Flow cytometry compensation tools for a host of GFP variants

GFP BrightComp eBeads Compensation Bead Kit.

The discovery in the early 1960s [1] and subsequent development of Green Fluorescent Protein (GFP) as a reporter gene has greatly advanced the study of gene expression, protein localization, and cell and tissue development in a multitude of disciplines. GFP, an intrinsically fluorescent protein originally isolated from the jellyfish *Aequoria victoria*, enables real-time examination in live cells of processes that have conventionally been observed through immunocytochemical snapshots in fixed specimens.

Although enhanced Green Fluorescent Protein (EGFP, Ex/Em = 488/510 nm) has emerged as the most widely used GFP derivative, a number of other GFP variants have been isolated or engineered, each with minor variations in extinction coefficient, quantum yield, and excitation and emission wavelengths [2]. Concerns that these variations have the potential to impact compensation values in flow cytometry experiments have led many researchers to require that the emission spectrum of the compensation control and the sample fluorophore be identical. This requirement necessitates the use of sample to collect compensation data, an arduous and sometimes costly addition to the experimental protocol. Here we show that the Invitrogen™ GFP BrightComp eBeads™ Compensation Bead Kit—designed to be used to collect compensation data for EGFP—can also be used with a number of popular variants of GFP.

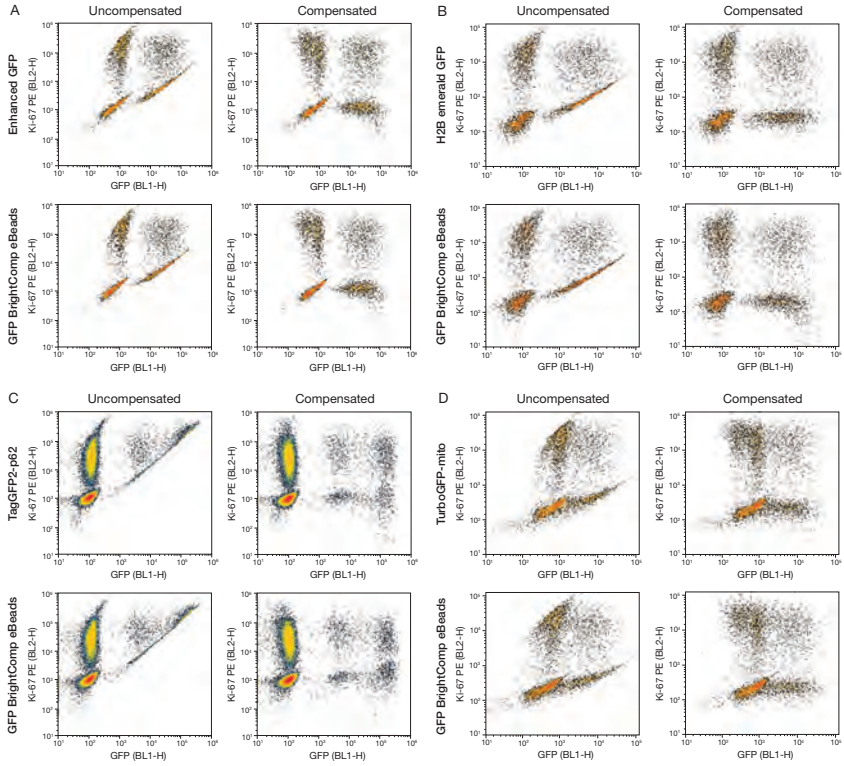

Figure 1. Flow cytometric analysis of GFP-expressing U2OS cells using either sample or GFP BrightComp eBeads Compensation Beads for compensation. U2OS cells expressing a variant of GFP were harvested, stained with Invitrogen™ LIVE/DEAD™ Fixable Far Red Dead Cell Stain (Cat. No. L10120), fixed, permeabilized, and then stained with Invitrogen™ Ki-67 Monoclonal Antibody (clone 20Raj1), PE conjugate (Cat. No. 12-5699-41). Data for each of the samples were acquired twice at the same voltages: first using the respective GFP-expressing U2OS cells for compensation (top row of each panel), and second using the Invitrogen™ GFP BrightComp eBeads™ Compensation Bead Kit (bottom row of each panel). Dual-parameter density plots (expressed GFP variant vs. PE-Ki-67 antibody staining) for each sample acquisition are presented without any compensation (uncompensated, left-hand columns of each panel) or with compensation with either cells or beads (compensated, right-hand columns of each panel). **(A)** For enhanced GFP expression, U2OS cells were transduced with an adenovirus containing enhanced GFP under the control of a CMV promoter (Vector Biolabs). **(B)** For emerald GFP expression, U2OS cells were transduced with Invitrogen™ CellLight™ Histone 2B-GFP (BacMam 2.0, Cat. No. C10594). **(C)** For TagGFP2 expression, U2OS cells were transduced with Invitrogen™ Premo™ Autophagy Sensor GFP-p62 (BacMam 2.0, Cat. No. P36240). **(D)** For TurboGFP expression, U2OS cells were transfected with the pTurboGFP-mito vector (Evrogen) using the Invitrogen™ Neon™ Transfection System (Cat. No. MPK5000). Compensation values for the LIVE/DEAD Fixable Far Red Dead Cell Stain were determined using 1:1 live and heat-killed U2OS cells labeled with the cell stain; compensation values for PE were determined using Invitrogen™ AbC™ Total Antibody Compensation Beads (Cat. No. A10513) labeled with PE anti–Ki-67 antibody. Samples were acquired on the Invitrogen™ Attune™ NxT Flow Cytometer at a flow rate of 200 µL/min; data were analyzed using the Attune NxT Software v2.6.

