DESCRIPTION
The Simply Cellular® anti-Human 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 human 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.

CHARACTERISTICS
Mean Diameter: 7-9µm
Particle Concentration: 2 x 10⁶ microspheres/mL

MATERIAL
Material Supplied
• Simply Cellular® anti-Human Compensation Standard microspheres

Material Required
• Cell samples
• Suspension solution
• Sample test tubes
• Fluorochrome-labeled human monoclonal antibodies

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-Human Compensation Standards for each fluorochrome used in your assays as follows:
1. Place one drop (~50µL) of Simply Cellular® anti-Human Compensation Standard microspheres into a test tube. Add one of the conjugated human 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-Human 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-Human Compensation Standards.

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


TRADEMARKS AND REGISTERED TRADEMARKS

1. Right Reference Standard™, QC Windows®, and Simply Cellular® are trademarks or registered trademarks of Bangs Laboratories.
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.

These products are for research use only and are not intended for use in humans or for in vitro diagnostic use.
## ORDERING INFORMATION

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<th>Cat. Code</th>
<th>Description</th>
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<tr>
<td>552</td>
<td>Simply Cellular® anti-Human Compensation Standard</td>
<td>5mL</td>
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## RELATED PRODUCTS

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<th>Cat. Code</th>
<th>Description</th>
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Order online anytime at [www.bangslabs.com](http://www.bangslabs.com).