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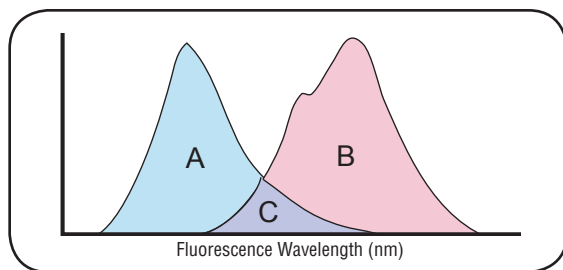


## BEADS ● ABOVE THE REST™

### Description

The Simply Cellular anti-Rat Compensation Standard is to be used in conjunction with hardware or software to remove spectral overlap from fluorochromes into secondary fluorescence detectors of a flow cytometer. Flow cytometers are designed to have a primary detector for each fluorochrome label (e.g. FL1- FITC, FL2- PE, FL3- Cy™5). Fluorescent signals emitted by fluorochromes can bleed or overlap into the secondary fluorescence detectors. In order to remove this overlap, the proper amount of signal must be subtracted from the secondary detector as a percentage of fluorescence intensity measured in the primary detector. This subtraction is performed by the electrical circuits prior to collecting sample data or by software when analyzing the list mode files. When the mean fluorescence of 2 populations of labeled standards are adjusted such that they have equal intensities in the secondary fluorescence detectors, then the data from the samples will be accurately compensated.

The Simply Cellular Compensation Standard is a mixture of 2 populations of microspheres that have the ability to bind rat monoclonal antibodies at high and low capacities, respectively. The microspheres are supplied in a sterile-filtered, pH buffered PBS solution containing surfactant and preservatives. As the operator labels these standards with the same antibody used to label the cell samples, the standards will exhibit spectral properties that closely match the cells being analyzed. These matching properties permit accurate adjustment of color compensation across the intensity range of the analysis.



*Fluorescence carryover (C) is the region of overlap of the two emission spectra (A,B).*

### Characteristics

Mean Diameter: 7-9µm  
 Particle Concentration: 2 x 10<sup>6</sup> microspheres/mL

### Material

#### Material Supplied

- Simply Cellular anti-Rat Compensation Standard microspheres

#### Material Required

- Cell samples
- Suspension solution
- Sample test tubes
- Fluorochrome-labeled rat monoclonal antibodies
- Vortex mixer
- Flow cytometer

## Procedure

Researchers are advised to optimize the use of particles in any application.

### Assays Using Conjugated Antibodies

Prepare a separate sample of Simply Cellular anti-Rat Compensation Standards for each fluorochrome used in your assays as follows:

1. Place one drop (~50µL) of Simply Cellular anti-Rat Compensation Standard microspheres into a test tube. Add one of the conjugated rat monoclonal antibodies used for labeling cells (the amount as per the manufacturer's instructions for cell labeling) and vortex the sample.
2. Incubate for 30 minutes with occasional agitation.
3. Wash 2 times and resuspend in 500µL of cell suspension solution. *Note:* To wash, centrifuge at low speed (<2000 RPM) for 2 minutes.

### Compensation: Data Collection and Instrument Adjustment

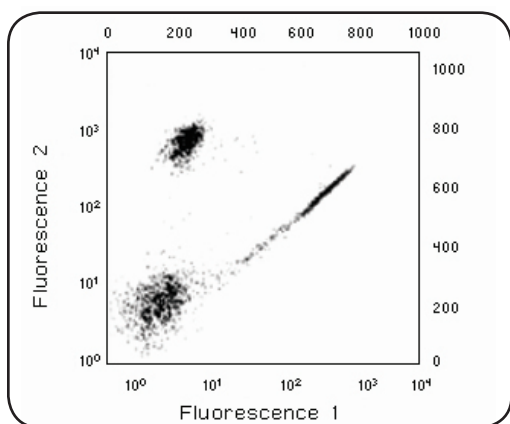
1. Perform routine set up of the analysis range (adjustment of PMT settings).
2. Perform compensation adjustments of each fluorescence channel separately.
3. Compensation
  - a. For software compensation, collect 10,000 events in a list mode file without gates and with the compensation circuits off. Gate the singlet population in the list mode files and make the appropriate adjustments in the software to make the 2 populations have equal intensities in the secondary fluorescence detectors.
  - b. For hardware compensation, gate on the singlet population and, while running, adjust the compensation circuits such that the 2 populations have equal intensities in the secondary fluorescence detectors.
  - c. *Alternative Hardware Compensation:* After washing, samples of Simply Cellular anti-Rat Compensation Standards labeled with different antibodies may be mixed together and analyzed. However, adjustments to the compensation circuits should be performed within an hour of mixing.
4. Validate compensation settings with cells labeled with the same conjugated antibodies.
5. If cells are not properly compensated, refer to the Notes section.

