Bangs Laboratories
BEADS ABOVE THE REST

Quantum™ Simply Cellular®

Product Data Sheet 814

DESCRIPTION

Quantum™ Simply Cellular® (QSC) microspheres are used in the quantitative analysis of cellular antigen expression. When stained with the same antibody that is used to label cells, they permit determination of the Antibody Binding Capacity (ABC) of the cells.

Each QSC kit consists of 5 bead populations - 1 blank and 4 with increasing levels of Fc-specific capture antibody. The coated populations are calibrated in terms of their ABC for monoclonal antibodies, and beads are labeled to saturation with the same monoclonal that is used to label cells. Beads are run in the same type of suspending solution / medium, on the same instrument, at the same instrument settings (PMT voltages, compensation settings), and on the same day as cell samples. Channel values for the bead populations are recorded in the lot-specific QuickCal® template that is provided, and a regression associating fluorescence channel value to the beads' ABC values is calculated. ABC values are assigned to stained cell samples using this standard curve. If monovalent antibody-to-surface receptor binding is presumed, then the ABC value = # surface receptors.

CHARACTERISTICS

Mean Diameter: ~6-9µm
Particle Concentration: 2 x 10^6 beads/mL (~100,000 beads / 50µL drop)

MATERIAL

Material Supplied

- Quantum™ Simply Cellular® kit: 5 bottles (4 populations coated with increasing amounts of capture antibody, 1 uncoated blank). Kits are available for use with mouse (Catalog Code 815), rat (Catalog Code 817), and human (Catalog Code 816) monoclonal antibodies. For example, the anti-Mouse kit is intended to bind mouse mAbs, not for the analysis of mouse cells.
- QuickCal® Template: Download from **www.bangslabs.com/quickcal** using the access code provided at the time of kit purchase.

Material Required

- Fluorochrome-conjugated monoclonal IgG antibodies. *Note:* Each kit is specific for antibodies produced in the noted host species.
  - Catalog Code 815: Quantum™ Simply Cellular® anti-Mouse for mouse mAbs
  - Catalog Code 816: Quantum™ Simply Cellular® anti-Human for human mAbs
  - Catalog Code 817: Quantum™ Simply Cellular® anti-Rat for rat mAbs
- Staining buffer (see *Notes*)
- Cell suspension solution
- Sample test tubes
- Flow cytometer
- Vortex mixer
- Centrifuge

BEFORE YOU BEGIN

As quantitative fluorescence analyses place the highest demands on cytometer performance, it is imperative that the instrument is performing optimally. To that end, a daily QC regimen is essential. In addition to the satisfactory performance of fluidics and optical systems, the linearity and resolution capabilities of fluorescence detectors must be ensured to achieve accurate and reproducible measurements. See the *Related Technical Literature* section of this data sheet for additional reading.

Sample preparation and staining procedures must be highly consistent to minimize variation in assignments, and we suggest the performance of titrations to ensure that saturating amounts of antibody are used to stain the beads and cells. Additionally, panel design should give consideration to fluorochrome selection in the context of expression level (fluorescence intensity, fluorochrome size to avoid steric effects), fluorescence carryover, etc.

GENERAL MICROSPHERE HANDLING

As QSC microspheres are coated with precise amounts of antibody, bottles must be handled carefully to preserve calibrated ABC values. Stock bottles must not be sonicated or vortexed, as repeated treatments over time may result in lessened or heterogeneous antibody binding. Microspheres must be stored in their original bottles at 2-8˚C. While the product will tolerate usual trips in and out of the refrigerator, quantitative products will be sensitive to lengthier excursions from 2-8˚C storage.

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. Additional tips for working with QSC kits and troubleshooting unexpected results may be found in Product Data Sheet 818.

Determining the Window of Analysis

The Window of Analysis is the intensity range that will be measured by the fluorescence detector of interest. It is defined by the test-specific fluorescence settings (PMT voltages) that result in the appearance of both unstained and brightly stained samples on scale (see figures below). To ensure that accurate ABC assignments are made, PMT voltages (fluorescence) and compensation settings must be the same for QSC beads and the cell samples of interest.

