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A comparison of GFP BrightComp eBeads Compensation Bead Kit performance against multiple GFP variants.

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ABSTRACT

Green Fluorescent Protein (GFP), a protein isolated from the jellyfish *Aequoria victoria* has proven to be a useful tool within multiple disciplines of biology since being identified in the 1960's[1]. By the 1990's, the use of fluorescent proteins in biomedical research had become prevalent, enabling the study of gene expression, cellular and tissue development. Although EGFP has emerged as the most widely employed form of GFP, there have been a number of GFP derivatives engineered, each with minor variations in extinction coefficient, quantum yield, and excitation and emission wavelengths[2]. Concerns that these variations can impact compensation values when performing flow cytometry has led many researchers to follow the imperative that the emission spectra of the compensation control and sample fluorophores must be identical. We show here that the Invitrogen ™ GFP BrightComp eBeads™ Compensation Bead Kit by, which contain dye embedded beads that were developed to compensate EGFP, can be used interchangeably with multiple variants of GFP, as their emission spectra do not vary significantly enough to impact compensation results in multiplexed flow cytometry experiments.

INTRODUCTION

- Convenient and simple to use beads for compensation of GFP.
- Effective at compensating multiple GFP variants, expression targets, and Transduction/Transfection methods.

MATERIALS AND METHODS

Incorporation of GFP variants into U2-OS cells and staining protocol

U-2OS cells expressing H2B emerald GFP were transduced with CellLight ™ Histone 2B GFP, with BacMam 2.0. U2-OS cells expressing TagGFP2 were transduced with Premo™ Autophagy Sensor GFP-p62, with BacMam 2.0. U-2OS cells expressing Enhanced GFP (Vector Biolabs #1060) were transduced with an Adenovirus under control of a CMV promotor. U2-OS cells expressing TurboGFP-Mito were transfected with pTurboGFP-mito (Evrogen #FP517), using the Invitrogen ™ Neon ™ Transfection System. All transductions and Transfections were carried out according to the manufacturers protocol

The cells were then harvested and stained with LIVE/DEAD™ Fixable Far Red Dead Cell Stain, fixed and permeabilized using the Invitrogen ™ eBioscience™ Foxp3 /Transcription Factor Staining Buffer Set. The permeabilized cells were then stained with Ki-67 PE (20Raj1).

Compensation Controls

The multiplexed samples were auto-compensated using the compensation controls described below. Multiplexed samples were acquired twice, one using the respective GFP variant to compensate and the other using the GFP BrightComp eBeads™ for compensation of the GFP respective GFP variant, both at the same voltages. The samples were acquired on Invitrogen™ Attune™ NxT Flow Cytometer at 200 µl/minute flow rate. Data were analyzed using the Attune NxT v2.6 software.

Dye or Fluorescent Protein	Compensation Control
LIVE/DEAD™ Fixable Far Red Dead Cell Stain	1:1 live and heat killed U2OS stained with LIVE/DEAD™ Fixable Far Red Dead Cell Stain
PE	AbC™ Total Antibody Compensation beads stained with Ki-67 PE
Emerald GFP	GFP BrightComp eBeads™ Compensation Bead Kit or CellLight ™ Emerald H2B GFP expressing U2-OS cells
TagGFP2	GFP BrightComp eBeads™ Compensation Bead Kit or Premo™ Autophagy Sensor GFP-p62 expressing U2-OS cells
Enhanced GFP	GFP BrightComp eBeads™Compensation Bead kit or Enhanced GFP U2-OS cells
Turbo GFP	GFP BrightComp eBeads™ Compensation Bead Kit or TurboGFP-mito expressing U2-OS cells

