invitrogen PRODUCT INFORMATION SHEET

UltraComp eBeads™ Spectral Unmixing Beads, UltraComp eBeads™ Plus Compensation Beads, and UltraComp eBeads™ Compensation Beads

Catalog Numbers U20250, 01-3333-41, 01-3333-42, 01-2222-41, and 01-2222-42

Pub. No. MAN0019374 Rev. C

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

Contents and storage

Product	Cat. No.	Size	Species reactivity	Storage
UltraComp eBeads™ Spectral Unmixing Beads	U20250	100 tests	Mouse, rat, hamster, rabbit, and human	2–8°C Do not freeze.
UltraComp eBeads™ Plus Compensation Beads	01-3333-41	25 tests	Mouse, rat, hamster, rabbit, and human	
	01-3333-42	100 tests		
UltraComp eBeads™ Compensation Beads	01-2222-41	25 tests	Mouse, rat, and hamster	
	01-2222-42	100 tests		

Product description

UltraComp eBeads™ Spectral Unmixing Beads:

UltraComp eBeads™ Spectral Unmixing Beads provide a consistent, accurate, and simple-to-use method for setting unmixing and compensation controls for fluorochrome-conjugated antibodies in conventional and spectral flow cytometry. The beads bind with antibodies of mouse, rat, hamster, rabbit, and human origin.

- Newest Generation: Low bead auto-fluorescence provides compatibility with UV to IR lasers.
- Best Compatibility: Works seamlessly with advanced spectral and conventional flow cytometry systems.
- Enhanced Performance in Spectral Flow Cytometry: Enable the most accurate unmixing and compensation among bead-based controls, ensuring high fidelity in multi-color experiments.

UltraComp eBeads™ Plus Compensation Beads:

UltraComp eBeads™ Plus Compensation Beads provide a consistent, accurate, and simple method for setting compensation controls for fluorochrome-conjugated antibodies in conventional flow cytometry. These beads bind with antibodies of mouse, rat, hamster, rabbit, and human.

- Broad Compatibility: Compatible with fluorochromes excited by lasers (355 nm, 405 nm, 488 nm, 532 nm, 561 nm, and 632–640 nm), including Super Bright 702, Super Bright 780, Brilliant Violet™ 711, and Brilliant Violet™ 786 conjugated antibodies.

UltraComp eBeads™ Compensation Beads:

UltraComp eBeads™ Compensation Beads provide a consistent, accurate, and simple method for setting compensation controls for fluorochrome-conjugated antibodies in conventional flow cytometry. These beads bind with antibodies of mouse, rat and hamster.

- Standard Compatibility: Compatible with fluorochromes excited by the most commonly used lasers (355 nm, 405 nm, 488 nm, 532 nm, 561 nm, and 632–640 nm), except for some far-red emitting 405 nm excitable dyes.
- Cell-Based Compensation: In some cases, compensation values for Super Bright™ 702, Super Bright™ 780, Brilliant Violet™ 711, or Brilliant Violet™ 786 conjugated antibodies may be higher in the violet 450/50 channel. For these situations, we recommend setting compensation with cells.

Each drop of beads contains two populations: a positive population that captures the fluorochrome-conjugated antibody used for cell staining, and a negative population that does not bind the antibody. This bimodal distribution is ideal for setting up single-color unmixing and compensation controls in multi-color flow cytometry experiments.

IMPORTANT! The UltraComp eBeads™ product line is not optimized for sorting workflows. For optimal performance in sorting applications, see the AbC™ Total Antibody Compensation Bead Kit (Catalog No. A10497).