GFP compensation beads are compatible with GFP variants

Although the GFP BrightComp eBeads Compensation Beads were developed to compensate for EGFP, they are compatible with several variants of GFP, including emerald GFP, TagGFP2, and TurboGFP (Figure 1), as well as AcGFP, a monomeric GFP isolated from *Aequorea coerulescens*. To demonstrate their compatibility, we analyzed U2OS cells expressing four different GFP derivatives and used either GFP-expressing cells from the actual sample or GFP BrightComp eBeads Compensation Beads to obtain compensation values.

Figure 1 shows the complete set of uncompensated and compensated dual-parameter density plots of four multiplexed samples, each compensated with either the particular GFP variant–expressing U2OS cells or GFP BrightComp eBeads Compensation Beads. In all cases, the multiplexed samples were acquired twice at the same voltages using the Invitrogen™ Attune™ NxT Flow Cytometer at a flow rate of 200 µL/min.

For each GFP variant tested, we found that the GFP BrightComp eBeads Compensation Beads can be used as a replacement for traditional compensation methods that require the use of sample. The emission spectra of these GFP variants are not significantly different from that of EGFP, and use of the GFP BrightComp eBeads Compensation Beads has been shown to not impact compensation results in multiplexed flow cytometry experiments. In addition to their use to compensate multiple GFP variants, these compensation beads are effective with both transduction and transfection methods and for a variety of expression targets, including GFP fusion proteins.

GFP BrightComp eBeads Compensation Beads are easy to use

The GFP BrightComp eBeads Compensation Beads provide a simple method for the compensation of GFP and its variants in flow cytometry experiments. Provided in a convenient dropper vial, these compensation beads are embedded-dye microspheres with a diameter of approximately 5 µm (actual size for each lot is listed on the vial label) that can be used to determine compensation values in samples with different levels of GFP expression. Each drop of beads contains negative control (unstained) beads, as well as beads stained with a dye that is excited with a blue (488 nm) laser and exhibits three intensity levels to match a variety of GFP expression levels, with an emission spectrum that is a nearly identical match to that of EGFP (Figure 2).

Simplify GFP compensation in your lab

The GFP BrightComp eBeads Compensation Beads provide a reliable, accurate, and simple-to-use technique for setting flow cytometry compensation when analyzing GFP-expressing samples. Here we show that these compensation beads can be used as a replacement for traditional methods that use sample to obtain compensation values. In addition, GFP BrightComp eBeads Compensation Beads are effective with multiple GFP variants, expression targets, and transduction and transfection methods.

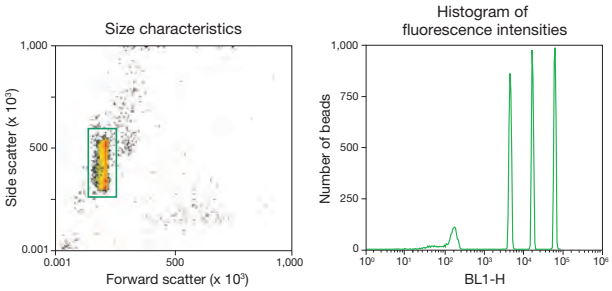

Figure 2. Size and fluorescence characteristics of the GFP BrightComp eBeads Compensation Beads. Fluorescent proteins are expressed in cells at varying levels, producing a range of fluorescence intensities. The Invitrogen™ GFP BrightComp eBeads™ Compensation Bead Kit (Cat. No. A10514) provides a suspension of beads that includes negative control (unstained) beads and beads stained at three levels of intensity with a dye that is a near-identical spectral match to GFP. Data were acquired on an Invitrogen™ Attune™ NxT Flow Cytometer using a 488 nm laser; emission was collected using a 530/30 nm bandpass filter for GFP.

Learn more about the diverse selection of flow cytometry compensation beads

Thermo Fisher Scientific offers a number of other flow cytometry compensation beads for use with a range of fluorophores. The Invitrogen™ UltraComp and Invitrogen™ OneComp eBeads™ Compensation Beads each contain a mixture of antibody-coated positive compensation beads and uncoated negative compensation beads in a single vial for quick and easy fluorescence compensation. The Invitrogen™ AbC Total Antibody and Invitrogen™ ArC Amine-Reactive Compensation Beads provide positive beads—which either bind all isotypes of the specific immunoglobulin or bind any of the amine-reactive dead cell stains in the Invitrogen™ LIVE/DEAD™ Fixable Dead Cell Stain Kits—and negative beads with no binding capacity or reactivity, for use in setting fluorescence compensation. These two components are provided in separate vials such that negative beads can be added after the positive beads are labeled, in order to avoid any transfer of fluorescence over time. Find out more about our wide selection of flow cytometry compensation tools at **thermofisher.com/brightcomp**. ■

References

1. Shimomura O, Johnson FH, Saiga Y (1962) *J Cell Comp Physiol* 59:223–229.
2. Day RN, Davidson MW (2009) *Chem Soc Rev* 38:2887–2921.

Product	Quantity	Cat. No.
GFP BrightComp eBeads™ Compensation Bead Kit	25 tests	A10514