BrightComp eBeads™ Compensation Beads

Catalog Numbers A10514, A54740, A54741, A54742, A54743

Pub. No. MAN0017432 Rev. B.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

The BrightComp eBeads™ Compensation Beads provide a consistent, accurate, and simple-to-use reagent for setting flow cytometry compensation when using green fluorescent protein (GFP), red fluorescent protein (RFP), yellow fluorescent protein (YFP), cyan fluorescent protein (CFP), or mCherry fluorescent protein.

The BrightComp eBeads™ Compensation Beads consist of modified microspheres to allow for easy compensation of samples with different levels of fluorescent protein expression. Each drop of beads contains negative beads and beads stained with a dye that is a near-identical, spectral match to fluorescent proteins at 3 levels of fluorescence intensity plus an unstained/negative population (Figure 1). The beads have a diameter of approximately 5 µm (actual size of beads for each lot is listed on the vial). The bead suspensions are supplied in a dropper vial for convenient application.

Contents and storage

Sufficient material is supplied for 25 assays following the protocol described in this user guide.

Table 1 BrightComp eBeads™ Compensation Beads

Amount	Composition	Storage [1]
1 mL	1.5×10^6 particles per intensity in 1 mL buffer (deionized water with 0.05% Tween™ 20 and 2 mM sodium azide)	2°C to 8°C Do not freeze

[1] When stored as directed, the beads are stable for at least 1 year.

Table 2 Excitation and emission

Catalog No.	Bead type	Excitation	Emission
A10514	GFP	488 (blue)	525/50
A54740	RFP	561 (yellow)	585/16
A54741	YFP	488 (blue)	530/30
A54742	CFP	405 (violet)	512/25
A54743	mCherry	561 (yellow)	620/15

Prepare BrightComp eBeads™ Compensation Beads

1. Label tubes for each of the following:
   - Tube1: BrightComp eBeads™ Compensation Beads
   - If additional antibody markers or fluorescent dyes are needed, label additional tubes for all other single color controls using antibody capture beads or cells.
2. Mix the BrightComp eBeads™ Compensation Beads by gently vortexing the dropper bottle for 10 seconds.
3. Add 1 drop of BrightComp eBeads™ Compensation Beads to Tube 1. Add additional single color control as needed to the other tubes.
4. Add 1 mL of PBS or other buffer to each tube and mix well.
5. Vortex the tubes before analyzing on the flow cytometer.
6. Perform compensation according to the preferred procedure for the flow cytometer in use.

Perform single color fluorescent protein compensation

1. Follow the sample preparation procedure described in "Prepare BrightComp eBeads™ Compensation Beads". Run Tube 1 and adjust the FSC/SSC voltage settings to place the FP particles on scale. Do not record any data.
2. Run FP-expressing cells. Adjust the PMT voltages to ensure that the cell sample expressing fluorescent protein is on-scale and achieves optimal separation from the negative cells. Do not record any data.
3. Repeat Step 2 for any other single color sample, ensuring that the PMT voltage for each channel is properly adjusted.
4. Place Tube 1 BrightComp eBeads™ Compensation Beads) on the flow cytometer for a second time. Set the gate around the negative peak, if software requires it, then run the beads and record data file. This sets the PMT voltages in each channel for compensation.

   Note: Due to the voltage adjustments made in Steps 2–3, one or more of the original four intensity peaks may no longer be on-scale.
5. Leaving Tube 1 on the instrument, run and record the beads after gating on the intensity peak that is greater than the intensity peak of the FP-expressing cells. This results in recording of the single color control on the FP detector.
6. Run each single-stained sample and record the results.
7. The PMT voltages in each channel are now set for compensation. Apply auto-compensation.
8. Run the cell samples. If needed, readjust the FSC/SSC settings, but do not adjust the fluorescent PMT settings.
9. Collect and record files for the experimental samples.

