UltraComp eBeads™ Compensation Beads and UltraComp eBeads™ Plus Compensation Beads

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

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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**.

Contents and storage

Product	Cat. No.	Size	Species reactivity	Storage
UltraComp eBeads™	01-2222-41	25 tests	mouse, rat, hamster	2-8°C Do not freeze.
	01-2222-42	100 tests		
UltraComp eBeads™ Plus	01-3333-41	25 tests	mouse, rat, hamster, rabbit, human	
	01-3333-42	100 tests		

Product description

UltraComp eBeads™ and UltraComp eBeads™ Plus compensation beads provide a consistent, accurate and simple-to-use method for setting compensation for fluorochrome-conjugated antibodies when running flow cytometry. UltraComp eBeads™ beads bind with antibodies of mouse, rat, and hamster origin and UltraComp eBeads™ Plus beads bind with antibodies of mouse, rat, hamster, rabbit, and human origin (see “Contents and storage” on page 1).

Each drop of beads contains two populations of beads by which to set compensation. The positive population of beads capture the fluorochrome-conjugated antibody used for cell staining. The negative population does not bind antibody. The resulting bimodal distribution can be used for single-colour compensation controls in multi-colour flow cytometry experiments.

UltraComp eBeads™ compensation beads are compatible with all fluorochromes excited by the most commonly used lasers (355-nm, 405-nm, 488-nm, 532-nm, 561-nm, and 632–640-nm) with the exception of some far-red emitting 405-nm excitable dyes. In some experiments, compensation values for Super Bright 702, Super Bright 780, Brilliant Violet 711, or Brilliant Violet 786-conjugated antibodies are higher in the violet 450/50 channel when using the UltraComp eBeads™ compensation beads as compared to single-color stained cells. In such circumstances, we recommend setting compensation with cells.

UltraComp eBeads™ Plus compensation beads are compatible with all fluorochromes excited by the most commonly used 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. It is recommended that cells be used for setting optimal single-color compensation values for 405 nm excitable fluorophors emitting in the 500 nm range.

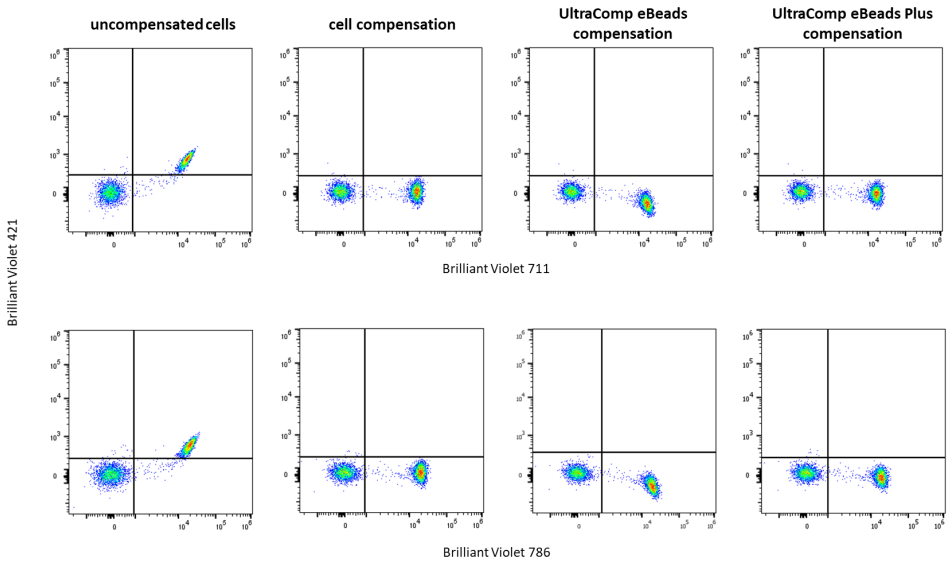

Figure 1 Compensation by UltraComp eBeads™ and UltraComp eBeads™ Plus compensation beads of 405-nm excitable, far red-emitting dyes is comparable to cells.

