Product Data Sheet 835

Simply Cellular® anti-Mouse for Violet Laser

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Bangs Laboratories, Inc.

BEADS ABOVE THE REST™

DESCRIPTION

Polymer-based calibration beads are commonly used for the routine set-up and QC of flow cytometers. They can be made to approximate the size and scatter characteristics of lymphocytes and other cells, and may be dyed or coated for use with a broad range of reagents and instruments. While these types of standards have almost universal application, there are special cases where a different bead matrix would be advantageous. Specifically, common polymer compositions (polystyrene, etc.) possess a significant absorbance band in the UV / Violet region. This can lead to seemingly higher detection thresholds and complicate bead-based compensation when using 405nm excitation.

The Simply Cellular® anti-Mouse Violet Laser standard features microspheres comprised of a proprietary matrix that exhibits low autofluorescence with violet excitation. Beads are suitable for labeling with mouse mAbs conjugated with violet fluorochromes, and for use as a compensation or general reference standard for detectors off of the violet laser. Beads are also suitable for use with other fluorochromes and lasers / detectors, e.g. 488nm, 633nm.

The Simply Cellular® anti-Mouse Violet Laser standard is supplied as 2 populations: 1 blank and one high binding anti-Mouse IgG (Fc-specific) population. They are supplied in aqueous suspension containing ProClin®.

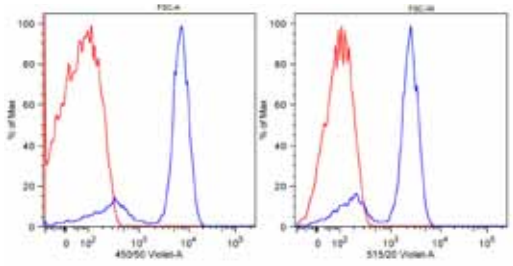


Figure 1: Unstained and CD3 Pacific Blue stained cells.

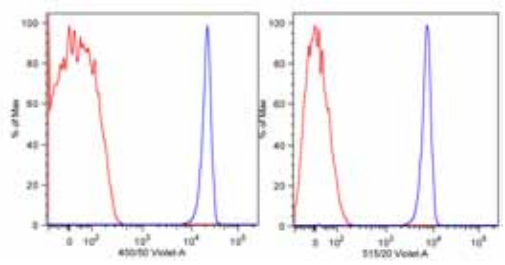


Figure 2: Blank and CD3 Pacific Blue stained microspheres.

CHARACTERISTICS

Mean Diameter:	~5-7µm
Particle Concentration:	2 x 10^6 beads/mL

MATERIAL

Material Supplied

- Simply Cellular® anti-Mouse Violet Laser standard: 2 bottles (1 blank, 1 labeled)

Material Required

- Bead staining buffer, e.g. PBS or other general buffer, pH 7.4
- Cell suspension solution
- Microcentrifuge tubes
- Flow tubes
- Pipets
- Rotator
- Vortex mixer
- Flow cytometer

PROCEDURE

Researchers are advised to optimize the use of particles in any application. Prepare all suspensions immediately prior to use. The standard should be analyzed on the same day and at the same PMT and compensation settings used to analyze cell samples.