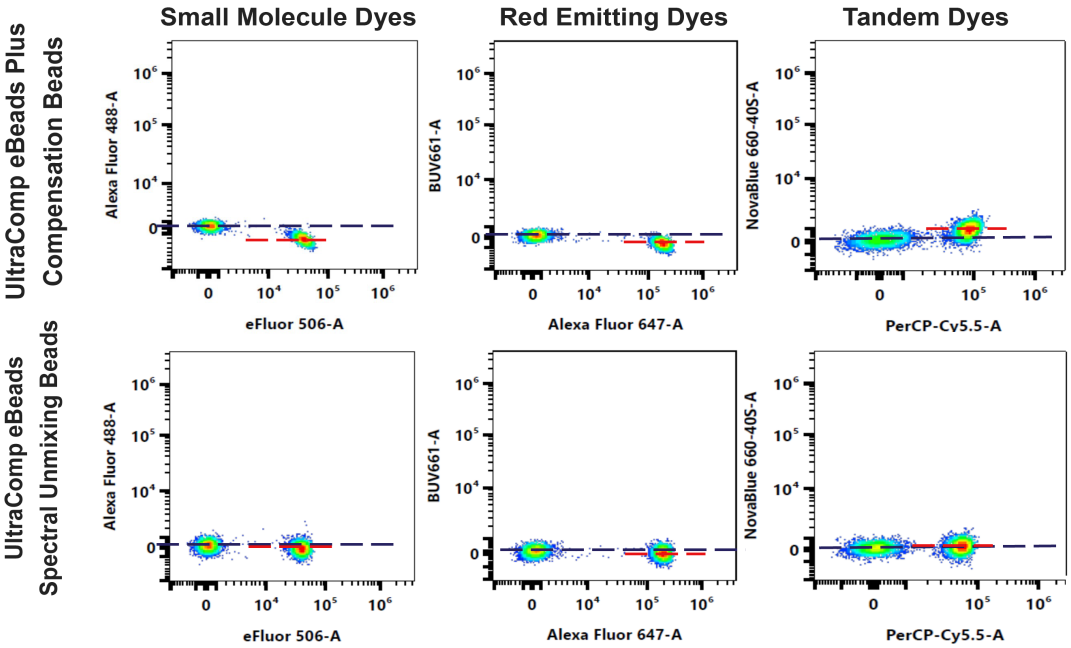

Figure 1 UltraComp eBeads™ Spectral Unmixing Beads improvements over UltraComp eBeads™ Plus. Representative flow cytometry dot plots of PBMCs stained with anti-CD4 antibodies in a 33-color, single-stained control panel. The samples were unmixed using either UltraComp eBeads™ Plus (Top) or UltraComp eBeads™ Spectral Unmixing Beads (Bottom) as single-color controls in a spectral flow cytometer. The plots demonstrate superior unmixing performance of UltraComp eBeads™ Spectral Unmixing Beads across different fluorophore combinations. This is evidenced by the closer alignment of the medians of negative (Black dotted line) and positive (Red dotted line) populations in UltraComp eBeads™ Spectral Unmixing Beads compared to UltraComp eBeads™ Plus.

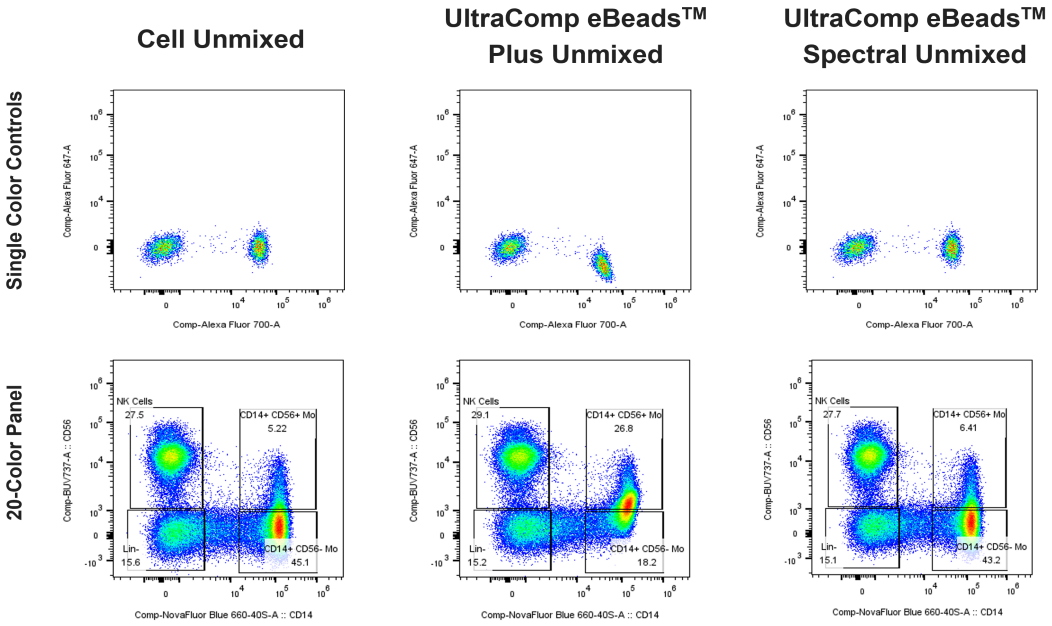

Figure 2 Unmixing sample using UltraComp eBeads™ Spectral Unmixing Beads. Spectral unmixing by UltraComp eBeads™ Spectral Unmixing beads of red emitting dyes is comparable to using matched cells as single-color unmixing controls and offers lower unmixing errors than the previous generation UltraComp eBeads™ Plus compensation beads. Human PBMCs stained with anti-CD4 antibodies in a 33-color, single-stained control panel (Top) or a 20-color human immunophenotyping panel (Bottom) were unmixed using either UltraComp eBeads™ Plus or UltraComp eBeads™ Spectral Unmixing Beads or Single stained PBMCs (Cells) as single-color controls in a spectral flow cytometer.

