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**B E A D S ● A B O V E T H E R E S T™**

Description

Quantum Simply Cellular kits are intended for use in the quantitation of cellular antigen expression in Antibody Binding Capacity (ABC) units. Additionally, Quantum Simply Cellular microspheres may serve as an accurate compensation standard when labeled with the same antibodies used to stain cell samples.

The Quantum Simply Cellular anti-Rat IgG kit is comprised of populations of uniform cell-sized microspheres with different calibrated binding capacities of goat anti-rat IgG (Fc-specific) on their surfaces. The kit includes 4 coated populations, each with a different ABC for rat monoclonal antibodies, and 1 blank population with no specific binding capacity for rat IgG. The microspheres are suspended in a sterile filtered, pH buffered solution containing surfactant and preservatives.

When the bead populations are labeled in the same manner as the cells to be analyzed, they provide a means for constructing a QuickCal calibration curve (ABC values versus fluorescence intensity), from which samples may be “read.” Cellular antigen expression may thus be quantified in ABC units. The calibration curve is also a useful means for determining instrument linearity.

Quantum Simply Cellular microspheres may also be used as a compensation standard when labeled with the same fluorochrome-conjugated antibodies used to stain cell samples.

Characteristics

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

Material

Material Supplied

- Quantum Simply Cellular anti-Rat IgG kit: 5 bottles included (4 coated, 1 uncoated blank)
- QuickCal® Template: Download from www.bangslabs.com using the access code provided at the time of kit purchase.

Material Required

- Fluorochrome-conjugated rat IgG monoclonal antibodies
- Cell suspension solutions
- Sample test tubes
- Flow cytometer
- Vortex mixer
- Centrifuge

Procedure

Researchers are advised to optimize the use of particles in any application. Prepare all suspensions immediately prior to use. The standards (including the Blank) should be analyzed on the same day and at the same fluorescent PMT and compensation settings used to analyze the samples you wish to quantitate. *Note:* Indirect staining of the Quantum Simply Cellular microspheres may be performed using a primary rat IgG antibody and a fluorochrome-conjugated anti-rat antibody when a fluorochrome-conjugated primary antibody is not available.

General Assay

1. Blank population: do not stain.
2. Combine 1 drop of each of the 4 labeled components and add 50µL of the same type of buffer or medium in which cell samples will be suspended and vortex. Do not sonicate.
3. Add 50% of the amount of fluorochrome-conjugated rat IgG monoclonal antibody recommended for 4,000,000 cells smoothly and rapidly to obtain the tightest distribution. *Note:* The amount of antibody needed to reach microsphere saturation may be assessed using small aliquots of Quantum 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.
4. Incubate in the dark for 30 minutes.
5. Add 2mL of cell suspension solution and centrifuge at 2500xG for 5 minutes.
6. Wash 2 times (centrifuge at 2500xG for 5 minutes) and resuspend to 500µL using the same solution as the cells to be analyzed.
7. Analyze the microspheres on the flow cytometer. Bead populations may be combined or run separately. A flow rate of 100-200 events per second is recommended. Typically, 1000 events are collected per bead population (i.e., 5 bead populations x 1000 events each).
8. Using a forward scatter versus side scatter dot plot, construct a live gate around the singlet population of microspheres and determine the peak (median) histogram channels of each of the 5 populations of microspheres in the corresponding fluorescent channel for entry into the QuickCal® spreadsheet. Unstained cells should be run at the same instrument settings as the bead standards. The ABC value of the unstained cell sample may be subtracted from the ABC values of your stained cell samples.
9. Log into www.bangslabs.com/products/quickcal to use Bangs Laboratories' quantitative software, QuickCal, to generate a calibration curve, determine the instrument detection threshold, and quantitate the ABC values of unknown samples. To access this free service, you will need the QuickCal access number affixed to your Quantum Simply Cellular kit. If internet access is not available, the following steps may be used to manually generate a calibration curve, determine the instrument detection threshold, and quantitate the ABC values of unknown samples.
 - a. *Establishing a Calibration Curve:*
 1. Establish a standard calibration curve by plotting the ABC (y-axis) versus the peak channel (x-axis) for each of the 4 antibody-binding microspheres. *Note:* If linear fluorescence is selected, a log-log plot of the data should give a 45° line. If log fluorescence is selected, the data should be plotted on a semi-log paper, and may not fall on a 45° line. The actual slope obtained will be characteristic of the particular log amplifier and PMT.
 - b. *ABC Detection Threshold Determination:*
 1. After completing the ABC calibration procedure and plotting the calibration curve, determine and record the peak (median) channel of the reference blank. Alternatively, you may wish to use the peak channel of your unstained cell sample.
 2. Use the calibration plot to determine the ABC value associated with the fluorescence of the reference blank (or unstained cell). This is the ABC detection threshold of the instrument at these instrument settings. The detection threshold is the lowest number of ABC units detectable above instrument noise.
 - c. *ABC Quantitation of Samples:*
 1. After completing the ABC calibration procedure described above and plotting the calibration curve, analyze the unknown samples on your flow cytometer. *Note:* To correctly quantitate the ABC of samples, the instrument settings used for the ABC calibration must remain exactly the same for sample analysis.
 2. Record the sample's peak (median or geo mean) channel value for each population.
 3. Use the calibration plot to determine the ABC value that corresponds to each of the sample's peak channels. The ABC value of the unstained cell sample may be subtracted from the ABC values of your stained cell samples.

