BANGS BEADS

FLOW CYTOMETRY SUPPLEMENT

2012-2013

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800.387.0672

BANGSLABS. COM



ABOUT BANGS LABORATORIES, INC.

Bangs Laboratories, Inc. was founded April 1, 1988, and has grown to include a product list containing more than 1,500 varieties of the best microspheres in the world. Bangs Laboratories is a manufacturer and supplier of specialty microsphere products for diagnostic, research, and flow cytometry applications.

CORPORATE LOCATIONS

Bangs is a wholly owned subsidiary of Polysciences, Inc. With corporate locations around the world, we are ready to meet your global needs.

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QUALITY POLICY

It is the policy of Bangs Laboratories, Inc. to provide products, on time, that meet our customers' quality and service requirements, and which exceed them whenever possible. To fulfill this policy, all Bangs employees are committed to a total quality assurance program, driven by the philosophy of preventing quality problems. To ensure that our products merit and earn customer satisfaction, Bangs Laboratories actively seeks a dynamic relationship with Customers, Employees, and Suppliers.

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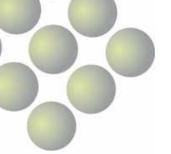


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In flow cytometry, a comprehensive quality assurance program is essential for achieving accurate and consistent results within a study, and generating comparable data between instruments and laboratories. Bangs Laboratories' flow cytometry supplement includes instrument standards that support the various aspects of quality assurance programs, from general instrument QC to study-specific standardization.

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Routine Quality Control

In the field, service engineers rely on microsphere standards to check and calibrate flow cytometers. Similar standards should be used by the facility as part of a comprehensive quality control program.

Microsphere standards aid in defining the instrument's capabilities and limitations in terms of sensitivity,

precision and accuracy, and provide a means for ensuring that the instrument is stable and suitable for use. They are also helpful in understanding the effects of extraneous factors such as temperature, humidity, and electronic noise.

The comparison of daily and historical QC data aids in the identification of random errors (due to electronic noise, air bubbles, etc.) and systematic errors (bias, shifts and trends caused by temperature variation, laser deterioration, misalignment, etc.) so that suitable corrective action may be taken.

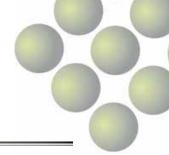
Fluorophore-labeled microspheres offer a convenient means to check general instrument status and



Figure 1: A flow cytometer.

monitor stability over time. When beads are run, the median channel values for pertinent fluorescence detectors are recorded, and data are monitored to identify outliers and trends. This type of check can alert the user to problems with the optical and fluidic systems (e.g. diminishing laser power or obstruction / leakage) and the effect of environmental factors such as temperature, humidity, and vibration on instrument performance.

When designing a flow cytometry QC program, the unique needs of the department or facility should be considered. Some products may satisfy multiple tasks, such as a single product used for basic daily QC and set-up of dedicated instruments. In other instances, combinations of products may be appropriate to meet the unique requirements of specific studies, or for instruments with shared use. Please feel free contact us to discuss what products are available to support the unique requirements of your QC program or study.



Routine Quality Control, continued

The below table is intended to provide a framework to aid in product selection for both initial instrument validation and continued management.

Purpose	Frequency	Products
General check of instrument stability/ status	Daily	Full Spectrum™ (multi) Ultra Rainbow Fluorescent Particles (multi) Fluorescent Reference Standards (single)
General check of instrument optical system	Daily	Full Spectrum™ (multi) Ultra Rainbow Fluorescent Particles (multi) Fluorescent Reference Standards (single)
Optical Alignment	Daily	Right Reference Standard™
Fluidics check	Daily	Surface-labeled fluorescent microspheres, e.g. QC Windows® QC3™ Fluorescent Reference Standards Quantum™ MESF
Optical System Sensitivity, Resolution and Linearity (for specific lasers/PMTs)	Weekly	Quantum™ MESF
Assessing Time Delay	Weekly	Time Delay Calibration Standard

Table 1: Instrument quality control suggestions.

FULL SPECTRUM™

Full Spectrum™ microspheres provide a convenient means for performing the initial daily QC check on 3-9+ color instruments. The microspheres are internally labeled with multiple fluorophores for use with common excitation and detection wavelengths within the visible spectrum.

Catalog	catalog Product Data		P	rice (\$US	S)
Code	- IIDECTINIUM		A 20 tests	B 100 tests	C 280 tests
885	Full Spectrum™	885	150	300	580

Routine Quality Control, continued

ULTRA RAINBOW FLUORESCENT PARTICLES (BY SPHEROTECH™)

Ultra Rainbow Fluorescent Particles are internally labeled with multiple fluorophores, enabling excitation at any wavelength from 365 – 650nm. Ultra Rainbow Fluorescent Particles may be used for general QC purposes for 3-9+ color instruments.

