

DESCRIPTION

Simply Cellular[®] standards are uniform, cell-sized microspheres with calibrated amounts of Fc-specific capture IgG on their surfaces. The microspheres have been characterized in terms of their Antibody Binding Capacity (ABC) for monoclonal IgG antibodies. ABC units are an effective tool for quantitating the level of surface expression of antigens, as determined by the number of antibodies bound. The microsphere standards are suspended in a sterile-filtered, pH buffered solution containing surfactant and preservatives.

Simply Cellular[®] microspheres may be labeled with monoclonal IgG antibodies using the same direct- or indirect-staining methods used with routine immunophenotyping samples. By labeling Simply Cellular[®] microspheres to saturation with fluorochrome-conjugated antibody, the antibody's effective F / P (fluorophore / protein) ratio may be determined. As a quality control reagent, Simply Cellular[®] microspheres may be used to monitor the fluorescence intensity and stability of fluorochrome-conjugated antibodies.

Additionally, Simply Cellular[®] standards may be used in conjunction with Bangs Laboratories' Quantum[™] MESF kits to determine the effective F/P ratio of fluorochrome-conjugated antibodies.

CHARACTERISTICS

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

MATERIAL

Material Supplied

Simply Cellular[®] anti- (Mouse, Rat or Human) IgG microspheres

Material Required

Fluorochrome-conjugated IgG monoclonal antibodies
Suspension solution
Sample test tubes
Vortex mixer
Flow cytometer

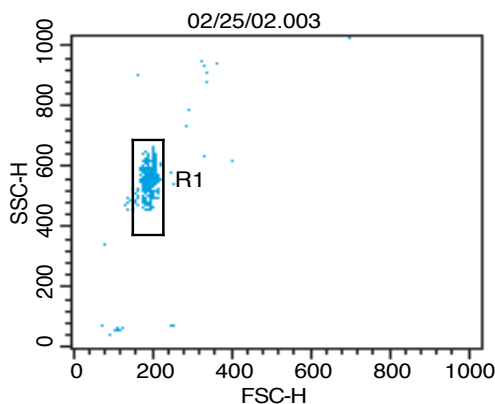


Figure 1

PROCEDURE

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

Preparation and Analysis

1. Manually shake the bottle to ensure a uniform suspension of microspheres. Do not vortex or sonicate the stock bottle.
2. Add one drop of Simply Cellular[®] microspheres to a test tube containing 100µL of the same type of buffer or medium in which the cell samples will be suspended.
3. Add the same amount of fluorochrome-conjugated IgG monoclonal antibody recommended for labeling 1,000,000 cells. *Note:* The amount of antibody needed to reach microsphere saturation may be assessed using small aliquots of Simply Cellular[®] microspheres and adding to them increasing amounts of the antibody, e.g. additional 50% antibody until less than a 10% increase in fluorescence intensity is obtained after adding the excess antibody. (See also PDS 814A QSC/SC Antibody Titration Protocol)
4. Incubate in the dark for 30 minutes.
5. Add 2mL of the cell suspension solution and centrifuge gently (<3000 RPM) for 3 minutes.
6. Discard the supernatant and resuspend the microspheres in 2mL of the cell suspension solution.
7. Wash the microspheres a second time (repeat Steps 5-6) and resuspend in 500µL of the cell suspension solution.
8. Analyze the microspheres on the flow cytometer. Using the forward scatter versus side scatter dot plot, construct a live gate around the single population of the microspheres. (Figure 1)
9. Create a fluorescence histogram (FL1, FL2, etc.) including only the events falling in the singlet gate of the forward scatter versus side scatter dot plot. Record the peak channel median (median, geo. mean, etc.) of the calibrated microbead population. Also, record the instrument settings (e.g. amplifier gains, PMT voltages, etc.)

QC of Fluorochrome-conjugated Antibodies

Prepare a separate test tube of Simply Cellular® microspheres as outlined in the above procedure for each fluorochrome-conjugated antibody to be QC'd. Always analyze the microspheres on the cytometer at the same instrument settings you recorded in Step 9 above. Record the peak channel of the Simply Cellular® microspheres. *Note:* Antibody QC is performed by comparing the daily peak channels of the Simply Cellular® microspheres labeled with the fluorochrome-conjugated antibody over time. A decline in the peak channel number may indicate the deterioration of the antibody or the associated fluorochrome.

Determination of Effective F/P Ratio

The F / P ratio (as determined by absorbance) is the number of fluorochromes conjugated to an antibody molecule. The effective fluorescence/protein ratio (effective F / P ratio) is the average fluorescence intensity in Molecules of Equivalent Soluble Fluorochrome (MESF) units per antibody molecule. It is the effective F / P ratio that is used to quantitate the fluorescence intensity of samples.

