StarBright Dyes are fluorescent nanoparticles, with superior brightness over conventional flow cytometry dyes. They have narrow excitation and emission peaks, hence low spillover, spreading and compensation, providing greater flexibility when building multiplex panels in flow cytometry. They are conjugated to numerous antibodies against surface markers found on human and mouse cells. High stability and batch consistency have been confirmed using the 5-laser and 27-detector ZE5 Cell Analyzer. The antibody conjugates also showed high reagent compatibilities, without a need for special staining buffers, even in multiplex panels with additional conjugates, and can be premixed and used at a later date. Furthermore, they reliably identify positive cells regardless of the staining buffer and conditions used, providing flexibility and helping researchers avoid false negatives. StarBright Dyes are new valuable tools to fulfill unmet needs in immunology-based flow cytometry.

**Materials and Methods**

**Excitation and emission spectra:** the StarBright Violet Dyes (SBV) spectra were generated using the Bio-Rad Spectraviewer available from bio-rad-antibodies.com/spectraviewer.

**Data collection and analysis:** data for these studies were collected on a 5-laser, 27-parameter ZE5 Cell Analyzer and analysis was performed using FlowJo 10.7.1 Software (Becton Dickinson & Company). Data for spectral signature and cellular analysis were collected on a Cytiva Aurora using SpectroFio Software.

**Ambient light stability:** conjugates and controls were stored at room temperature and exposed to ambient light for 4 and 14 days. Red blood cell lysed human blood was stained with the treated samples and compared to aliquots stored at 4°C in the dark. Cells were stained in FACS buffer (1x DPBS, 1% BSA) for 1 hr at room temperature. Cells were washed x3 and resuspended in FACS buffer prior to data acquisition.

**Staining buffer and fixation compatibility:** red blood cell lysed human blood was stained with CD4 SBV515 and FACS buffer (1x DPBS, 1% BSA), Bio-Rad Stain Buffer, Bio-Rad Fixation Buffer, or Brilliant Stain Buffer (Becton Dickinson & Company). Cells were stained in a 96-well plate for 1 hr at room temperature, washed x3 and resuspended in the appropriate buffer for data collection. For fixation, following surface staining, cells were fixed in either Bio-Rad Fixation Buffer or 2% paraformaldehyde prior to data acquisition.

**Cocktail storage:** red blood cell lysed human blood was stained with FACS buffer (1x DPBS, 1% BSA) for StarBright Dye conjugates. The antibody cocktails were either premixed for 7 or 28 days and stored at 4°C. The aged and fresh cocktails were compared. Cells were stained for 1 hr at room temperature, washed x3 and resuspended in staining buffer prior to data acquisition.

**StarBright Dyes Have Narrow Emission Spectra and Minimal Spillover**

**StarBright Dye Stability and Reagent Compatibility**

**Unique Spectral Signature of StarBright Dyes Allows Multiplexing of Dyes with Similar Emission**

**StarBright Dyes Stored as a Master Mix Cocktail**

**Conclusions**

- StarBright Dyes offer superior brightness with narrow excitation and emission spectra.
- They have unique spectral signatures and can be used in combination with dyes with similar spectra in spectral flow.
- They have similar or less spillover than competitors’ dyes.
- They are compatible with commercially available staining buffers without the addition of a special reagent when used in multiplex panels.
- They retain brightness and correct population identification when fixed.
- They can be stored as a master mix for up to 2 months at 4°C.

**Bio-Rad** is a trademark of Bio-Rad Laboratories, Inc. in certain jurisdictions. All trademarks used herein are the property of their respective owner.