StarBright Red Dyes: Superior Dyes for Flow Cytometry

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The StarBright™ Dye range is expanding as we release StarBright Red Dyes excited by the 640 nm laser. They have narrow excitation and emission peaks with unique spectral signatures, hence low spillover, spreading, and compensation, providing greater flexibility when building multiplex panels in flow cytometry. StarBright Dyes have been conjugated to numerous antibodies against surface markers found on human and mouse cells, as well as streptavidin. StarBright Dyes show high reagent compatibility without a need for special cell staining buffers, even in multiplex panels. Furthermore, they can reliably identify positive cells regardless of the staining buffer and conditions used. StarBright Dyes come in a variety of brightness, providing more flexibility and helping researchers build higher plex panels.

Streptavidin conjugates have been used to construct tetramers to identify antigen specific T cells and with biotinylated primary antibodies to identify cell populations in flow cytometry. StarBright Dyes are new valuable tools to fulfill unmet needs in immunology-based flow cytometry.

StarBright Red (SBR) Dye Excitation and Emission Spectra

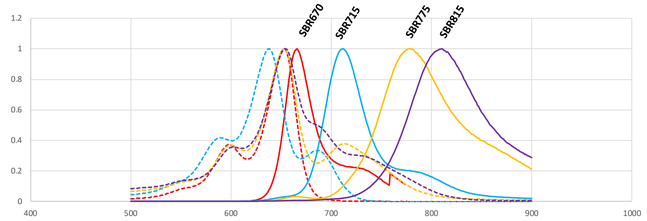

Fig. 1. Excitation and Emission spectra for StarBright Red Dyes. Dotted lines are excitation spectra and solid lines emission spectra. StarBright Red 670, 715, 775, 815

Materials and Methods

Staining conditions for multiplex panel: Human peripheral blood mononuclear cells (PBMCs) were incubated with a cocktail containing eight antibodies or a single antibody, for compensation control tubes. Cells were stained in a 96-well plate for 1 hr at 4°C , washed four times in FACS buffer (PBS + 3% FBS, 0.09% sodium azide), and resuspended in FACS buffer. 4',6-diamidino-2-phenylindole (DAPI) was added 5 minutes prior to acquisition. All antibodies were titrated for optimal concentration.

Staining for stain index: Peripheral blood mononuclear cells (PBMCs) were stained with StarBright conjugated anti-human CD4 for 1 hour at 4°C, washed three times, and resuspended in FACS buffer.

PBMC stimulation for tetramer staining: HLA-A*02:01 PBMCs were stimulated for 9 days with EBV BMLF-1 Pepmix (JPT Peptide Technologies) prior to staining in RPMI complete (RPMI, 10% FBS, 1%NEAA, 1/%, Penstrep). Cells were stained in a 96-well plate for 1 hour at 4 °C, washed four times then resuspended in FACS buffer.

Tetramer assembly: Tetramers were assembled using biotinylated monomer HLA-A*02:01 loaded with either EBVBMLF-1 or a negative peptide (TB-0011-M and TB0029-M respectively, MBL international) and either a SBV510-SA or PE-SA. The biotinylated monomers and fluorophore-SA conjugates were assembled per the NIH tetramer protocol. (https://tetramer.yerkes.emory.edu/support/protocols#10)

Stain index (SI) calculation: Cells were gated for single cells, scatter, lymphocytes, and the positive and negative populations within the lymphocyte gate. The median fluorescence intensity (MFI) of the negative and positive populations and the robust standard deviation (rSD) for the negative populations were quantified. The stain index was calculated with the following formula: $SI = (MFIpos - MFIneg)/(2XSDneg)$

Data collection and analysis: Spectral signatures and similarity index were collected on a Cytek 5-laser 64 channel Aurora (Cytek Biosciences). Data for the multiplex and tetramer studies were collected on a 5-laser, 30-parameter ZE5 Cell Analyzer. 150,000 cells were collected for the multiplex panels and 60,000 cells for the single-stained controls and CD4 samples. Analysis was performed using FlowJo 10.8 (BD Biosciences).

Table 1. Bio-Rad reagents used in the multiplex panel.

Multiplex

Target	Fluorophore	Catalog number
CD3	SBV760	MCA463SBV760
CD4	SBR775	N/A
CD8	SBY800	N/A
CD19	SBR815	N/A
CD20	FITC	MCA1710F
CD25	PE	BioLegend 302605
CD127	PECF594	BDBioscience 562397
Live/Dead	PURE BLU™ DAPI	1351303

Multiplex with tetramer

Target	Fluorophore	Catalog number
CD3	FITC	MCA6147F
CD8	APC	MCA1226APC
EBV BMLF-1 Tet	SBV510	N/A
EBV BMLF-1 Tet	PE	N/A
Negative Tet	SBV510	N/A
Negative Tet	PE	N/A

Antibodies shown in red are pre-launch material. APC, allophycocyanin; DAPI, 4',6-diamidino-2-phenylindole; FITC, fluorescein isothiocyanate PE, phycoerythrin; SBV, StarBright Violet.

