Minimize nonspecific interactions using Super Bright Staining Buffer

Introduction

Polymer-based dyes, such as Invitrogen™ Super Bright fluorochromes and Brilliant Violet™ fluorochromes, have been reported to exhibit nonspecific interactions when using more than one polymer dye–conjugated antibody together in the same flow cytometry experiment. These nonspecific interactions result in mutually exclusive immunostained populations that appear as under-compensated data. To correct this, Invitrogen™ Super Bright Staining Buffer (Cat. No. SB-4400-42) should be included during staining each time when more than one Super Bright fluorochrome–conjugated antibody is used or if Super Bright fluorochrome–conjugated antibodies are used in combination with Brilliant Violet fluorochrome–conjugated antibodies (Figure 1). The Super Bright Staining Buffer is comparable to a polymer-staining buffer from another supplier (Figure 2).

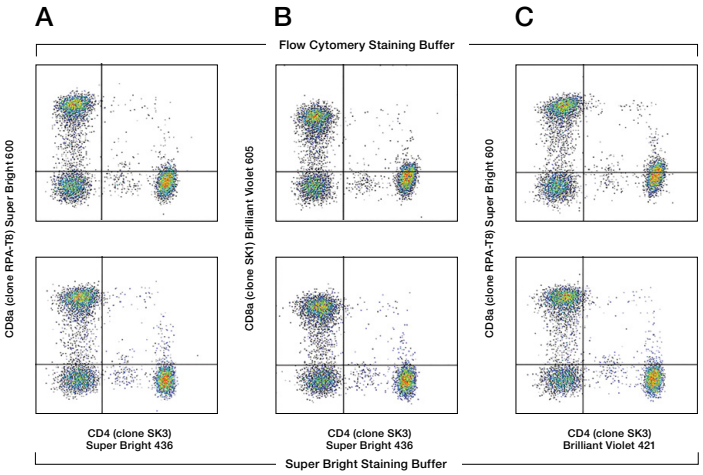

Figure 1. Super Bright Staining Buffer mitigates nonspecific polymer dye interactions. The top plots depict use of the Invitrogen™ eBioscience™ Flow Cytometry Staining Buffer (Cat. No. 00-4222), whereas the bottom plots show use of the Super Bright Staining Buffer. Flow Cytometry Staining Buffer or the Super Bright Staining Buffer was added to human peripheral blood cells prior to staining with **(A)** Anti-CD8a Super Bright 600 (Cat. No. 63-0088-42), and Anti-CD4 Super Bright 436 (Cat. No. 62-0047-42), **(B)** Anti-CD8 Brilliant Violet 605 and Anti-CD4 Super Bright 436, or **(C)** Anti-CD8a Super Bright 600 and Anti-CD4 Brilliant Violet 421.

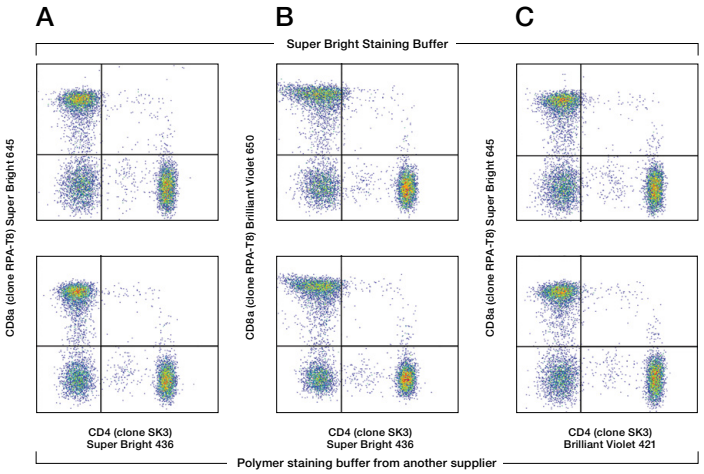

Figure 2. Super Bright Staining Buffer comparison to a polymer staining buffer from another supplier. Super Bright Staining Buffer (top plot), or a polymer staining buffer from another supplier (bottom plot) was added to human peripheral blood cells prior to staining with **(A)** Anti-CD8a Super Bright 645 (Cat. No. 64-0088-42) and Anti-CD4 Super Bright 436, **(B)** Anti-CD8a Brilliant Violet 650 and Anti-CD4 Super Bright 436, or **(C)** Anti-CD8a Super Bright 645 and Anti-CD4 Brilliant Violet 421.

invitrogen

General notes

- Super Bright Staining Buffer is provided in a convenient 5 µL/test format
- Super Bright Staining Buffer is compatible with traditional fluorochromes and Invitrogen™ eBioscience™ Fixable Viability eFluor™ dyes
- Super Bright Staining Buffer can result in increased background signal in the 450/50 bandpass detector (used to collect signal for Invitrogen™ eBioscience™ eFluor 450 dye and Super Bright 436) on the violet laser if used at higher than recommended amounts
- Super Bright Staining Buffer is not compatible with Invitrogen™ UltraComp eBeads™ Compensation Beads (Cat. No. 01-2222); if using UltraComp eBeads Compensation Beads as a compensation tool, solely use Invitrogen™ eBioscience™ Flow Cytometry Stain Buffer (Cat. No. 00-4222) for any antibody dilutions
- Super Bright Staining Buffer is compatible with red blood cell (RBC) lysis reagents such as Invitrogen™ eBioscience™ 1-Step Fix/Lyse (Cat. No. 00-5333) and Invitrogen™ eBioscience™ 10X RBC Lysis Buffer (Multi-species) (Cat. No. 00-4300)
- Super Bright Staining Buffer can also be used at the appropriate test concentration when preparing bulk (multitest) antibody cocktails
  - It is critical to add the Super Bright Staining Buffer first to the appropriate volume of Flow Cytometry Staining Buffer and mix well, prior to adding conjugated antibodies; cocktails should be made and used fresh, as storing cocktails can increase nonspecific polymer-dye interactions

Protocol

Materials

- Super Bright Staining Buffer
- 12 x 75 mm round-bottom test tubes
- Flow Cytometry Staining Buffer
- Primary antibodies (directly conjugated to fluorochromes)

Experimental procedure

1. Add 5 µL of Super Bright Staining Buffer to each tube. Super Bright Staining Buffer can be added directly to tubes or to previously aliquoted cells in tubes. When adding buffer to the cells, mix well by pipetting up and down or gently vortexing the sample.
2. Add appropriate amounts of each fluorochrome-conjugated antibody, including Super Bright dye– and traditional fluorochrome-conjugated antibodies, to the tubes containing Super Bright Staining Buffer.
3. Mix well after addition of each antibody by pipetting up and down or gently vortexing the sample.
   - Note: If a cocktail of antibodies is prepared in bulk, it should be used fresh to minimize nonspecific polymer–dye interactions.
4. If cells were not previously added to the tubes, aliquot 100 µL of cells to the buffer-antibody cocktail promptly.
5. Mix samples well by pipetting up and down or gently vortexing.
6. Incubate for 30 minutes in the dark at 2–8°C.
7. Wash the cells by adding 2 mL/tube of Flow Cytometry Staining Buffer. Centrifuge at 400–600 x *g* for 5 minutes. Discard supernatant.
8. Repeat Step 7.
9. Resuspend cells in an appropriate volume of Flow Cytometry Staining Buffer.
10. Analyze samples by flow cytometry, or if staining for intracellular targets, proceed with **"Best Protocols: Staining Intracellular Antigens for Flow Cytometry"**.

Find out more at **thermofisher.com/superbright**

ThermoFisher SCIENTIFIC