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SPHERO™ Technical Note STN-18 Rev A 071015

INTRODUCTION TO AN EASY-TO-USE TECHNIQUE FOR THE SETTING OF FLOW CYTOMETER COMPENSATION USING COMPtrol ANTIBODY CAPTURE BEADS AS A SUBSITITUTE FOR CELLS

INTRODUCTION

When setting up multicolor flow cytometry experiments proper compensation is extremely important since these experiments provide complex data. Compensation helps correct spectral overlap to match the various fluorophores used during cell staining, after which the data becomes easier to interpret. Compensation using cells for single color staining provides autofluorescence levels that are the same as those obtained during multicolor staining and is independent of the antibody host or isotype. However, valuable cellular material and antibodies targeting dimly expressed antigens or rare cellular populations create difficulties when using this approach. Furthermore, native cells are difficult to standardize and introduce additional variability. Compensation procedures using antibody capturing beads overcome some of these limitations. However, many bead kits are host specific and do not cover the full range of isotypes. In addition, high backgrounds upon violet laser or red laser excitation are observed for the vast majority of capture bead kits. As a result, Spherotech offers the COMPtrol line of antibody capture beads.

The COMptrol beads offer:

- low autofluorescence regardless of excitation wavelegth or detection bandpass
- and the enormous breadth of compatible hosts and isotypes makes COMPtrol capture beads a truly universal compensation tool.

COMPtrol Antibody Capture Bead kit provides both positive and negative microspheres that can be used to set compensation for multicolor flow cytometry experiments. The positive population captures conjugates in single color stains and recognizes all mouse and rat isotypes, most hamster isotypes and rabbit polyclonal IgG. The negative population provides autofluorescence similar to unstained cells across the different excitation/emission wavelengths..

PRODUCT DESCRIPTION

The COMPtrol Antibody Capture Bead Kit, Cat. No. CMIgP-30-2K, contains two 5mL vials of 3.0-3.4µm beads in suspension at a concentration of approximately 1 x 10^7 beads/mL. COMPtrol Antibody Capture Negative Beads acts as a negative control and does not bind fluorochrome-conjugated antibodies. The COMPtrol Antibody Capture Positive Beads contains beads coated with an IgG that will bind all mouse and rat isotypes, as well as Syrian and Armenian hamster IgG and rabbit polyclonal antibodies.

PROCEDURE FOR USE

Reagents

The COMPtrol Antibody Capture Bead Kit contains the
following:

- COMPtrol Antibody Capture Negative Beads – 5 mL vial, P/N CMIgP-30-5B
- COMPtrol Antibody Capture Positive Beads – 5 mL vial, P/N CMIgP-30-5H

The beads are suspended in a storage buffer containing 0.016 M PBS, 0.2% BSA and 0.02% Sodium Azide.

NOTES:

1. These reagents contain 0.02% sodium azide. Sodium azide under acid conditions yields hydrazoic acid, an extremely toxic compound. Azide compounds should be flushed with running water while being discarded. These precautions are recommended to avoid deposits in metal piping in which explosive conditions can develop. If skin or eye contact occurs, wash excessively with water.
2. Ensure the beads are completely resuspended by sonication or vortexing before use. They may settle over extended periods of time.
3. Minimize exposure of reagents to light during storage or use.
4. Use Good Laboratory Practice (GLP) when handling this reagent.

Reagent Preparation

Vortex and/or sonication is required for proper resuspension of the individual kit components before use.

Materials Required But Not Supplied:

- Appropriately sized test tubes
- Flow cytometer
- Vortex Mixer
- Water Bath Sonicator
- Centrifuge
- Staining Buffer – 1 x PBS at pH 7.4 with 0.02% Sodium Azide and 0.2% BSA

Storage Conditions and Stability

These reagents are stable to 1 year after receipt when stored at 2-8°C. Do not freeze. Minimize exposure to light. Opened vials must be refrigerated after use.

Procedure for Bead Staining

1. Add one drop of negative beads and one drop of positive beads to each test tube.
2. Place single color antibody conjugates into individual, labeled test tubes at the antibody concentration used for your application and vortex immediately.
3. Incubate at room temperature in the dark for 20 minutes.
4. Add 1 mL of buffer to each test tube, vortex and centrifuge at 300 x G for 6 minutes. For best results, use the staining buffer described in the "Materials Required But Not Supplied" section for this step.
5. Decant the supernatant and resuspend the beads in 600 µL of staining buffer.

Compensation Setup on Cytometer

1. Create an acquisition protocol with a FS (Lin) vs. SS (Lin) dot plot and either single color histograms or dual color dot plots for each relevant fluorescence channel. On the FS vs. SS dot plot, create a region to capture the singlet bead population and gate all fluorescence plots/ histograms based on this region. Set the protocol to collect 10,000 beads.

NOTE: Verify that the discriminator is low enough to detect the singlet bead population.

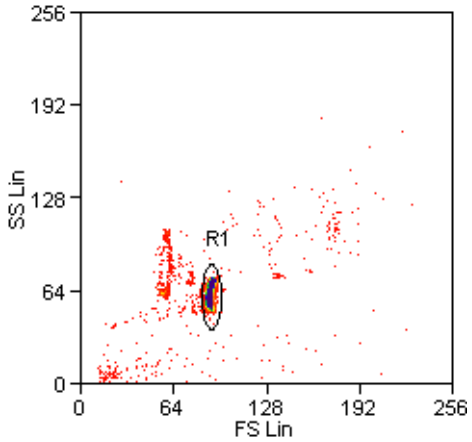
2. Using cytometer settings optimized for your application, run each of the stained bead samples and ensure that the positive signals are on scale.
3. Generate a compensation matrix using acquisition and/or analysis software.

NOTE: If large amounts of doublets and triplets are visible in the FS vs SS histogram, sonicate the stained sample for 60 seconds and reanalyze.

SUMMARY

For multicolor applications, compensation must be optimized to obtain consistent and allow for proper data interpretation. COMPtrol beads are designed to capture antibodies with conjugated fluorophores to provide detectable signals. They allow for multiple fluorophores to be used in an experiment, and when combined with acquisition and analysis software, compensation values are provided. These values provided in the form of a compensation matrix correct the emission overlap so that various fluorophore combinations can be used to achieve the best results.

Figures 1: FS vs SS histogram



Figures 2: Fluorescence histograms