eBioscience™ Super Bright Staining Buffer

Catalog Numbers SB-4400-42 and SB-4400-75

Pub. No. MAN0018677 **Rev.** A.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

eBioscience™ Super Bright Staining Buffer is designed for use as a supplement to Flow Cytometry Staining Buffer in immunofluorescent staining protocols of cells in suspension. eBioscience™ Super Bright Staining Buffer is only necessary when using more than one polymer dye-conjugated antibody in the same sample to prevent nonspecific polymer interactions, which can result in data appearing under-compensated. eBioscience™ Super Bright Staining Buffer is provided in a convenient 5 µL/test format and is compatible with traditional fluorochromes, Brilliant Violet dyes, and standard flow cytometry protocols.

Product specifications

Concentration[1]	5 µL/test
Storage	Store at 2–8°C.
Application	Flow cytometry.
Testing	This product is tested by flow cytometric analysis of normal human peripheral blood cells or mouse splenocytes.
Batch code	See product label.
Use by	See product label.
Related product	Flow Cytometry Staining Buffer (Product No. 00-4222).

[1] A test is defined as the amount of buffer to be used in a final volume of 100 µL.

Important product information

- eBioscience™ Super Bright Staining Buffer is not compatible with UltraComp eBeads™ Compensation Beads (Cat. No. 01-2222). If using UltraComp eBeads™ Compensation Beads as a compensation tool, solely use Flow Cytometry Stain Buffer (Cat. No. 00-4222) for any antibody dilutions.
- eBioscience™ Super Bright Staining Buffer is provided in a convenient 5 µL/test format.
- eBioscience™ Super Bright Staining Buffer is compatible with traditional fluorochromes and Live/Dead and Fixable Viability eFluor™ dyes.
- eBioscience™ Super Bright Staining Buffer is compatible with RBC lysis protocols, such as 1-step Fix/Lyse (Cat. No. 00-5333) and 10X RBC Lysis Buffer (multi-species) (Cat. No. 00-4300).
- eBioscience™ Super Bright Staining Buffer can also be used at the appropriate test concentration when preparing bulk (multi-test) antibody cocktails.

Workflow

Materials required

- eBioscience™ Super Bright Staining Buffer (Cat. No. SB-4400-42 or SB-4400-75)
- 12 × 75 mm round-bottom test tubes
- Flow Cytometry Staining Buffer (Cat. No. 00-4222)
- Primary antibodies (directly conjugated to fluorochromes)

Procedure

1. Add 5 µL of eBioscience™ Super Bright Staining Buffer to each tube. Staining buffer can be added directly to tubes or to previously aliquoted cells in tubes. If adding to 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 and traditional fluorochrome-conjugated antibodies, to the tubes containing eBioscience™ 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 × *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* (available on our website).

Limited product warranty

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