Catalog Product Data	Price (\$US)				
Code	Description	Sheet	A 20 tests	B 100 tests	C 280 tests
610	Ultra Rainbow Fluorescent Particles, ~3.8µm (3.61 – 3.99µm)	610	150	300	580
611	Ultra Rainbow Fluorescent Particles, ~10.2µm (8.1 – 12.0µm)	611	150	300	580

FLUORESCENCE REFERENCE STANDARDS

Single-color Fluorescence Reference Standards are labeled with specific fluorochromes to exhibit the same spectral characteristics as labeled cells. These standards may be used to QC a specific path of the optical system (laser / filter / PMT), to optimize filter and mirror sets for fluorophores, and to establish a test-specific Target Channel Value for instrument set-up.

Catalon	Catalog Product Da		F	rice (\$US	S)
Code	Description	Sheet		B 100 tests	C 280 tests
890	Certified Blank™	890	150	300	580
910	Autofluor™	890	150	300	580
897	Acridine Orange	890	150	300	580
886	Alexa Fluor® 488	892	150	300	580
887	Alexa Fluor® 647	892	150	300	580
901	Allophycocyanine (APC)	890	150	300	580
914	APC-Cy™7	890	150	300	580
898	Chlorophyll	890	150	300	580
895	Cy™5	890	150	300	580
896	Dansyl Chloride	890	150	300	580
906	DAPI	890	150	300	580
913	Far-Out Red	890	150	300	580
891	Fluorescein	890	150	300	580
851	Fluorescein, 2µm	890	150	300	580
903	Fura 2	890	150	300	580
894	Hoechst 33342	890	150	300	580

continued



Routine Quality Control, continued

Catalog Production Prod		Product Data	Price (\$US)		
Code	Description	Description Sheet		B 100 tests	C 280 tests
902	Indo 1	890	150	300	580
900	Oxazine 1	890	150	300	580
908	PE-Cy™5	890	150	300	580
889	PE-Cy™7	890	150	300	580
909	PE-TR	890	150	300	580
892	Propidium Iodide	890	150	300	580
899	R-Phycoerythrin	890	150	300	580
907	Rhodamine 123	890	150	300	580
904	Rhodamine B	890	150	300	580
905	T.M. Rhodamine	890	150	300	580
893	Texas Red [®]	890	150	300	580
915	Violet Laser (Glacial Blue)	890	150	300	580

Alignment

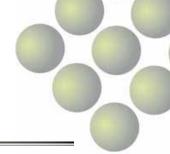
Microspheres with narrow fluorescence coefficients of variation (CVs) are used for alignment. Fixed alignment instruments are verified periodically, while manual instruments are aligned on a daily basis. Some applications, such as DNA content analysis, will also dictate daily alignment verification.

Although reference fluorescence CVs are provided on alignment bead Certificates of Analysis, it is important that instrument-specific tolerances be established. Tolerances should be established on an optimally aligned instrument, i.e. immediately following a service visit. Keep in mind that fluorescence CV is dependent upon flow rate, concentration, and the optical system of the instrument.

RIGHT REFERENCE STANDARD™

Each standard consists of a population of microspheres surface-labeled with a single fluorochrome at a given intensity level (low, medium, high). With their narrow fluorescence CVs, Right Reference Standard™ products are suitable for alignment purposes. These standards may also be used to QC a specific path of the optical system (laser / filter / PMT), and to establish a unified fluorescence range for a particular detector.

,	•		
Catalog	Description	Product Data	Price (\$US)
Code	Describtion	Sheet	B – 100 tests
510	Right Reference Standard™ Fluorescein Low Level	510	255
511	Right Reference Standard™ Fluorescein Medium Level	510	255
512	Right Reference Standard™ Fluorescein High Level	510	255
513	Right Reference Standard™ Phycoerythrin Low Level	510	255
514	Right Reference Standard™ Phycoerythrin Medium Level	510	255
515	Right Reference Standard™ Phycoerythrin High Level	510	255
516	Right Reference Standard™ PE-Cy™5 Low Level	510	255
517	Right Reference Standard™ PE-Cy™5 Medium Level	510	255
518	Right Reference Standard™ PE-Cy™5 High Level	510	255
519	Right Reference Standard™ APC Low Level	510	255
520	Right Reference Standard™ APC Medium Level	510	255
521	Right Reference Standard™ APC High Level	510	255



Linearity

The accurate measurement of fluorescence signal is imperative for applications in quantitative fluorescence cytometry, such as surface marker expression or telomere length determination. To this end, the linear response of the PMTs should be assessed regularly.