### Recommendations

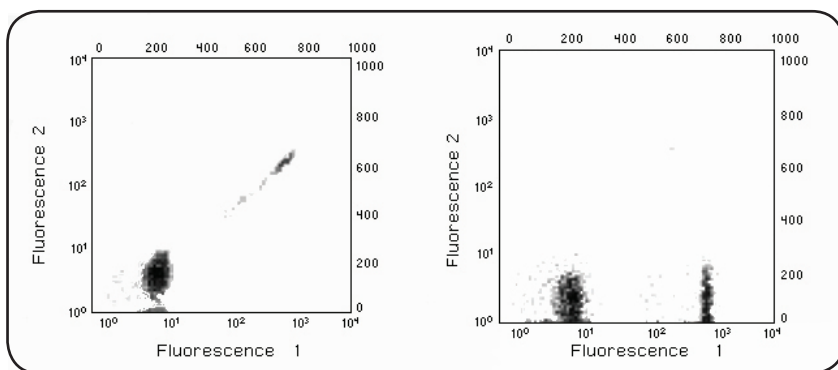
For consistency of data across instruments and time, it is recommended that a unified analysis range (Unified Window of Analysis) be used. The Unified Window of Analysis may be achieved by setting the PMT's of the detectors with Bangs' Right Reference Standard™ or QC Windows® when performing your daily set-up.

### Expected Values

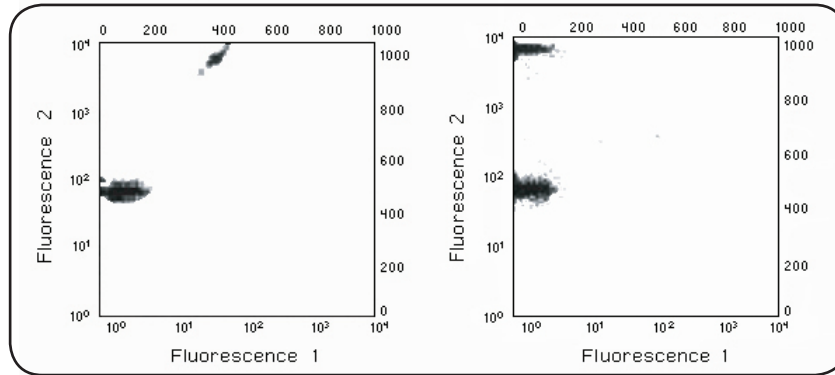
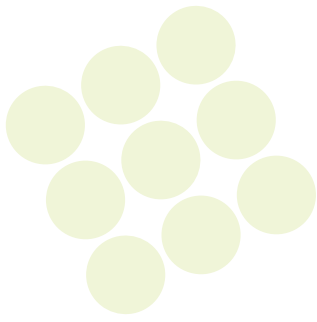
The following data are examples of how the fluorescence signals of the cells will be compensated when the instrument is adjusted using labeled Simply Cellular anti-Rat Compensation Standards.



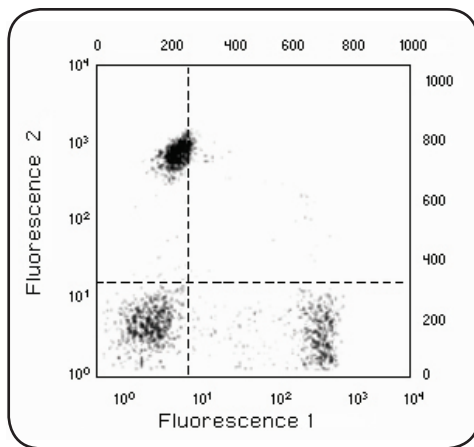
Uncompensated lymphocytes labeled with CD4-PE and CD8-FITC



