

# GFP BrightComp eBeads™ Compensation Beads

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## Product information

The GFP BrightComp eBeads™ Compensation Beads provide a consistent, accurate, and simple-to-use reagent for setting flow cytometry compensation when using green fluorescent protein (GFP).

The GFP BrightComp eBeads™ Compensation Beads consist of modified microspheres to allow for easy compensation of samples with different levels of GFP expression. Each drop of beads contains negative beads and beads stained with a dye that is a near-identical, spectral match to GFP at 3 levels of intensity (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.

Table 1. Contents and storage

Material	Amount	Composition	Storage*
GFP BrightComp eBeads™ Compensation Beads	1 mL	1 × 10 <sup>6</sup> particles per intensity in 1 mL buffer (PBS with 0.05% Tween 20™ and 2 mM sodium azide)	<ul style="list-style-type: none"> <li>• 2–8°C</li> <li>• DO NOT FREEZE</li> </ul>
<p><b>Number of assays:</b> Sufficient material is supplied for 25 assays based on the protocol described in this user guide.</p> <p>* When stored as directed, this kit is stable for at least 1 year.</p>			

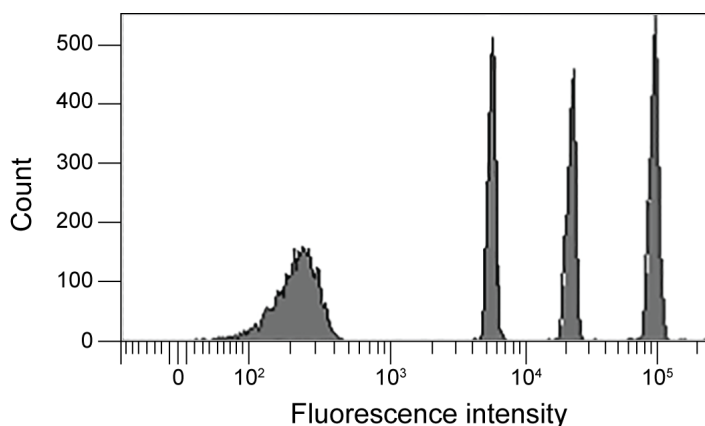
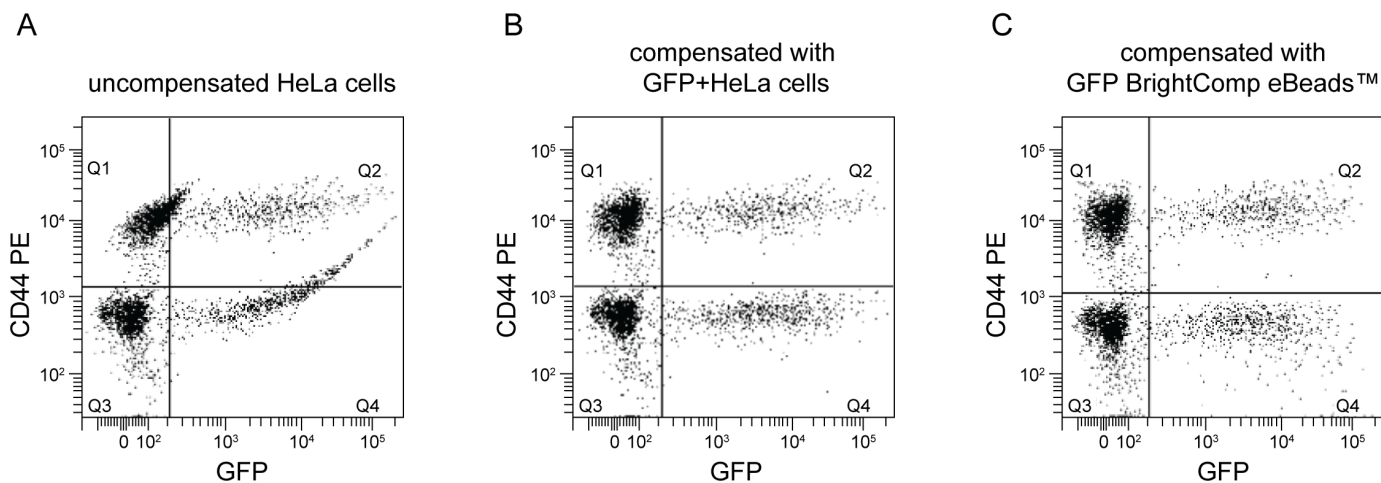


Figure 1. Multiple intensities of GFP BrightComp eBeads™. Fluorescent proteins can be expressed at varying levels, resulting in the detection of a range of fluorescent intensities. When setting compensation, selection of the bead peak with a higher intensity than the experimental sample is recommended. Data were acquired on a BD™ LSR II flow cytometer using a 488-nm laser. Emission was collected using 525/50 nm for GFP.



**Figure 2.** GFP BrightComp eBeads™ 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™ (C) show the same degree of compensation as the samples compensated using the GFP-expressing HeLa cells (B).

## Methods

### Guidelines for single color fluorescent protein compensation

1. Label a tube for each of the following:
  - Tube 1: GFP 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 GFP BrightComp eBeads™ Compensation Beads by gently vortexing for 10 seconds.
3. Add 1 drop of GFP 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.

**Note:** For general guidelines for compensation set-up, see page 2.

## General compensation set-up

- principles
1. Follow the compensation procedure described on page 2. Run Tube 1 and adjust the FSC/SSC voltage settings to place all four populations on scale. Do not record any data.
  2. Run GFP-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 (GFP 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 GFP-expressing cells. This results in recording of the single color control of the GFP detector.
6. Run each single-stained sample and record.
7. Now the PMT voltages in each channel are 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 experiment samples.

# Documentation and support

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**Note:** For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

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Revision	Date	Description
A.0	02 November 2017	New user guide

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