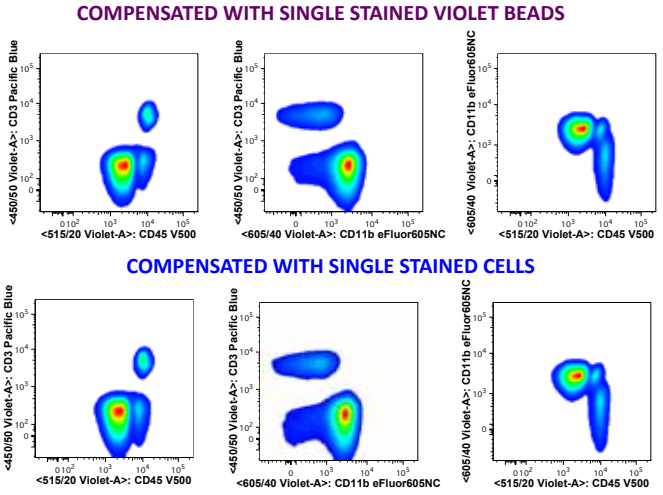
Preparation and Analysis

1. Manually shake the bottle to ensure a uniform suspension of microspheres. Do not vortex or sonicate.
2. Add one drop of Simply Cellular® microspheres to a microcentrifuge tube and add 100µL bead staining buffer, e.g. PBS pH 7.4 or other general buffer.
3. Add the same amount of fluorochrome-conjugated mouse IgG mAb recommended for labeling 1e+6 cells.
4. Incubate in the dark for 30 minutes, either at RT or in the refrigerator.
5. Add 1mL of the staining buffer and centrifuge gently (<3000 RPM) for 3 minutes.
6. Discard the supernatant and resuspend the microspheres in 1mL staining buffer.
7. Wash the microspheres a second time (repeat steps 5-6) and resuspend in 500µL of the same type of buffer / medium in which cells will be run.
8. The Blank population may now be added to the prepared tube of stained microspheres.
9. Analyze the microspheres on the flow cytometer. Using the FSC / SSC dot plot, construct a live gate around the singlets population.
10. Create appropriate fluorescence dot plots or histograms, depending on the specific application of the standard.

Compensation

1. Prepare a stained bead sample for each antibody conjugate. After final

washes are complete, labeled populations may be combined with the blank population to establish compensation settings for each detector.
2. Create a bivariate dot plot for the fluorescence channels of interest.
3. Adjust the PMT settings such that the blank population is positioned in the lower left corner / quadrant 3 of the dot plot.
4. Adjust the color compensation settings such that beads labeled with Ab / Fluorophore 1 appear in quadrant 1, in line with the Blank microspheres, and beads labeled with Ab / Fluorophore 2 appear in line with the Blank microspheres in quadrant 4.



Fluorescence Reference Standard

1. Prepare a stained bead sample using the antibody conjugate of interest. After final washes are complete, the labeled population may be combined with the blank population, or separate tubes may be run for the labeled and blank beads.
2. The prepared standard may be used as a general reference for stained cell samples, or for instrument set-up, i.e. to establish The Window of Analysis for the fluorescence detector of interest. The Window of Analysis is the intensity range that will be measured for the specific fluorescence detector. It is defined by establishing test-specific settings (PMT voltages) that result in both unstained and brightly stained samples appearing on scale.

NOTES

1. Bead samples should be protected from light during preparation, and used immediately. For best results, prepared beads samples should not be stored for later use.
2. Do not freeze bead suspensions or samples. Freezing is expected to lead to irreversible aggregation of beads.
3. A daily QC regimen is essential for ensuring the satisfactory performance of the fluidics and optical systems, and the linearity and resolution capabilities of the fluorescence detectors.
4. Standardization is essential for achieving consistent results and generating comparable data between studies and over time. The program should be comprehensive, encompassing reagents, sample preparation protocols, instrument configuration, and, for qualitative analyses, fluorescence intensity units.

REGISTERED TRADEMARKS

1. Simply Cellular® is a registered trademark of Bangs Laboratories, Inc.
2. ProClin® is a registered trademark of Rohm and Haas Company.

RELATED TECHNICAL LITERATURE

1. *Flow Cytometry Supplement*
2. BSS 008 - *Flow Quality Control and Standardization*
3. BSS 007- *Flow Cytometry Instrument Quality Assurance / Quality Control*
4. BSS 025 - *Quantitative Cytometry*

STORAGE AND STABILITY

Store at 2-8°C. Do not freeze. Prepare samples immediately before use.

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

ORDERING INFORMATION

Cat. Code	Description	Sizes
835	Simply Cellular® anti-Mouse for Violet Laser	1mL or 5mL

RELATED PRODUCTS

Cat. Code	Description	Sizes
550	Simply Cellular® anti-Mouse Compensation Standard	5mL
551	Simply Cellular® anti-Rat Compensation Standard	5mL
552	Simply Cellular® anti-Human Compensation Standard	5mL
820	FITC / PE Compensation Standard	1mL, 5mL, or 14mL

Order online anytime at **www.bangslabs.com.**