RESULTS

Figure 1. Gating strategy

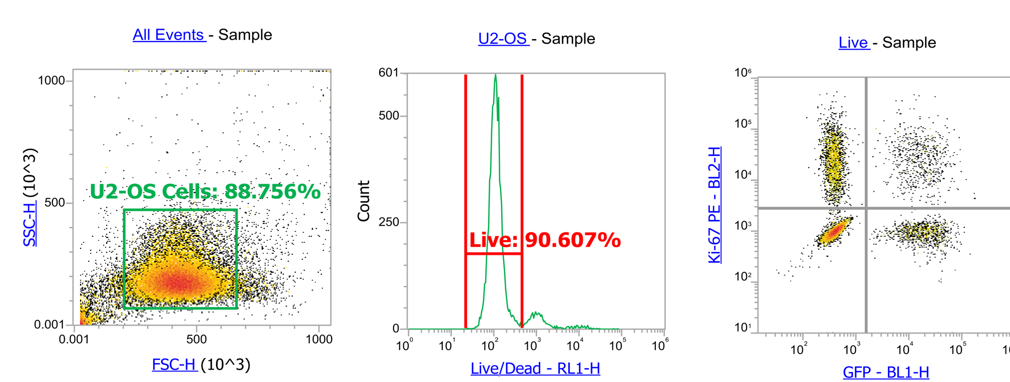

Figure 1: U2-OS cells were gated on Forward Scatter-Height vs. Side Scatter-Height and a histogram display of live/dead was generated . A dual parameter density plot of GFP vs. Ki-67 PE was generated from the live cells.

Figure 2. GFP BrightComp eBeads™ Compensation Beads

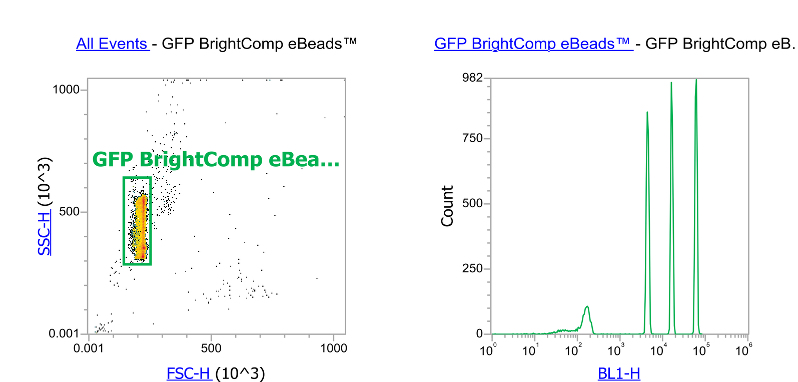

Figure 2: Fluorescent proteins can be expressed at varying levels, resulting in the detection of a range of fluorescent intensities. GFP BrightComp eBeads™ Compensation Bead Kit comes with 3 different bead intensities as well as blank beads for compensation of GFP at varying levels. Data was acquired on a Attune NxT using a 488 nm laser. Emission was collected using 530/30 nm for GFP.

Figure 3. Flow Cytometric Analysis Using GFP BrightComp eBeads™ vs. multiple GFP variants for compensation.

