



# Super Bright polymer dyes

## Bright dyes for the violet laser

### Featuring:

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

Invitrogen™ eBioscience™ Super Bright dyes are a line of bright fluorochromes, based on a polymer dye and its tandem dye, 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 reagents from other suppliers.

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

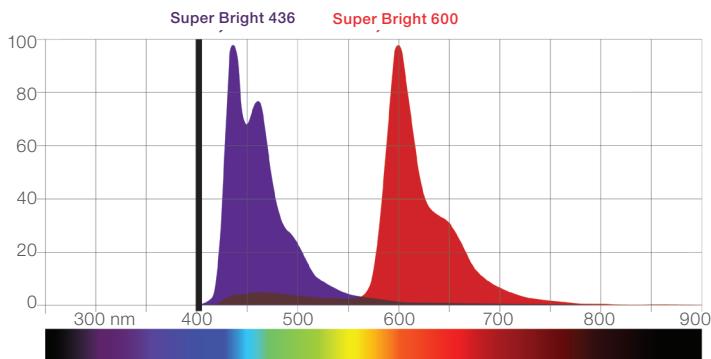


Figure 1. Emission spectra of Super Bright dyes.

## Super Bright 436

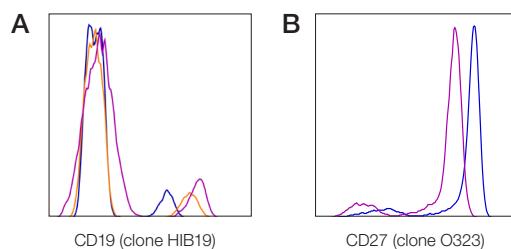
Super Bright 436 has an excitation maximum of 414 nm and an emission peak of 436 nm. We recommend using a 450/50 bandpass filter or equivalent, similar to Invitrogen™ eFluor™ 450 conjugates. This polymer dye is significantly brighter than eFluor 450 conjugates, and is an alternative for Brilliant Violet™ 421 conjugates 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 when exposed overnight to ambient light.

## Super Bright 600

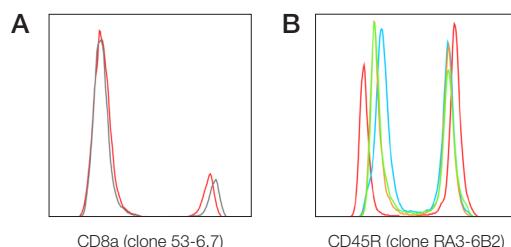
Super Bright 600 is a tandem dye consisting of Super Bright 436 and an acceptor dye that emits a fluorescence signal at 600 nm. It can be detected using a 610/20 bandpass filter or equivalent. This tandem polymer dye is comparable in brightness to Brilliant Violet™ 605 conjugates (Figure 3). Super Bright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

## Super Bright Staining Buffer

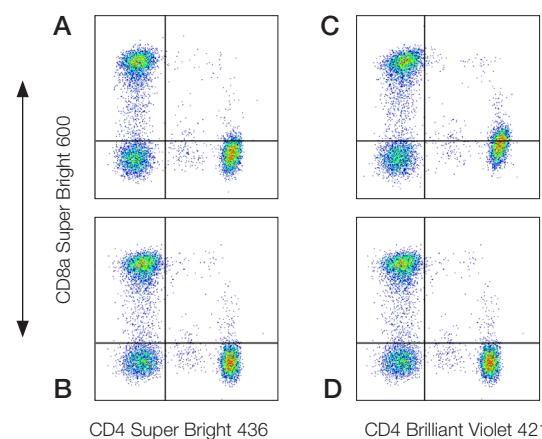
Super Bright dyes can be used in flow cytometric applications similarly to traditional fluorophores. However, if multiple Super Bright conjugates are combined in the same panel, the use of Invitrogen™ eBioscience™ Super Bright Staining Buffer (Cat. No. SB-4400) is recommended to minimize any nonspecific interaction that may occur between these polymer-based dyes (Figure 4). No special buffer is required when using a single Super Bright conjugate 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  $\mu$ L/test, making it more convenient for use when preparing cocktails.



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



**Figure 3. Staining performance and post-fixation stability.** (A) Direct comparison of mouse splenocytes stained with CD8a antibody (clone 53-6.7) conjugated to either Super Bright 600 (red histogram) or Brilliant Violet 605 dye (gray histogram), at the same antibody concentration. (B) Mouse splenocytes were stained with CD45R antibody (clone RA3-6B2) conjugated to Super Bright 600 and either left unfixed (red histogram), or were fixed in Invitrogen™ eBioscience™ IC Fixation Buffer for 30 minutes (blue histogram), 24 hours (orange histogram), or 3 days (green histogram).



**Figure 4. Super Bright Staining Buffer minimizes nonspecific interactions.** Human peripheral blood cells were stained with CD8a antibody (clone RPA-T8) conjugated to Super Bright 600 and CD4 antibody (clone SK3) conjugated to Super Bright 436 (A and B) or Brilliant Violet 421 dye (C and D). Cells were stained in the presence of Invitrogen™ eBioscience™ Flow Cytometry 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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