides a powerful tool to simplify and standardize laboratory workflow and maximize lab efficiency by eliminating repetitive pipetting and human-error errors. The dried cocktail will deliver easy-to-use standardization of multicolor flow cytometry data across multiple instruments and set the stage for its application in diverse areas of flow cytometry-based research.

- The single-color CD4 conjugates reported here across the various BD, ultraviolet, and blue laser lines demonstrate comparable performance between the dried and liquid reagents with minimal impact on reagent brightness.

- A 7-color Treg panel containing five BD Horizon Brilliant™ magnets has been dried down and proven to be comparable to the liquid panel with no significant impact on the resolution of the Treg populations.

- To further demonstrate the feasibility of the drying technology, a 7-color Treg panel was performed using the BD FACSDiva™ software. Population statistics are shown as percent of parent population for the respective subsets. Results from the panel showed the resolution for both dried-down and liquid cocktails are comparable with a minor percentage drop between the respective Treg subsets.

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