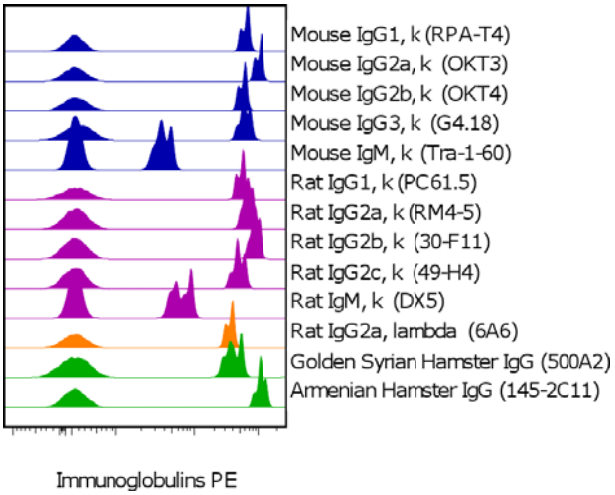
OneComp™ eBeads Compensation Beads

Catalog Number: 01-1111

For Research Use Only. Not for use in diagnostic procedures.



Staining of OneComp eBeads with 13 different PE-conjugated monoclonal antibodies including one of each subclass commonly used in flow cytometry. Beads were stained with 0.25 ug of each antibody and analyzed by flow cytometry. Each histogram represents one staining antibody (clone and isotype indicated at right).

Product Information

Contents: OneComp™ eBeads Compensation Beads

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Concentration: 1 drop (50 uL)/test

Formulation: aqueous buffer, 0.09% sodium azide, may contain carrier protein/stabilizer

Temperature Limitation: Store at 2-8°C. Do not freeze.

LOT **Batch Code:** Refer to vial

Use By: Refer to vial

Contains sodium azide

Description

OneComp eBeads react with antibodies of mouse, rat and hamster origin, and are immunoglobulin light chain-independent. The beads are spherical particles that can be stained with individual fluorochrome-conjugated antibodies for use as single-color compensation controls.

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 antibody. 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 cross-react to some antibodies of rabbit origin, but have not been extensively tested for this reactivity. 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 (633-635 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.

Applications Reported

OneComp eBead has been reported for use in flow cytometric analysis.

Applications Tested

OneComp eBeads have been tested for binding of fluorochrome-conjugated antibodies by flow cytometric analysis. This can be used at 1 drop (50 µL) per test. Refer to protocol for further information.

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14 March 2017 Rev. 10

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Related Products

00-4222 eBioscience™ Flow Cytometry Staining Buffer

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invitrogen

OneComp eBeads™

Protocol: OneComp eBeads™

Other Materials Needed

- 12x75 mm round bottom test tubes
- Primary antibodies (directly fluorochrome conjugated)
- Flow Cytometry staining Buffer (Thermo Fisher Cat. No. 00-4222)

Experimental Procedure

Preparation of Single-Color Compensation Controls

1. Label a tube for each of the 4 fluorochromes that will be used in the experiment.
2. Mix beads by vigorously inverting at least 10 times or pulse-vortexing.
3. Add 1 drop of OneComp eBeads to each tube.
4. Add 1 test or less of antibody conjugate to each tube.
   - 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 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.
5. Mix briefly by flicking or pulse-vortexing.
6. Incubate at 2-8°C for 15-30 minutes in the dark.
7. Add 2 mL of Flow Cytometry Staining Buffer to each tube and centrifuge at 400-600 x *g* for 3-5 minutes.
8. Decant supernatant and add 0.2-0.4 mL of Flow Cytometry Staining Buffer to each tube.
9. Mix briefly by flicking or pulse-vortexing before analysis.

General Compensation Setup Principles

10. Run unstained cells on cytometer. Determine appropriate FSC/SSC settings and fluorescence detector (PMT) voltages for the cells.
11. Run a sample of beads to adjust FSC/SSC to visualize beads (this can even be a single stained bead). It is OK to adjust the FSC/SSC to get the beads in view.
12. 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.
13. Run each single-stained bead sample to perform compensation setup and record files for compensation controls. For compensation setup, it is recommended to set a FSC/SSC gate around the major singlet population and use this for further fluorescence analysis.
14. Readjust FSC/SSC setting for cell samples, but do not adjust settings for fluorescence detectors.
15. Collect and record experimental samples.

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Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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