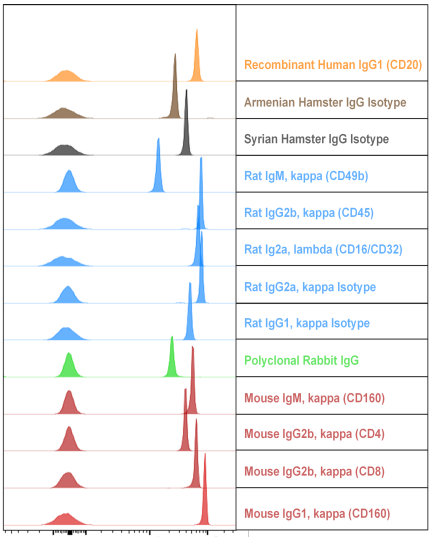

Figure 3 UltraComp eBeads™ Spectral Unmixing Beads bind up to 13 different antibody isotypes. Staining of UltraComp eBeads™ Spectral Unmixing Beads with 13 different R-PE-conjugated monoclonal antibodies, including one of each subclass commonly used in flow cytometry. The beads were stained with each antibody, then analyzed by flow cytometry. The x-axis shows the median fluorescence intensity of the stained beads in the R-PE detector. Each histogram represents one staining antibody.

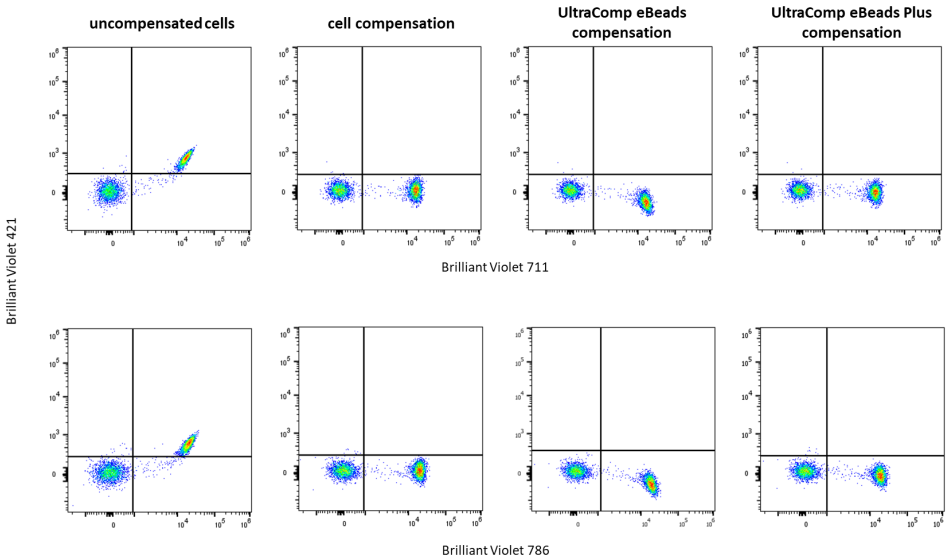

Figure 4 Compensation by UltraComp eBeads™ and UltraComp eBeads™ Plus compensation beads of 405 nm excitable, far red-emitting dyes is comparable to cells. Human peripheral blood mononuclear cells (PBMCs) were stained with anti-CD19 Brilliant Violet 421 and either anti-CD4 Brilliant Violet 711 or anti-human CD4 Brilliant Violet 786. Before analysis, single-stain samples were prepared to set compensation. 1×10^6 cells or 1 drop of either UltraComp eBeads™ or UltraComp eBeads™ Plus compensation beads were incubated with the antibody for 20 minutes, then washed with 1X PBS with 1% BSA.

Required materials not provided

- 12 x 75-mm round bottom test tubes
- Primary antibodies (directly fluorochrome conjugated)
- Flow Cytometry Staining Buffer (Cat. No. 00-4222) or other suitable staining buffer

Note: UltraComp eBeads™ Spectral Unmixing Beads, UltraComp eBeads™ Compensation Beads, and UltraComp eBeads™ Plus Compensation Beads are compatible with standard staining buffers that contain PBS or HBSS, proteins such as BSA or FBS, and sodium azide.