StarBright Red Dyes Have Unique Spectral Signatures

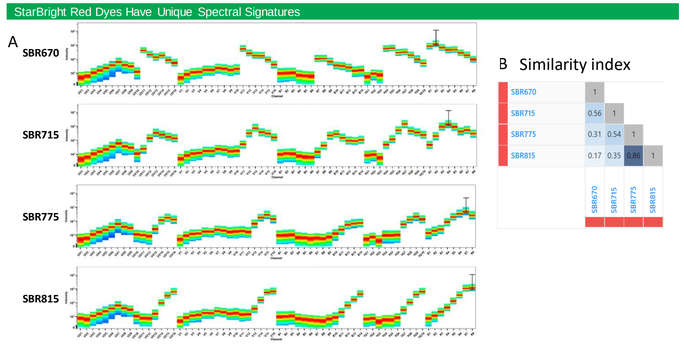

Fig. 2. Spectral signatures of StarBright Red (SBR) Dyes. A, SBR Dyes have a unique signature when viewed on a spectral analyzer. **B,** SBR Dyes have a low similarity index, thus can be used in multiplex panels. Data were collected and analyzed on a Cytek Aurora (Cytek Biosciences).

StarBright Red Used in a Multiplex Panel

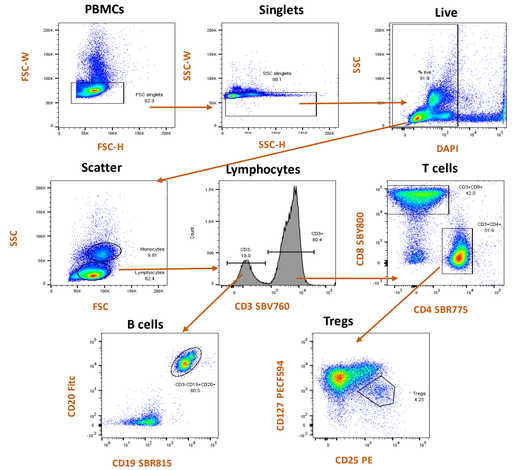

Fig. 3. StarBright Red Dyes in multiplex with other dyes do not require special buffers. Human PBMCs were stained with an 8-color panel and data were analyzed on a 5-laser 30-parameter ZE5 Cell Analyzer.

StarBright Dyes have a Variety of Brightness Across the Spectrum

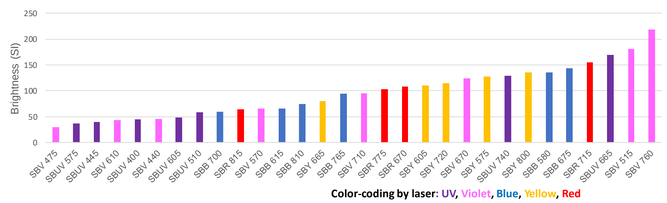

Fig. 4. StarBright Dyes have a broad range of brightness, providing greater flexibility for building multiplex panels. SI is calculated based on StarBright anti-CD4 staining of human PBMCs. Data were acquired on a ZE5 Cell Analyzer.

StarBright Streptavidin Conjugates Used to Build Tetramers to Identify Antigen Specific T Cells

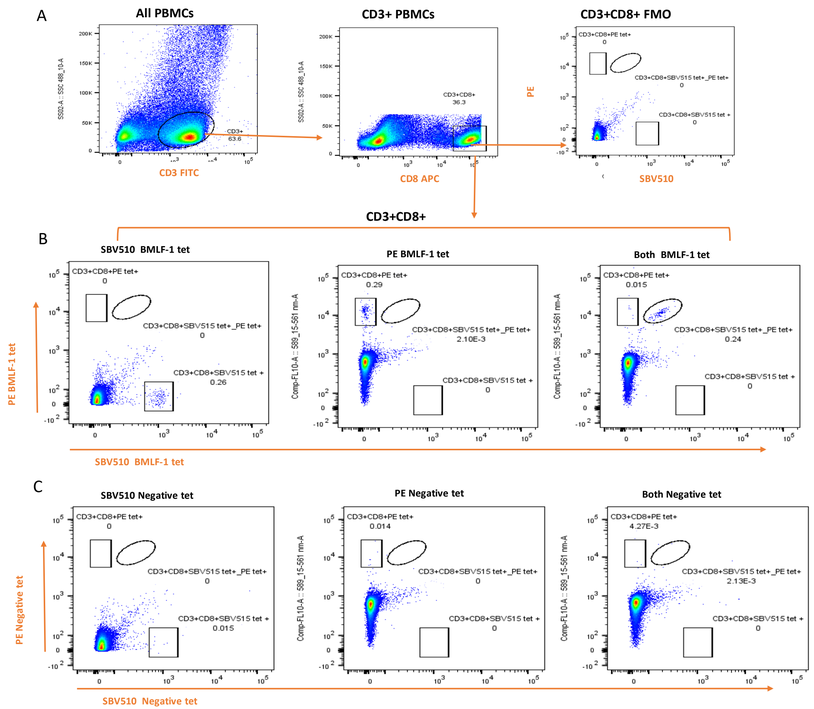

Fig. 4. StarBright Dyes in combination with MHC I monomers form tetramers and identify EBV BMLF-1 specific T cell responses. PBMCs were stimulated for 9 days with EBV BMLF-1 Pepmix (JPT Peptide Technologies) and stained with anti-CD3, CD8 in the presence or absence of tetramers. (A) All PBMCs were gated on CD3+ and CD8+, CD3+CD8+ cells visualized in SBV510 vs. PE. (B) PBMCs were stained with anti-CD3, CD8, and either singly with SBV510 BMLF-1 tet, PE BMLF-1 tet, or both . (C) PBMCs were stained with anti-CD3, CD8, and either singly with SBV510 Negative tet, PE Negative tet, or both.

Conclusions

- StarBright Red Dyes have narrow excitation and emission spectra and unique spectral signatures
- StarBright Dyes can be multiplexed without special stain buffers
- StarBright Dye streptavidin conjugates can be used to build MHC tetramers to identify antigen specific T cells
- StarBright Dyes are new valuable tools to fulfil unmet needs and provide greater flexibility for researchers building higher plex panels

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