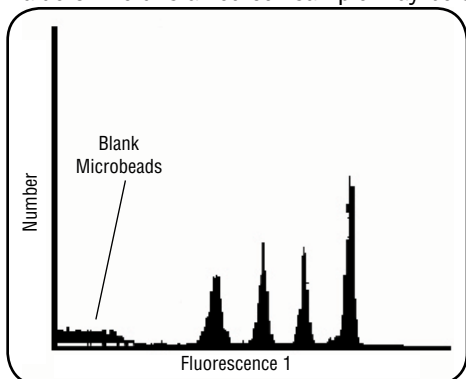


Figure 1: The fluorescence histogram of Quantum Simply Cellular microspheres.

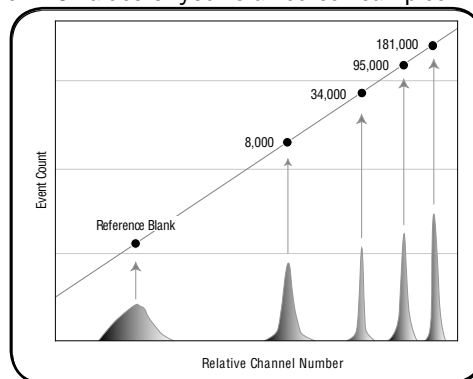


Figure 2: The calibration curve from which the ABC values of samples may be obtained.

For Use as a Compensation Standard

Quantum Simply Cellular microspheres labeled with fluorochrome-conjugated antibodies will have the same spectral properties as cells labeled with the same antibodies and will therefore provide an accurate compensation standard, which will cover the intensity range of different labeled cells.

1. Prepare the Quantum Simply Cellular microspheres as per the procedure above, preparing a separate tube for each fluorochrome-conjugated antibody to be used.
2. Mix the Quantum Simply Cellular microspheres labeled with the different fluorochrome-conjugated antibodies in a single test tube and perform instrument compensation following your routine laboratory procedures.

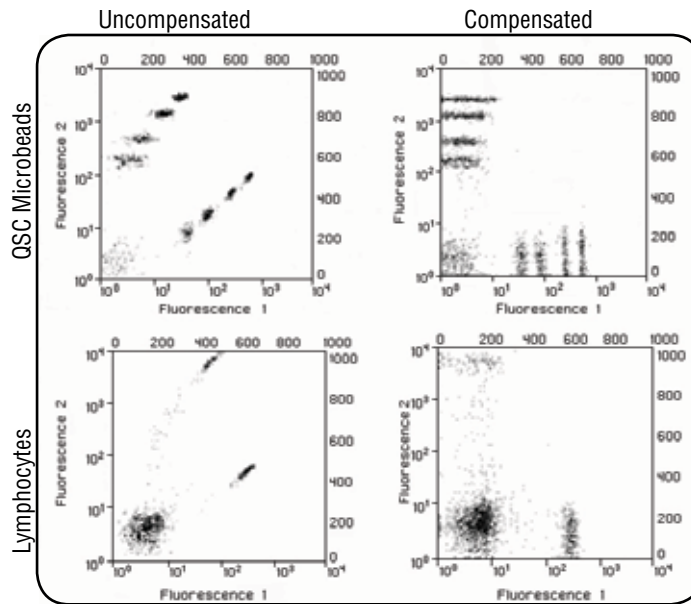


Figure 3. The Quantum Simply Cellular microspheres used as a compensation standard with respect to lymphocytes labeled with the same antibodies, CD4-FITC and CD8-PE (BDIS).