Fluorochrome-labeled microspheres of differing intensities are used to generate a standard curve relating channel values to standardized fluorescence intensity units. Our QuickCal® analysis template calculates a regression and reports the regression coefficient (r²), which should be as near as possible to 1.0. Deviations may indicate the need for maintenance or calibration of components of the instrument's optical system.

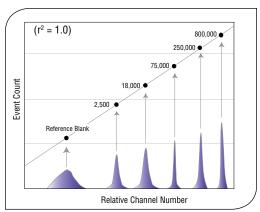


Figure 2: Use of Quantum™ MESF beads as a linearity tool.

QUANTUM™ MESF

Our Quantum™ MESF kits contains one blank population and a series of either four fluorescent microsphere populations labeled with varying amounts of Alexa Fluor® 488, Alexa Fluor® 647, APC, Cy™5, PE-Cy™5, or R-PE, or five fluorescent microsphere populations labeled with varying amounts of FITC. The assignment of fluorescence intensity in Molecules of Equivalent Soluble Fluorochrome (MESF) units is performed through direct comparison of fluorescence measurements from solutions of the pure fluorochrome with those from microspheres surface-labeled with the same fluorochrome. A free QuickCal® analysis template (see pages 23 -24) is provided with each kit to aid in determining expression levels of cells, and for evaluating instrument linearity and detection threshold.

Catalog		Product Data	Price (\$US)		
Code	Description	Sheet		B 100 tests	C 280 tests
488	Quantum™ Alexa Fluor® 488 MESF	888	358	693	1123
555	Quantum™ FITC-5 MESF	855	358	693	1123
555p	Quantum™ FITC-5 MESF (Premix)	856	358	693	1123
827	Quantum™ R-PE MESF	827	358	693	1123
828	Quantum™ PE-Cy™5 MESF	828	358	693	1123
822	Quantum™ Cy™5 MESF	822	358	693	1123
647	Quantum™ Alexa Fluor® 647 MESF	889	358	693	1123
823	Quantum™ APC MESF	823	358	693	1123



Flow cytometers generate a tremendous amount of data for each cell that is analyzed, and this is particularly true for instruments equipped with more than one laser. To be meaningful, the information that is collected by the detectors off of each laser must be integrated and attributed to the proper cell. Provided that time delays are in calibration, the instrument is able to "track" the cell as it passes by each laser for correct data assimilation and reporting.

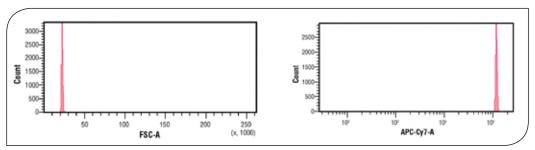


Figure 3: The highly uniform Time Delay Calibration Standard exhibits red / far-red emission.

TIME DELAY CALIBRATION STANDARD

Bangs' Time Delay Calibration Standard is intended for use in assessing the delay between blue and red lasers. It features ~6µm microspheres dyed with a fluorophore that is excited with 488nm or 635nm lasers, and exhibits red / far-red emission.

Catalog	Catalog Product Data		P	rice (\$U	S)
Code	Hescrintion	Sheet	A 1mL	B 5mL	C 7mL (2)
830	Time Delay Calibration Standard 831		150	300	580

In flow cytometry, comprehensive standardization is particularly important for longitudinal studies and studies involving multiple instruments or centers. Efforts to standardize should encompass reagent, protocols, instrument configuration, and, for qualitative analysis, fluorescence intensity units.

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General Instrument Set-Up

In flow cytometry, consistent instrument set-up and standardization are essential for achieving consistent results and generating comparable data. The program should be comprehensive, encompassing reagents, protocols, instrument configuration, and, for qualitative analyses, fluorescence intensity units.

Our microsphere-based tools for set-up and standardization can serve a significant role in supporting flow cytometry programs, and establishing a framework for the comparison of data from different instruments, laboratories, and over time.

Instrumer	nt Standardization
Instrument configuration	PMT voltages Compensation settings
Assay S	Standardization
Protocols	Sample collection
Reagents	Antibodies
Fluorescence scale	Window of Analysis Fluorescence intensity units

Table 2: Standardization suggestions.