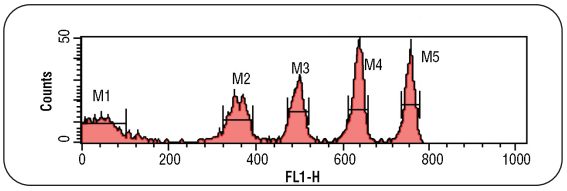

Figure 1: Labeled QSC microspheres.

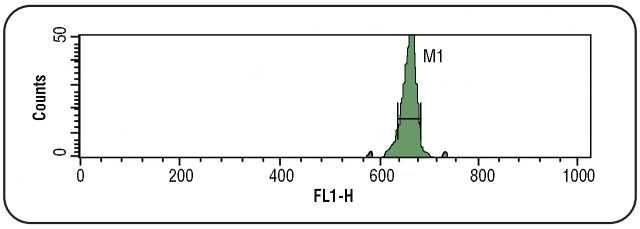

Figure 2: Labeled cell sample.

General Assay

1. Manually shake the bottle to ensure a uniform suspension of microspheres. Do not vortex or sonicate the stock bottle.
2. Blank population: Do not stain.
3. Stain each labeled population (1 – 4) separately for best resolution.
4. Add one drop of QSC microspheres to a microcentrifuge tube, add 50µL staining buffer (See *Notes*), and gently tap or flick the tube. Do not sonicate. Prepare one tube for each antibody capture population (1 – 4).
5. Add the amount of fluorochrome-conjugated mAb recommended for 2 x 10^6 cells smoothly and rapidly to obtain the tightest distribution, and gently tap or flick the tube. *Note:* The amount of antibody needed to reach microsphere saturation may be assessed using small aliquots of QSC microspheres and adding to them increasing amounts of the antibody, e.g. additional 50% antibody until less than a 10% increase is obtained after adding the excess antibody.
6. Incubate in the dark for 30 minutes. *Note:* Incubations may be performed at room temperature or in the refrigerator. It is not necessary to perform them on ice.
7. Add 1mL buffer and centrifuge at 2500 x G for 5 minutes.
8. Wash 2 times (centrifuge at 2500 x G for 5 minutes) and resuspend in 500µL of the same type of buffer or medium as cells. Immediately transfer the beads to a flow tube. Stained bead populations may be combined and run in a single tube, or run individually. The blank population may be added to the stained populations or run individually.
9. Analyze the microspheres on the flow cytometer at the test-specific instrument settings (PMT voltages and compensation). 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.
10. Using a FSC / SSC dot plot, construct a live gate around the singlets population. In the appropriate fluorescence histogram, apply a half-height / full-width gate to each population.
11. QSC microspheres should exhibit 5 populations, with the blank population falling in the first decade when the flow cytometer is properly adjusted. The resolution, or spacing between the populations, will be a function of the effective F/P ratio of the specific antibody, the specific fluorochrome, method of staining (direct / indirect), and the efficiency of instrument response.
12. Record the Geo Mean or Median channel value for each population for entry into the QuickCal® spreadsheet.
13. Stain and run cell samples at test-specific settings, and record their Geo Mean or Median channel values. Cells and QSC beads must be run on the same day, on the same instrument, in the same type of suspending solution, and at the same instrument settings (PMT voltages, compensation settings) to ensure accurate and reproducible assignments. When recording channel values, the same statistic must be used for beads and cells.
14. QuickCal®: Log into **www.bangslabs.com/quickcal** to download a lot-specific QuickCal® analysis template. The Access Number is printed on a label that is affixed to the product. Detailed instructions for use and troubleshooting tips are provided in Product Data Sheet # 819, *QuickCal® v. 3.0 Data Analysis Program.*
15. Enter bead channel values in the appropriate cells at the top of the template. A regression associating each bead's fluorescence channel value to its pre-assigned ABC value will be calculated automatically. A curve will appear in the calibration plot, and the regression coefficient (r^2) and detection threshold will be reported. (*Note:* QuickCal® templates are specific to both the QSC lot and the instrument scale. Depending on scale, either a logarithmic or semi-log regression will be calculated.)
16. R^2 values as near as possible to 1.0 are desired. Sub-optimal linearity will impact the accuracy and reproducibility of assignments that are made to cells and may indicate: a.) a problem with the run; b.) use of the wrong template resolution; or c.) the need for instrument maintenance.
17. The blank bead population is not used to construct the curve; rather, it is read from the curve, and its ABC value is reported as the Detection Threshold. If unstained cells possess lower autofluorescence than the blank bead population, they may be used for determining the Detection Threshold.
18. Enter channel values from labeled cell samples in the appropriate cells in the lower portion of the template. An ABC value will be assigned to each population.
19. The ABC value of either unstained cells or isotype controls may be subtracted from the cells' ABC values.