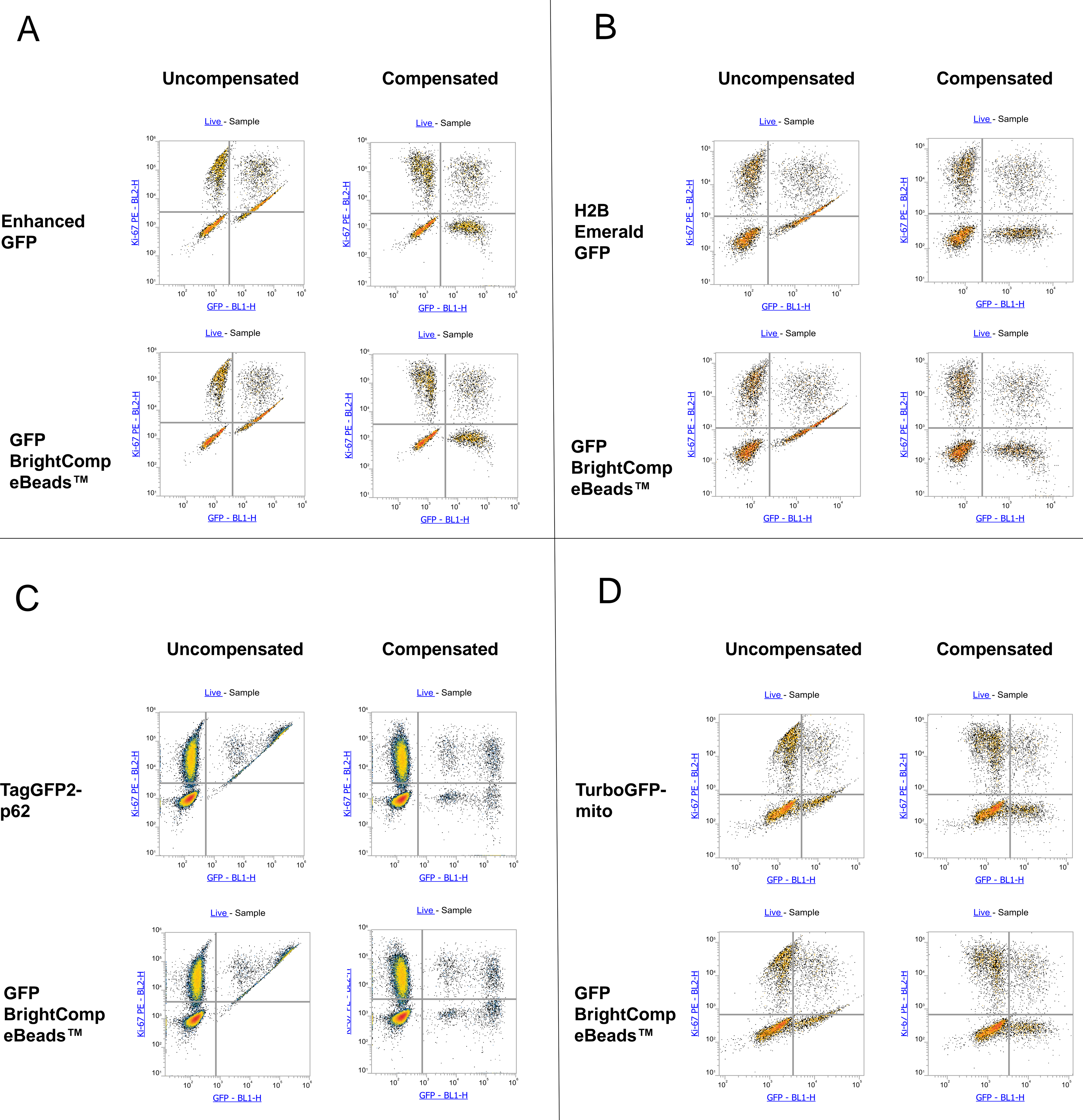

Figure 3: Multiplexed samples were run twice for each respective GFP variant. Once using the respective GFP expressing U2-OS cells for compensation and another using the GFP BrightComp eBeads™.

- Quadrant A: Uncompensated and compensated dual parameter density plots of Enhanced GFP and Ki-67 when compensating with Enhanced GFP expressing U2-OS cells vs. GFP BrightComp eBeads™
- Quadrant B: Uncompensated and compensated dual parameter density plots of H2B emerald GFP and Ki-67 when compensating with H2B Emerald GFP expressing U2-OS cells vs. GFP BrightComp eBeads™
- Quadrant C: Uncompensated and compensated dual parameter density plots of Premo™ Autophagy Sensor TagGFP2-p62 and Ki-67 when compensating with Premo™ Autophagy Sensor TagGFP2-p62 expressing U2-OS cells vs. GFP BrightComp eBeads™
- Quadrant D: Uncompensated and compensated dual parameter density plots of TurboGFP-mito GFP and Ki-67 when TurboGFP-mito compensating with Turbo GFP expressing U2-OS cells vs. GFP BrightComp eBeads™

CONCLUSIONS

Here we have shown that GFP BrightComp eBeads™ Compensation Beads can be used as a replacement for traditional compensation methods which employ the use of sample to compensate. In addition, GFP BrightComp eBeads™ Compensation Beads are effective at compensating across multiple GFP variants, expression targets, and transduction/transfection methods.

REFERENCES

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2. Day and Davidson, (2009) "The fluorescent protein palette: tools for cellular imaging" *Chemical Society Reviews* 2009 Oct; 38(10): 2887-2921

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