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


ABSTRACT

Green Fluorescent Protein (GFP), a protein isolated from the jellyfish Aequorea victoria has proven to be a useful tool within multiple disciplines of biology since being identified in the 1960's. 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 variants developed, each with minor variations in extinction coefficient, quantum yield, and excitation and emission wavelengths. 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, 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 METHOD

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

U2-OS cells expressing H2B emerald GFP were transduced with CellLight™ Histone 2B GFP, with BacPAK 2.0. U2-OS cells expressing TagGFP2 were transduced with Premo™ Autophagy Sensor GFP, p62, with BacPAK 2.0. U2-OS cells expressing Enhance GFP (Vector Biolabs #1060) were transduced with an Adenovirus under control of a CMV promoter. U2-OS cells expressing Turbo GFP-Mito were transfected with pTurboGFP-mito (Eorgen FP517), using the Invitrogen™ Neon™ Transfection System. All transfections and Transfections were carried out according to the manufacturer's protocol.

The cells were then harvested and stained with LIVE/DEAD™ Fixable Far Red Dead Cell Stain, fixed and permeabilized using the Invitrogen™ eBioscience™ Fix/Perm/Transcription Factor Staining Buffer Set. The permeabilized cells were then stained with K67 PE (20Raj1).

Compensation Controls

The multiplexed samples were auto-compensated using the compensation software described below. Multiplexed samples were acquired twice, once using the respective GFP variant to compensate and the other using the GFP BrightComp eBeads™ Compensation Bead Kit 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 millisecond flow rate. Data were analyzed using the Attune™ NxT v2.6. software.

RESULTS

Figure 1. Gating strategy

Figure 2. GFP BrightComp eBeads Compensation Beads

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

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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