affymetrix eBioscience

Super Bright polymer dyes

Bright dyes for the violet laser

Featuring

- Super Bright 436
- Super Bright 600
- Super Bright Staining Buffer

The eBioscience™ Super Bright dyes are a line of bright fluorochromes, based on a polymer and its tandems, that are excited by the violet laser (405 nm). All Super Bright formats are named for their emission wavelength (Figure 1). These dyes are optimized for use in flow cytometry and may allow for better discrimination of dim populations due to their brightness. Certain dyes may display less nonspecific interaction with other polymer dyes as compared to similar competitor reagents.

The Super Bright polymer dyes are fully compatible with other commonly used fluorescent molecules, eBioscience buffers and fixatives, and UltraComp eBeads™ microspheres. These features, combined with our broad portfolio of biological content, easily enable dye selection for optimized flow cytometry multicolor antibody panel design and allow you to expand the utility of your violet laser.

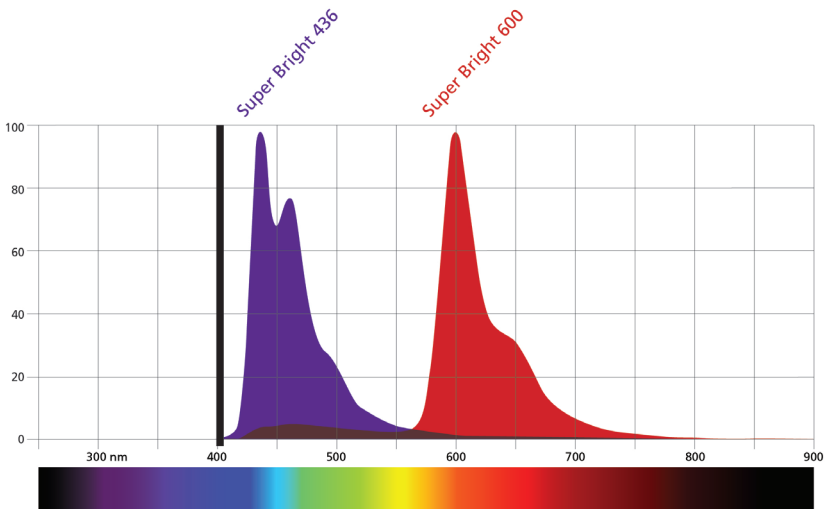

Figure 1. Super Bright emission spectra.

Super Bright 436

Super Bright 436 has an excitation maximum of 414 nm and an emission peak of 436 nm. We recommend using a 450/50 bandpass filter or equivalent, similar to eFluor™ 450. This polymer dye is significantly brighter than eFluor 450, and is an alternative for Brilliant Violet™ 421 with similar resolution of positive and negative populations (Figure 2). Stability studies indicate that Super Bright 436 exhibits a minimal loss of fluorescence when cells are exposed to formaldehyde fixative for up to three days, or overnight to ambient light.

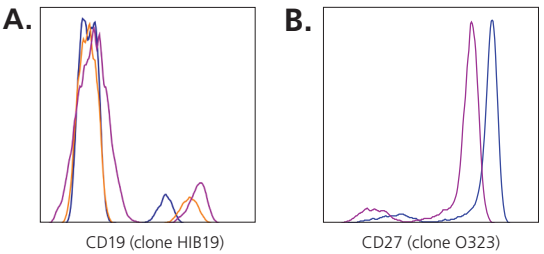

Figure 2. Fluorescence intensity comparison.
(A) Human peripheral blood cells were stained with Anti-CD19 (clone HIB19) conjugated to either Super Bright 436 (purple histogram), eFluor 450 (blue histogram), or Brilliant Violet 421 (orange histogram) using the manufacturer's recommended volume per test.
(B) Anti-human peripheral blood cells were stained with Anti-CD27 (clone O323) conjugated to either Super Bright 436 (purple histogram) or Brilliant Violet 421 (blue histogram).

Super Bright 600

Super Bright 600 is a tandem dye consisting of Super Bright 436 and an acceptor dye that emits at 600 nm. It can be detected using a 610/20 bandpass filter or equivalent, similar to eVolve™ 605. This tandem polymer dye is comparable in brightness to Brilliant Violet™ 605 (Figure 3) and is brighter than eVolve 605. Super Bright 600 is stable for up to three days when stored in a formaldehyde fixative solution (Figure 3).

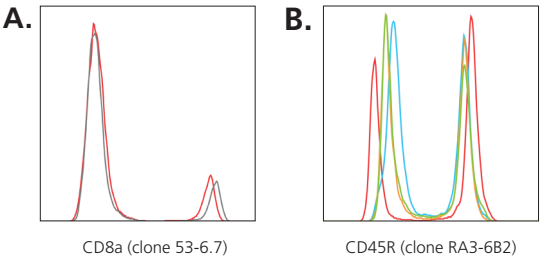

Figure 3. Staining performance and post-fixation stability.
(A) Direct comparison of mouse splenocytes stained with Anti-CD8a (clone 53-6.7) conjugated to either Super Bright 600 (red histogram) or Brilliant Violet 605 (gray histogram), at the same concentration of antibody.
(B) Mouse splenocytes were stained with Anti-CD45 (clone RA3-6B2) Super Bright 600 and either left unfixed (red histogram), or were fixed in IC Fixation buffer for 30 minutes (blue histogram), 24 hours (orange histogram), or three days (green histogram).

Super Bright Staining Buffer

Super Bright dyes can be used in flow cytometric applications similarly to traditional fluorophores. However, if multiple Super Bright-conjugated antibodies are combined in the same panel, the use of Super Bright Staining Buffer (Cat. No. SB-4400) is recommended to minimize any nonspecific interaction that may occur between these polymer-based dyes (Figure 4). No special buffer is required when using a single Super Bright-conjugated antibody within a panel. When using Super Bright dyes in combination with other polymer dyes, such as Brilliant Violet dyes, the Super Bright Staining Buffer can still be used to prevent dye–dye interaction. Super Bright Staining Buffer is formulated at 5 µL/test, making it convenient for use when preparing cocktails.

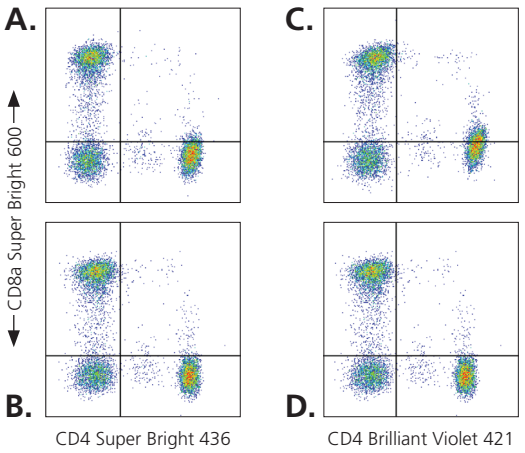

Figure 4. Super Bright Staining Buffer minimizes non-specific interactions.
Human peripheral blood cells were stained with Anti-CD8 (clone RPA-T8) Super Bright 600 and Anti-CD4 (clone SK3) conjugated to Super Bright 436 (A and B) or Brilliant Violet 421 (C and D). Cells were stained in the presence of Flow Staining Buffer only (A and C) or Super Bright Staining Buffer was added to cells prior to addition of antibodies (B and D).

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