StarBright Dyes: Improved Panel Design with New StarBright Blue, StarBright Yellow, and StarBright Red Dyes
S. Sanderson and M. Blundell

StarBright™ Blue (SBB), StarBright Yellow (SBY), and StarBright Red (SBR) Dyes are the latest bright dyes designed for flow cytometry from Bio-Rad®. They expand the range of StarBright Dyes, which also includes StarBright UltraViolet (SBUV) and StarBright Violet (SBV) Dyes, across all five common laser lines. These superior dyes deliver tuneable brightness and spectral properties, greater stability, minimal background staining, improved lot-to-lot reproducibility, and spectral consistency.

The SBB and SBY Dyes are bright and allow an expansion in the number of dyes excited by the 488 nm and 640 nm lasers, respectively. SBY Dyes are bright, true 561 nm excitable dyes with reduced excitation from the 488 nm laser.

Data shown here from the five laser ZE5 Cell Analyzer demonstrate the benefits of using new StarBright Dyes in multiplexing panels. Firstly, we show how StarBright Dyes from the entire StarBright Dye range, alongside some traditional fluorescent dyes, can be used in a multiplex panel, allowing the identification of many peripheral blood subsets with high resolution and without the requirement of special staining buffers. In addition, we show, in a smaller panel, that SBY Dyes improve the resolution of cell populations, compared to using traditional 561 nm excitable dyes, by reducing compensation and spreading.

Bright with reduced spillover, multiplexing compatible with no requirement for a special buffer, and high stability make StarBright Dyes perfect for all flow cytometry experiments regardless of panel size and protocol.

Materials and Methods

Staining conditions. Red blood cell lysed human peripheral blood was blocked with 10% human serum. Cells were incubated with an antibody panel or a single antibody, for compensation control tubes. Cells were stained in a 36-well plate for 1 hr at room temperature, washed 3X in FACS Buffer (PBS + 1% BSA) and resuspended in FACS Buffer. Propidium Iodide (PI) (#1351101, Bio-Rad) was added 5 min prior to acquisition.

Multiplex panels. Antibodies used in the large panel are shown in Table 1. All antibodies in the panels were titrated to determine the optimal staining concentration prior to use.

Data collection and analysis. Cells were acquired on a 5-laser, 30-parameter ZE5 Cell Analyzer with option A, 355 nm laser upgrade (Bio-Rad). 300,000 cells were acquired for the multiplex panels and 60,000 cells for the single stained controls. Analysis was performed using FCS Express 7 Software (DeNovo Software).

Conclusions

• The StarBright Dye range is expanding to include dyes excited by the 488, 561 and 640 nm lasers, offering bright dyes with narrow excitation and emission spectra (Figure 1).
• StarBright Dyes can be used together in multiplexing panels without the requirement for a special buffer (Figure 2).
• StarBright Yellow Dyes improve the resolution of cell populations, compared to using traditional 561 nm excitable dyes in a panel (Figure 3).
• StarBright Dyes are an excellent choice for inclusion in multiplexing panels.

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