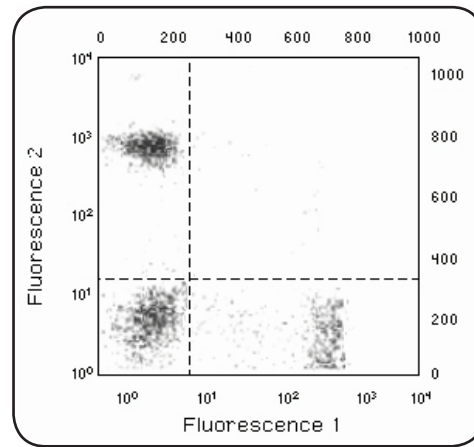
Uncompensated (left) and compensated (right) Simply Cellular® anti-Rat Compensation Standards labeled with CD8-FITC



Uncompensated (left) and compensated (right) Simply Cellular® anti-Rat Compensation Standards labeled with CD4-PE



Validation of FITC Compensated lymphocytes



Validation of FITC and PE compensated lymphocytes

## Notes

If cells are not properly compensated, follow these steps:

- Drain and fill the flow cell several times to eliminate air bubbles and debris.
- Wash fluidics system by running a fresh solution of 10% household bleach. Follow manufacturer's instructions.
- Check system for pressure leaks.
- Check the properties of diluent and sheath fluid (such as pH).
- Check alignment of the instrument.
- Consult your service engineer.

## References

1. **Schwartz, A., E. Fernandez-Repollet.** 1993. Development of clinical standards for flow cytometry. *Clinical Flow Cytometry*, Ann NY Acad Sci. 677: 28-39.
2. **Shapiro, H.M.** 1995. *Practical flow cytometry, 3rd Ed.* New York: Wiley Liss, Inc.
3. **Schwartz, A., G.E. Marti, R. Poon, J.W. Gratama, E. Fernandez-Repollet.** 1998. Standardizing flow cytometry: a classification system of fluorescence standards used for flow cytometry. *Cytometry*, 33: 106-114.

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2. Cy™, including Cy5, is a trademark of GE Healthcare Limited. These products are manufactured under license from Carnegie Mellon University under U.S. Patent Number 5,268,486 and related patents.



## Storage and Stability

Store at 2-8°C. Freezing may result in irreversible aggregation and loss of binding activity. Stable for 12 months from date of purchase, provided the product is handled in accordance with the manufacturer's recommendations. The reagent should be kept in its opaque bottle.

## Safety

This particle suspension contains sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azides. Upon disposal of material, flush with a large volume of water to prevent azide accumulation. Please consult the Material Safety Data Sheet for more information.

**This product is for research use only and is not intended for use in humans or for *in vitro* diagnostic use.**

## Ordering Information

Catalog Code	Description	Size
551	Simply Cellular® anti-Rat Compensation Standard	5mL

## Related Products

Catalog Code	Description	Sizes
550	Simply Cellular® anti-Mouse Compensation Standard	5mL
510	Right Reference Standard™ Fluorescein, Low Intensity	5mL
511	Right Reference Standard™ Fluorescein, Medium Intensity	5mL
512	Right Reference Standard™ Fluorescein, High Intensity	5mL
513	Right Reference Standard™ Phycoerythrin, Low Intensity	5mL
514	Right Reference Standard™ Phycoerythrin, Medium Intensity	5mL
515	Right Reference Standard™ Phycoerythrin, High Intensity	5mL
516	Right Reference Standard™ PE-Cy™5, Low Intensity	5mL
517	Right Reference Standard™ PE-Cy™5, Medium Intensity	5mL
518	Right Reference Standard™ PE-Cy™5, High Intensity	5mL
519	Right Reference Standard™ APC, Low Intensity	5mL
520	Right Reference Standard™ APC, Medium Intensity	5mL
521	Right Reference Standard™ APC, High Intensity	5mL
845	QC Windows® (FITC/PE)	1mL, 5mL, or 14mL
846	QC Windows® (FITC/PE/PE-TR)	1mL, 5mL, or 14mL
847	QC Windows® (FITC/PE/PE-Cy™5)	1mL, 5mL, or 14mL
848	QC Windows® (FITC/PE/PE-Cy™5, APC)	1mL, 5mL, or 14mL

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