affymetrix eBioscience

Compensation Beads for Flow Cytometry

Research Use Only

Introduction

OneComp and UltraComp eBeads® react with antibodies of mouse, rat and hamster origin, and are immunoglobulin light chain independent. Each drop of beads contains two populations: a positive population that will capture any mouse, rat, or hamster antibody; and a negative population that will not react with antibodies. When a fluorochrome-conjugated antibody is added to the beads, both positive and negative populations result. This bimodal distribution can be used for single-color compensation controls in multicolor flow cytometry experiments.

OneComp eBeads® are designed for use in compensation with all fluorochromes excited by blue (488 nm), green (532 nm), yellow-green (561 nm), and red (633–640 nm) lasers. This product is compatible with eFluor® 450 but is not optimized for compensation of other fluorochromes excited by a violet (405 nm) laser.

UltraComp eBeads® are compatible with all fluorochromes excited by blue (488 nm), green (532 nm), yellow-green (561 nm), red (633–635 nm), ultraviolet (355 nm) or violet (405 nm) lasers.

Protocol

Materials

- Compensation beads: OneComp eBeads® (cat. no. 01-1111) or UltraComp eBeads® (cat. no. 01-2222)
- Unstained cells
- Primary antibodies (directly conjugated)
- Flow Cytometry Staining Buffer (cat. no. 00-4222)
- 12 x 75 mm round-bottom test tubes

Experimental Procedure

Step I: Preparation of Single-Color Compensation Controls

1. Label a tube for each fluorochrome that will be used in the experiment.
2. Mix beads by vigorously inverting at least 10 times or pulse-vortexing.
3. Label each tube and pulse vortex 10 times.
4. Add 1 drop of OneComp or UltraComp eBeads® to each tube.
5. Add 1 test or less of antibody conjugate to each tube and mix.

***NOTE**: UltraComp eBeads® are compatible with standard staining buffers that contain PBS or HBSS, proteins such as bovine serum albumin (BSA) or FBS, and sodium azide. No other additives should be used. For more information, please contact Technical Support.*

***NOTE**: A test is defined as the amount (µg) of antibody that will stain a cell sample in a final volume of 100 µL. If high background is observed on the negative-bead population, less antibody can be used. For these cases, it is recommended to use 0.125 µg or less. Because the binding of the antibody to the positive bead is not dependent on the antibody's specificity, it is not necessary to*

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use the antibody at its optimal concentration. For most antibodies, appropriate compensation values will result when 0.03–1.0 µg of antibody is used in a test.

6. Mix well by flicking, inverting vigorously, or pulse vortexing.
7. Incubate at 2-8ºC for 15-30 minutes in the dark.
8. Add 2 mL of Flow Cytometry Staining Buffer to each tube and centrifuge at 400-600 *x g* for 3-5 minutes.
9. Decant supernatant and add 0.2-0.4 mL of Flow Cytometry Staining Buffer to each tube.
10. Mix briefly by flicking or pulse vortexing before analysis.

Step II: General Compensation Setup Principles

1. Run unstained cells on cytometer. Determine appropriate Forward scatter (FSC) and Side scatter (SSC) settings and fluorescence detector (photomultiplier tube, or PMT) voltages for the cells.
2. Run a sample of beads to adjust FSC/SSC to visualize beads (this can even be a single stained bead). It is acceptable to adjust the FSC/SSC to get the beads in view.
3. Run each single-stained bead sample to assure the positive peaks are on scale. PMT voltages should be decreased (as minimally as possible) for any positive bead peak that is off-scale. Do not record any data until all single-stained beads have been reviewed.
4. Run each single-stained bead sample to perform compensation setup and record files for compensation controls.
5. Readjust FSC/SSC settings for cell samples and acquire experimental samples.
6. Collect and record experimental samples.

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