For Use as a Compensation Standard

QSC microspheres labeled with fluorochrome-conjugated antibodies will have the same spectral properties as cells labeled with those antibodies. They will thus serve as an accurate compensation standard that will cover the intensity range of cells with different expression levels.

1. Prepare the QSC microspheres as described above, preparing a separate set for each fluorochrome-conjugated antibody to be used.
2. Mix the QSC sets in a single tube and perform fluorescence compensation following your routine laboratory procedures.

NOTES

1. Beads may be stained using a general staining buffer, e.g. PBS or other buffer, as needed. For example, some cell media contain fixatives such as formaldehyde, which can be detrimental to the bead matrix. In these instances, beads may be stained in a fixative-free buffer.
2. Beads are not intended to be fixed, even if fixed cells are being run.
3. Our calibrations for this product feature direct staining with a labeled monoclonal Ab. Some customers use indirect staining out of necessity; the caveat is that there could be a greater than 1:1 ratio of reporter antibody to primary mAb, which would artificially increase the signal. However, this is sometimes a necessity and we take results to be semi-quantitative, and certainly useful to assess expression within a specific study.
4. Standardization is essential for quantitative fluorescence analyses. This includes bead and cell sample preparation, general handling, instrument settings, etc.
5. Quantitative analyses demand optimal instrument performance, including linearity and resolution capabilities.
6. Binding capacities (i.e. ABC values) vary for bead populations from one Lot to the next. It may be necessary to perform a titration with a new kit if results are not as expected, e.g. if bead peaks have shoulders or are more broad than usual, if cell samples have higher than normal ABC values, or if regression coefficients are unusally low.
7. Product Data Sheet 818 has additional information about working with QSC kits as well as tips for troubleshooting unexpected results.

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(3):504-509.
3. **Lenkei, R., B. Andersson.** 1995. Determination of the antibody binding capacity of lymphocyte membrane antigens by flow cytometry in 58 blood donors. *J Immunol Meth*, 183(2):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*, 26(4):265-274.
5. **Borowitz, M.J., J. Shuster, A.J. Caroll, 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. A pediatric oncology group study. *Blood*, 89(11):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(2):106-114.

TRADEMARKS AND REGISTERED TRADEMARKS

1. Quantum™, QuickCal®, 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.

RELATED TECHNICAL LITERATURE

1. PDS 818 - *Quantum™ Simply Cellular® and Quantum™ MESF Tips and Techniques*
2. PDS 819 - *QuickCal®, v. 3.0 Data Analysis Program*
3. BSS 004 - *Compensation Standards*
4. BSS 008 - *Flow Quality Control and Standardization*
5. BSS 007 - *Flow Cytometry Instrument Quality Assurance / Quality Control*
6. BSS 025 - *Quantitative Cytometry*

STORAGE AND STABILITY

Store at 2-8˚C. Do not freeze and do not sonicate. Prepared samples may be vortexed briefly, if necessary (e.g. single pulse), 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.

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

ORDERING INFORMATION

Cat. Code	Description	Sizes
815	Quantum™ Simply Cellular® anti-Mouse IgG	1mL, 5mL, or 14mL
816	Quantum™ Simply Cellular® anti-Human IgG	1mL, 5mL, or 14mL
817	Quantum™ Simply Cellular® anti-Rat IgG	1mL, 5mL, or 14mL

RELATED PRODUCTS

Cat. Code	Description	Sizes
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

PRODUCTS FOR DAILY SET-UP AND QC

Cat. Code	Description	Sizes
725	Quantum™ QC	5mL
855	Full Spectrum™	1mL, 5mL, or 14mL
610	Ultra Rainbow Fluorescent Particles, ~3.8µm	1mL, 5mL, or 14mL
611	Ultra Rainbow Fluorescent Particles, ~10.2µm	1mL, 5mL, or 14mL

PRODUCTS FOR COMPENSATION

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

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