1. Calibrate the flow cytometer in terms of MESF units using the appropriate Quantum™ MESF kits available from Bangs Laboratories. (See the Quantum™ MESF Product Data Sheet for detailed calibration instructions.)
2. Prepare the Simply Cellular® microspheres as outlined in the above procedure.
3. Determine the fluorescence intensity in MESF units of the saturated Simply Cellular® microspheres according to the calibration procedures in Step 1.
4. Determine the effective F/P ratio of the antibody by dividing the MESF value of the saturated Simply Cellular® microspheres by the ABC value of the microspheres, as indicated on the bottle label.

Effective F / P Ratio:

$$\frac{\text{MESF of saturated Simply Cellular microspheres}}{\text{ABC value of Simply Cellular microspheres}}$$

Recommendation

For consistency 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 Quantum™ QC when performing your daily instrument set-up.

NOTES

1. Proper storage and handling are essential to maintaining the calibrated binding capacities of the Quantum™ Simply Cellular® microspheres. Vigorous shaking of the reagent bottle prior to use is necessary to ensure a uniform suspension of microspheres. When dispensing the standards from the dropper bottle(s), turn the bottle upside down and shake gently to remove any air bubbles trapped in the tip before squeezing the bottle to dispense the product.
2. The fluorescence resolution (CV%) of labeled Simply Cellular® microspheres will vary with the fluorochrome-conjugated antibody used. Such variations are expected. Larger CV%'s may be indicative of poor antibody quality (e.g., non-uniform fluorochrome-conjugation).
3. Prior to acquiring data for counts, the flow cell should be free of debris. This can be accomplished by running a 10% solution of household bleach (follow instrument manufacturer's recommendations) for 5 minutes followed by distilled water for another 5 minutes. Should this fail, follow these steps:
 - Prepare and run a fresh sample.
 - 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, M. Velilla, B. Ocasio. 1990. *Calibration of flow cytometers to directly measure numbers of antibody binding sites on cells*. Abstracts, for the XIV International Meeting of the Society for Analytical Cytology, Asheville, NC; Cytometry Suppl 4, Abstract 463B.
2. Randlev B, Huang L-C, Watatsu M, Marcus M, Lin A, Shih S-J. (2010) *Validation of a quantitative flow cytometer assay for monitoring HER-2/neu expression level in cell-based cancer immunotherapy products*. *Biologicals*; 38(2):249-259.
3. McBean RS, Wilson B, Liew Y-W, Hyland CA, Flower RL. (2015) *Quantitation of Lan antigen in Lan+, Lan+(w) and Lan- phenotypes*. *Blood Transfus*; 13:662-5
4. Phillips A, Boghaert E, Vaidya K, Mitten M, Norvell S, Falls H, ...Reilly A (2016) *ABT-414, an Antibody-Drug Conjugate Targeting a Tumor-Selective EGFR Epitope*. *Mol Cancer Ther* 15(4):661-669
5. Tillotson B, Cho YK, Shusta E, (2013) *Cells and Cell Lysates: A Direct Approach for Engineering Antibodies Against Membrane Proteins Using Yeast Surface Display*. *Methods* 60(1):27-37

TRADEMARKS AND REGISTERED TRADEMARKS

1. Quantum™, QuickCal®, Right Reference Standard™, and Simply Cellular® are trademarks or registered trademarks of Bangs Laboratories, Inc.
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.
3. Alexa Fluor® is a registered trademark of Life Technologies Corporation.

STORAGE AND STABILITY

Store at 2-8°C. Do not freeze and do not sonicate. Prepared samples may be vortexed briefly, if necessary to increase % singlets. Stable for 12 months from date of purchase, provided the product is handled in accordance with the manufacturer's recommendations. Store in reagent's 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 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

Cat. Number	Description	Sizes
810	Simply Cellular® anti-Mouse IgG	1ml, 5mL, or 14mL
812	Simply Cellular® anti-Human IgG	1ml, 5mL, or 14mL
813	Simply Cellular® anti-Rat IgG	1ml, 5mL, or 14mL

RELATED PRODUCTS

Cat. Number	Description	Size
512	Right Reference Standard™ Fluorescein, High Intensity	5mL
515	Right Reference Standard™ Phycoerythrin, High Intensity	5mL
518	Right Reference Standard™ PE-Cy™5, High Intensity	5mL
521	Right Reference Standard™ APC, High Intensity	5mL
488	Quantum™ Alexa Fluor® 488 MESF	1mL, 5mL, or 14mL
647	Quantum™ Alexa Fluor® 647 MESF	1mL, 5mL, or 14mL
823	Quantum™ APC MESF	1mL, 5mL, or 14mL
822	Quantum™ Cy™5 MESF	1mL, 5mL, or 14mL
555	Quantum™ FITC-5 MESF	1mL, 5mL, or 14mL
555p	Quantum™ FITC-5 MESF (Premix)	1mL, 5mL, or 14mL
828	Quantum™ PE-Cy™5 MESF	1mL, 5mL, or 14mL
827	Quantum™ R-PE MESF	1mL, 5mL, or 14mL
725	Quantum™ QC	5mL

Order online anytime at www.bangslabs.com.