Note: Since BrightComp eBeads™ Compensation Beads use dyes with near identical, but not exact, fluorescence spectra compared to the fluorescent proteins, some manual adjustment of the auto compensation may be needed. Typically, adjustment is not needed in a well-designed panel, and are minor if necessary.

Example of typical results

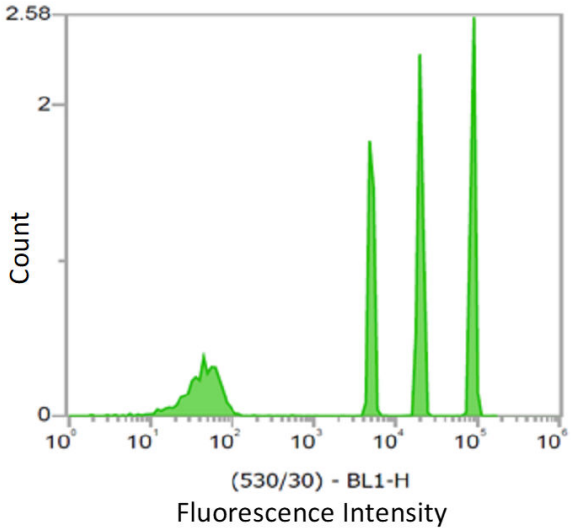

Figure 1 Multiple intensities of GFP BrightComp eBeads™ Compensation Beads.

Fluorescent proteins can be expressed at varying levels, resulting in the detection of a range of fluorescent intensities. When setting compensation, selection of the bead peak with a higher intensity than the experimental sample is recommended. Data were acquired on an Attune NxT flow cytometer using a 488-nm Laser. Emission was collected using 525/50 nm for GFP.

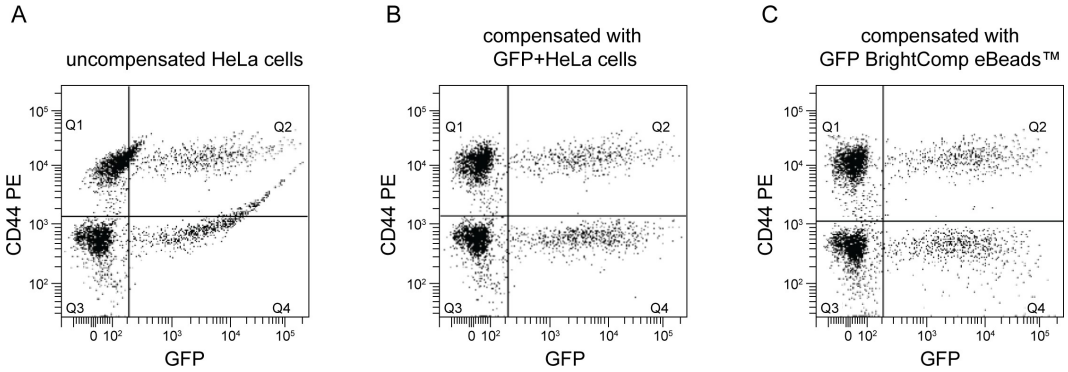

Figure 2 GFP BBrightComp eBeads™ Compensation Beads can be used to compensate for GFP.

(A–C) HeLa cells, a human cervical cancer cell line, were transduced with CellLight™ Histone 2B-GFP, BacMam 2.0, resulting in GFP expression. Cells were subsequently harvested and stained with rat anti-human/mouse CD44-PE antibody, then analyzed on a BD LSR II flow cytometer using a 488-nm laser. Emission was collected using 525/50-nm and 585/42-nm filters and the samples were autocompensated using BD FACSDiva™ software, v6.2. Samples compensated with GFP BrightComp eBeads™ Compensation Beads (C) show the same degree of compensation as the samples compensated using the GFP-expressing HeLa cells (B).

Limited product warranty

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Revision	Date	Description
B.0	5 October 2022	Addition of RFP, YFP, CFP, and mCherry compensation bead products.