IMPORTANT! Do not use beads with Super Bright™ Staining Buffer, Brilliant Staining Buffer, CellBlox™ Plus, or other additives. For more information, contact Technical Support.

Prepare single-color controls

Note: If using 96 well plates, then adjust wash and redispersion volumes and decanting and mixing methods as required.

1. **Label tubes:** Label a tube for each antibody conjugate to be used in the experiment.

2. **Mix beads:** Mix the compensation or unmixing beads by vigorously inverting at least 10 times or by pulse vortexing.

3. **Add beads:** Add 1 drop of compensation or unmixing beads to each tube (there is no need to add staining buffer).

4. **Add Antibody Conjugate:** Add 1 test or less of antibody conjugate to each tube.

   Note: A test is defined as the amount (µg) of antibody that will stain a cell sample in a final volume of 100 µL. If high background is observed on the negative bead population, use 0.125 µg or less antibody. It is not necessary to use the antibody at its optimal concentration. For most antibodies, appropriate compensation or unmixing values result when 0.03–1.0 µg of antibody is used in the test.

5. **Mix well:** Mix well by flicking or pulse-vortexing.

6. **Incubate:** Incubate at 2–8°C for 15–30 minutes in the dark.

7. **Wash:** Add 2–3 mL of Flow Cytometry Staining Buffer to each tube, then centrifuge at 400–600 × *g* for 3–5 minutes.

8. **Decant supernatant:** Decant the supernatant, then add 0.2–0.4 mL of Flow Cytometry Staining Buffer to each tube.

9. **Mix before acquisition:** Mix by flicking or pulse-vortexing before analysis.

   Note: When developing a new spectral flow cytometry panel, we recommend the user predetermine what the best single-color controls (cells vs. UltraComp eBeads™ Spectral Unmixing Beads) are for each antibody marker in the panel. UltraComp eBeads™ Spectral Unmixing Beads have been designed for Spectral Flow cytometry and result in better unmixing performance than UltraComp eBeads™ Plus Compensation Beads (see Figure 1 and Figure 2), but single-color control optimization may still be required

   Note: Unmixing performance may vary depending on buffers and protocol used. We highly recommend that finalized buffers and protocol are used when evaluating single-color control performance.

Setup spectral unmixing

1. **Run unstained cells:** Determine the appropriate FSC/SSC settings and fluorescence detector voltages or gains for the cells. Follow the instrument manufacturer's recommendations if adjusting voltages or gains.

2. **Run a sample of unstained beads:** Beads should be on scale for most cell types, but if not, adjust the FSC/SSC settings to visualize the bead population and apply a gate to the majority population for use in the spectral unmixing setup.

3. **Run each single-color control sample:** Ensure the positive peaks are on scale. If not, check and adjust the instrument settings and/or re-titrate the single-color controls with the antibody.

4. **Collect single-color control data:** Run each single-color control sample, set an FSC/SSC gate around the major singlet population, and record the single-color controls. This will involve capturing the emission spectrum for each fluorophore to enable unmixing.

5. **Create unmixing matrix:** Use software to create an unmixing matrix based on the spectral data collected from the single-stained samples.

6. **Collect and record experimental samples:** Apply the unmixing matrix to the experimental data to separate the signals from different fluorophores.

Setup compensation

1. **Run unstained cells:** Determine appropriate FSC/SSC settings and fluorescence detector voltages for the cells. Follow the instrument manufacturer's recommendations if adjusting voltages or gains.

2. **Run a sample of beads:** Run a sample of beads to adjust FSC/SSC to visualize the beads (this can even be a single-stained bead). If needed, adjust the FSC/SSC to get the beads in view. Apply a gate to the majority population for use in compensation setup.

3. **Run each single-colored control sample:** Ensure that the positive peaks are on scale and adjust the voltages or gains, if necessary, and/or re-titrate the single-color controls with the antibody.

4. **Collect single-color control data:** Run each single-color control, set an FSC/SSC gate around the major singlet population, and record files for compensation controls.

5. ***(Optional)* Apply compensation:** Compensation matrix can be generated and applied to the experiment at this step using the instrument's compensation wizard, in case real time visualization of compensated data is required.

6. If required, readjust the FSC/SSC setting for cell samples. Do not adjust the settings for fluorescence detectors.

7. Collect and record experimental samples.

Limited product warranty

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN0019374 B.0

Revision	Date	Description
C	20 August 2025	Adding SKU U20250 and additional edits.
B.0	8 September 2020	Baseline version used for revision of UltraComp eBeads™ Compensation Beads and UltraComp eBeads™ Plus Compensation Beads Product Information Sheet.

The information in this guide is subject to change without notice.

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