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 Standards™ or QC Windows® when performing your daily instrument setup.

Expected Values

Quantum Simply Cellular microspheres should exhibit 5 populations, with the blank population falling in the first decade when the flow cytometer is properly adjusted. The distance of the 4 labeled populations from the blank will be a function of the effective F/P ratio of the specific antibody and the efficiency of instrument response.

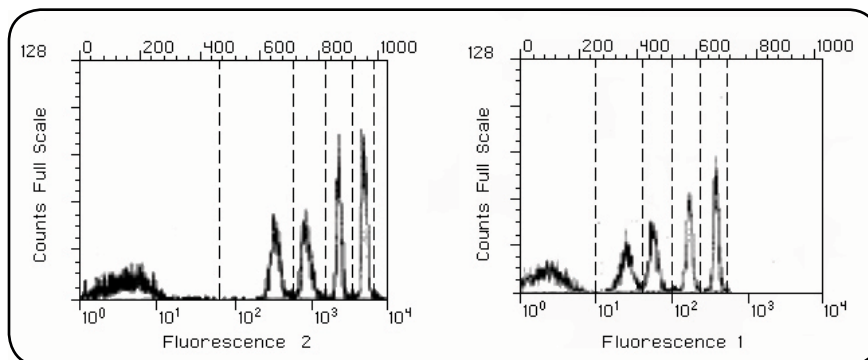


Figure 4. The different fluorescence response obtained with Quantum Simply Cellular microspheres labeled with 2 different antibodies, CD4-PE and CD4-FITC (Sigma).

Specific antibodies may not result in the population resolution normally observed with Quantum Simply Cellular microspheres labeled with other antibodies. Such results may reflect the quality of the specific antibodies (e.g., non-uniform fluorochrome-conjugation).

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), invert the bottle and shake gently to remove any air bubbles trapped in the tip before squeezing the bottle to dispense the product (~50µL drops).
2. 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.** 1993. Development of clinical standards for flow cytometry. *Ann NY Acad Sci* 677: 28-39.
2. **Zagursky, R.J., D. Sharp, K.A. Solomon, A. Schwartz.** 1995. Quantitation of cellular receptors by a new immunocytochemical flow cytometric technique. *BioTechniques*, 18: 504-509.
3. **Lenkei, R., B. Anderson.** 1995. Determination of the antibody binding capacity of lymphocyte membrane antigens by flow cytometry in 58 blood donors. *J Immunol Meth*, 183: 267-277.
4. **Denny, T.N., D. Stein, T. Mui, A. Scolpino, B. Holland.** 1996. Quantitative determination of surface antibody binding capacities of immune subsets present in peripheral blood of healthy adult donors. *Cytometry (Communications in Clinical Cytometry)*, 26: 265-274.
5. **Borowitz, M.J., J. Shuster, A.J. Carroll, M. Nash, A.T. Look, B. Camitta, D. Mahoney, S.J. Lauer, D.J. Pullen.** 1997. Prognostic significance of fluorescence intensity of surface marker expression in childhood B-precursor acute lymphoblastic leukemia. *Blood*, 89: 3960-3966.
6. **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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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 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
817

Description
Quantum™ Simply Cellular® anti-Rat IgG

Sizes
1mL, 5mL, or 14mL

Related Products

Catalog Code

Description

Sizes

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
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
555	Quantum™ FITC-5 MESF	1mL, 5mL, or 14mL
555p	Quantum™ FITC-5 MESF (Premix)	1mL, 5mL, or 14mL
827	Quantum™ R-PE MESF	1mL, 5mL, or 14mL
828	Quantum™ PE-Cy™5 MESF	1mL, 5mL, or 14mL
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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