Mouse peripheral blood mononuclear cells (PBMCs) were stained with mouse anti-CD19 Brilliant Violet 421 and either mouse anti-CD4 Brilliant Violet 711 or mouse anti-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.

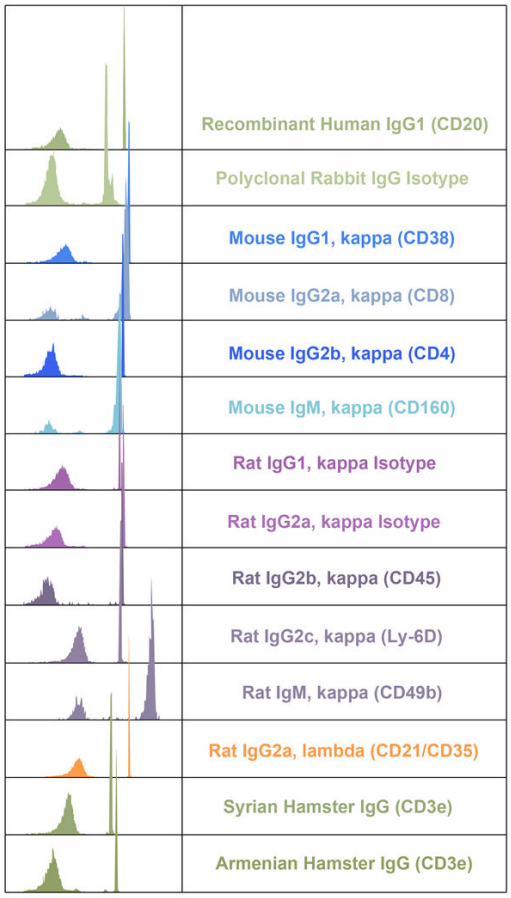

Figure 2 UltraComp eBeads™ Plus Compensation Beads bind up to 14 different antibody isotypes.

Staining of UltraComp eBeads™ Plus Compensation Beads with 14 different R-PE-conjugated monoclonal antibodies, including one of each subclass commonly used in flow cytometry. The beads were stained with 0.25 µg of each antibody, then analyzed by flow cytometry. Each histogram represents one staining antibody (clone and isotype indicated at right).

Other materials needed

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

Note: UltraComp eBeads™ 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. Do not use UltraComp eBeads™ and UltraComp eBeads™ Plus compensation beads with Super Bright Staining Buffer or other additives. For more information, contact Technical Support.

Prepare single-color compensation controls

1. Label a tube for each antibody conjugate that will be used in the experiment.
2. Mix the compensation beads by vigorously inverting at least 10 times or by pulse-vortexing.
3. Add 1 drop of compensation beads to each tube (there is no need to add staining buffer).
4. 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, we recommend that you use 0.125 µg or less antibody. It is not necessary to use the antibody at its optimal concentration. For most antibodies, appropriate compensation values result when 0.03–1.0 µg of antibody is used in the test.
5. Mix well by flicking or pulse-vortexing.
6. Incubate at 2–8°C for 15–30 minutes in the dark.
7. Add 2–3 mL of Flow Cytometry Staining Buffer to each tube, then centrifuge at 400–600 × *g* for 3–5 minutes.
8. Decant the supernatant, then add 0.2–0.4 mL of Flow Cytometry Staining Buffer to each tube.
9. Mix by flicking or pulse-vortexing before analysis.

Setup compensation

1. Run unstained cells on the cytometer. Determine appropriate FSC/SSC settings and fluorescence detector (PMT) voltages for the cells.

2. 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-stained bead sample to ensure that the positive peaks are on scale. Decrease the PMT voltages for any positive bead peak that is off-scale. Do not reduce voltages more than is necessary to bring positive beads on-scale. Do not record any data until all single-stained beads have been reviewed and all voltages are set.

4. Run each single-stained bead sample to perform compensation setup and record files for compensation controls. For compensation setup, we recommend that you set a FSC/SSC gate around the major singlet population, then use this for further fluorescence analysis.

5. Readjust the FSC/SSC setting for cell samples, but do not adjust the settings for fluorescence detectors.

6. Collect and record experimental samples.

Limited product warranty

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