# Compensation and instrument beads

## Compensation beads for flow cytometry

Emission profiles of fluorophores are broad, which can result in overlapping profiles that require compensation for signal correction. Compensation can be set using beads, particularly when cell samples are limited or when a positive population is needed.

## The latest generation of compensation beads

Build flow cytometry panels with more accurate compensation using new Invitrogen™ UltraComp eBeads™ Plus Compensation Beads. When a fluorophore-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. In addition to all the features of the first-generation Invitrogen™ UltraComp eBeads™ product, UltraComp eBeads Plus Compensation Beads now offer:

- Increased species reactivity including rabbit- and human-origin antibodies (Figure 1)
- Compatibility with fluorophores excited by ultraviolet (355 nm), violet (405 nm), blue (488 nm), green (532 nm), yellow–green (561 nm), and red (633–640 nm) lasers
- Better compensation resolution for antibodies conjugated with Invitrogen™ eBioscience™ Super Bright 780, Brilliant Violet 711, or Brilliant Violet 786 dyes

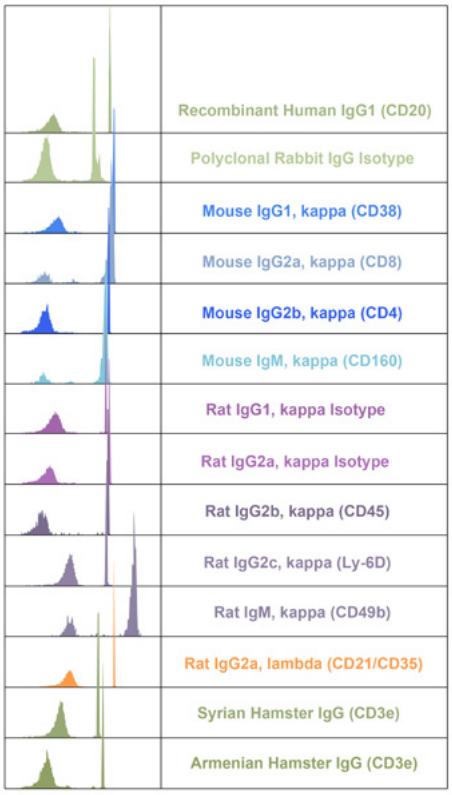


**Figure 1. Staining of UltraComp eBeads Plus Compensation Beads with 14 different Invitrogen™ PE dye–conjugated monoclonal antibodies, including one of each subclass commonly used in flow cytometry.** Beads were stained with 0.25 µg of each antibody and analyzed by flow cytometry. Each histogram represents one staining antibody.

## Invitrogen™ antibody compensation beads

| | UltraComp eBeads™ Plus beads | UltraComp eBeads™ beads | OneComp eBeads™ beads | AbC™ Total Antibody Compensation Bead Kit* | ArC™ Amine Reactive Compensation Bead Kit | GFP BrightComp eBeads™ beads |
|---|---|---|---|---|---|---|
| Application | Immunophenotyping | | | | Cell viability assay | GFP expression; beads are present at 3 levels of GFP-like intensity |
| Reactivity | Human, rabbit, hamster, mouse, and rat antibodies | Hamster, mouse, and rat antibodies with recognition of the kappa and lambda chains | | Hamster, mouse, rabbit, and rat antibodies | Invitrogen™ LIVE/DEAD™ fixable dead cell stains* | GFP isoforms |
| Format | One vial: dispense as a single drop | | | 1 vial positive beads, 1 vial negative beads | | One vial: dispense as a single drop |
| Laser compatibility | Compatible with most standard lasers, UV to 633 nm; improved for polymer dye use from the violet laser | Compatible with most standard lasers, UV to 633 nm | Compatible with most standard lasers, but not with UV or violet lasers | Compatible with most standard lasers, UV to 633 nm | | 488 nm |
| Quantity | 25 tests or 100 tests | | | | | 25 tests |
| Cat. No. | 01-3333-41<br>01-3333-42 | 01-2222-41<br>01-2222-42 | 01-1111-41<br>01-1111-42 | A10513<br>A10497 | A10628<br>A10346 | A10514 |

* Also applicable to similar amine-reactive dyes.

