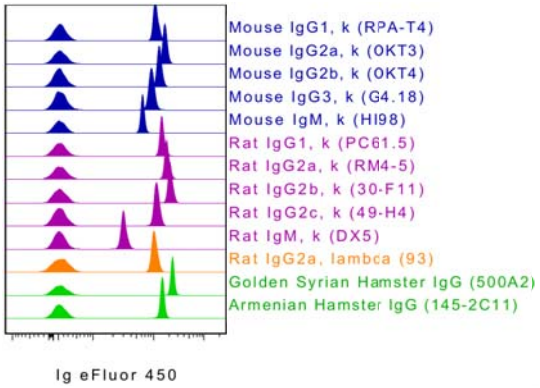
UltraComp™ eBeads Compensation Beads

Catalog Number: 01-2222
RUO: For Research Use Only. Not for use in diagnostic procedures.



Staining of UltraComp eBeads® with 13 different eFluor® 450-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: UltraComp™ eBeads Compensation Beads
REF **Catalog Number:** 01-2222
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

UltraComp eBeads® react with antibodies of mouse, rat and hamster origin, and are immunoglobulin light chain-independent. They are designed for use in compensation with all fluorochromes excited by ultraviolet (355 nm) violet (405 nm), blue (488 nm), green (532 nm), yellow-green (561 nm), and red (633-640 nm) lasers. 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.

UltraComp eBeads are compatible with all fluorochromes excited by an ultraviolet (355 nm) or violet (405 nm) laser. They should be used with standard staining buffers which contain PBS or HBSS, protein such as BSA or FBS, and sodium azide. Do not use UltraComp eBeads with Super Bright Staining Buffer or other additives. For more information, please contact technical support.

Applications Reported

UltraComp eBeads has been reported for use in flow cytometric analysis.

Applications Tested

UltraComp 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.

UltraComp eBeads are compatible with standard staining buffers which contain PBS or HBSS, protein such as BSA or FBS, and sodium azide. Do not use UltraComp eBeads with Super Bright Staining Buffer or other additives. For more information, please contact technical support.

Tel: 888.999.1371 or 858.642.2058 • Fax: 858.642.2046 • thermofisher.com/ebioscience • info@ebioscience.com

UltraComp™ eBeads Compensation Beads

Catalog Number: 01-2222

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

Related Products

01-1111 OneComp™ eBeads Compensation Beads

Tel: 888.999.1371 or 858.642.2058 • Fax: 858.642.2046 • thermofisher.com/ebioscience • info@ebioscience.com

invitrogen

UltraComp eBeads™

Protocol

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 fluorochrome 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 UltraComp eBeads™ to each tube.
4. Add 1 test or less of antibody conjugate to each tube.

   Note: *UltraComp eBeads™ are compatible with standard staining buffers which contain PBS or HBSS, protein such as BSA or FBS, and sodium azide. Do not use UltraComp eBeads™ with Super Bright Staining Buffer or other additives. 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, it is recommended to use 0.125 µg or less antibody. 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 well by flicking, inverting vigorously, 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

1. Run unstained cells on cytometer. Determine appropriate FSC/SSC settings and fluorescence detector (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 OK to adjust the FSC/SSC to get the beads in view. Apply a gate to the majority population for use in compensation setup.
3. Run each single-stained bead sample to assure the positive peaks are on scale. PMT voltages should be decreased for any positive bead peak that is off-scale. Do not reduce voltages more than is necessary to bring positive beads on-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. For compensation setup, it is recommended to set a FSC/SSC gate around the major singlet population and use this for further fluorescence analysis.
5. Readjust FSC/SSC setting for cell samples, but do not adjust settings for fluorescence detectors.
6. Collect and record experimental samples.

Documentation and support

Customer and technical support

Visit **thermofisher.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support, including:
  - Product FAQs
  - Software, patches, and updates
- Order and web support
- Product documentation, including:
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

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

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **thermofisher.com/support**.

Corporate entity: Life Technologies | Carlsbad, CA 92008 USA | Toll Free in USA 1.800.955.6288

For support visit **thermofisher.com/support** or email **techsupport@lifetech.com**

thermofisher.com

23 January 2017

ThermoFisher SCIENTIFIC