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## Counting beads

Absolute cell counting is a method for quantifying cell concentration or the absolute count of cells in a sample. Benefits of our absolute counting beads include:

- Wide range of fluorophores to fit a broad spectrum (Figure 2)
- Simple protocols that work with multiple cell types
- Increased consistency and reliable results
- Accommodates most cell sizes with increased percentage of singlets

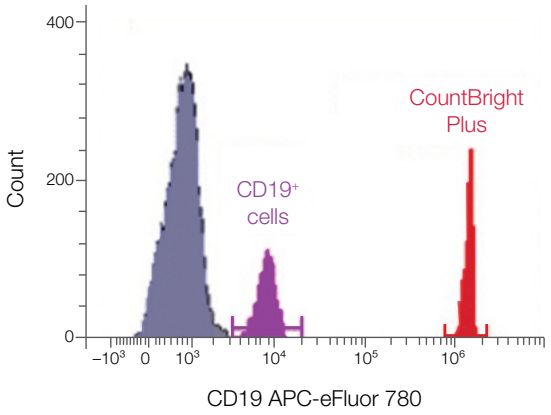


**Figure 2. CountBright Plus beads can be used with a broader range of fluorophores.** CountBright Plus beads (red) can be detected simultaneously with cells stained with Invitrogen™ CD19 APC-eFluor™ 780 antibody (pink) in lysed whole blood when excited with an IR laser (808 nm) with an 840/20 nm emission filter.

### Invitrogen™ absolute counting beads

| | CountBright™ Plus beads* | AccuCheck™ beads | | LIVE/DEAD™ *Bac*Light™ Bacterial Viability and Counting Kit** |
|---|---|---|---|---|
| **Parameters measured** | Cell concentration in sample | • Cell concentration in sample<br>• Pipetting accuracy | | • Viability<br>• Bacterial concentration in sample |
| **Sample type** | Any type | Whole blood | | Bacteria |
| **Bead size** | 4 µm | Bead A<br>6.40 µm | Bead B<br>6.36 µm | 6 µm |
| **Range** | Ex: UV–800 nm<br>Em: 385–860 nm | Bead A<br>Ex: 488 nm<br>Em: 575–585 nm | Bead B<br>Ex: 635 nm<br>Em: 660–680 nm | Ex: 488 nm<br>Em: 617 nm, 498 nm |
| **Cat. No.** | C36995 | PCB100 | | L34856 |

* The original CountBright Absolute Counting Beads are still available, but not compatible with IR-excitable fluorophores.
** Stains all cells, so a pure bacterial sample is required for accurate results.

## Calibration and size beads

Instrument calibration is critical to collecting and analyzing accurate experimental data. Our beads are designed to help ensure robust flow cytometer performance.

### Invitrogen™ calibration beads

| | Size calibration | | Instrument control | Alignment control |
|---|---|---|---|---|
| **Product** | Flow Cytometry Size Calibration Kit | Flow Cytometry Sub-micron Particle Size Reference Kit | Rainbow Calibration Particles | Alignflow™ Flow Cytometry Alignment Beads |
| **Use** | Size reference | Size reference | Routine calibration of flow cytometers | Calibrate laser alignment |
| **Emission** | No fluorescence | Green fluorescence | 400–680 nm | 3 types: 400–470 nm (for UV lasers), 515–660 nm (for blue lasers), or 645–680 nm (for red lasers) |
| **Bead size** | 6 sizes, 1.0–15 µm range | 6 sizes, 0.02–2.0 µm range | 3.0–3.4 µm | 2 sizes, 2.5 µm or 6.0 µm diameter |
| **Cat. No.** | F13838 | F13839 | A34305 | 2.5 µm: A16502, A16500, A16501<br>6.0 µm: A16505, A16503, A16504 |

Contact your sales representative:

Find out more about flow cytometry beads and controls at **thermofisher.com/flow-controls**

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