Purpose	Frequency	Products
Standardized instrument set-up	Daily, or between runs if settings are changed	QC Windows® QC3™
Standardized compensation settings for multicolor analyses	Daily, or between runs if settings are changed	FITC / PE Compensation Standard Simply Cellular® Compensation Standard Quantum™ Simply Cellular®

Table 3: Instrument set-up product suggestions.



Flow cytometers are highly configurable, and results can vary considerably with different instrument settings. Establishing a common "Window of Analysis" for each detector, with upper and lower fluorescence limits defined, allows reference populations to be positioned in approximately the same place on the scale. This type of standardized instrument set-up ensures consistency of results from specific instruments and enables meaningful

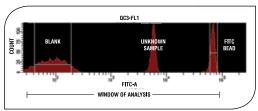


Figure 4: QC3™ showing the Window of Analysis.

data comparison between instruments. Standardized instrument set-up using our QC Windows® or QC3™ products can ameliorate differences in range, relative scale, and reporting units, as well as daily fluctuation due to electronic noise and ambient temperature and humidity.

QC WINDOWS®

QC Windows® kits for instrument set-up include a blank and one or more bead population(s) surface-labeled with fluorochromes. The kits allow the establishment of upper and lower fluorescence thresholds, the Window of Analysis, for each detector, permitting meaningful data comparison between instruments and over time.

Catalog Code	Description	Product Data	Price (\$US)		
		Sheet	A 20 tests	B 100 tests	C 280 tests
845	QC Windows® (FITC / PE)	845	303	508	1044
846	QC Windows® (FITC / PE / PE-TR)	845	398	673	1196
847	QC Windows® (FITC / PE / PE-Cy [™] 5)	845	398	673	1196
848	QC Windows® (FITC / PE / PE-Cy™5, APC)	845	453	754	1277

QC3™

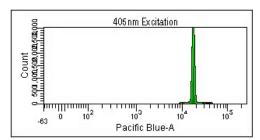
QC3™ kits include one or more bead population(s) surface-labeled with fluorochromes for defining an upper fluorescence threshold for each detector, permitting meaningful data comparison between instruments and over time.

Catalog Code	Description F	Product Data	Price (\$US)		
		Sheet	A 20 tests	B 100 tests	C 280 tests
841	QC3™ (FITC / PE)	841	276	447	817
842	QC3™ (FITC / PE / PE-TR)	841	325	574	959
843	QC3™ (FITC / PE / PE-Cy™5)	841	325	574	959
844	QC3™ (FITC / PE / PE-Cy™5, APC)	841	325	574	959

General Instrument Set-Up, continued

FULL SPECTRUM™

Full Spectrum™ microspheres are used to perform the initial daily QC check on 3-9+ color instruments. The microspheres are internally labeled with multiple fluorophores for use with common excitation and detection wavelengths within the visible spectrum.



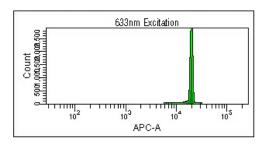


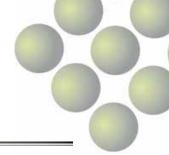
Figure 5: Histograms from Full Spectrum™.

Catalog	P	Product Data	Price (\$US)			
Code	Description '	Sheet	A 20 tests	B 100 tests	C 280 tests	
885	Full Spectrum™	885	150	300	580	

ULTRA RAINBOW FLUORESCENT PARTICLES (BY SPHEROTECH™)

Ultra Rainbow Fluorescent Particles are internally labeled with multiple fluorophores, enabling excitation at any wavelength from 365 – 650nm. Ultra Rainbow Fluorescent Particles may be used for general QC purposes for 3-9+ color instruments.

Catalog	Description	Product Data	Price (\$US)		
Code		Sheet	A 20 tests	B 100 tests	C 280 tests
610	Ultra Rainbow Fluorescent Particles, ~3.8µm (3.61 – 3.99µm)	612	150	300	580
611	Ultra Rainbow Fluorescent Particles, ~10.2µm (8.1 – 12.0µm)	612	150	300	580



Compensation

Microsphere standards offer a convenient means for establishing compensation settings in multicolor flow cytometry. Our FITC / PE Compensation Standard is suitable for analyses using these common fluorophores. For analyses relying on other or additional fluorochromes, customized compensation bead sets may be easily developed by labeling aliquots of our Simply Cellular® Compensation Standard or Quantum™ Simply Cellular® microspheres with the same antibody / fluorochrome conjugates that are used to label cells.

FITC / PE COMPENSATION STANDARD

The FITC / PE Compensation Standard includes four microsphere populations: an Autofluor™ population, and single populations surface labeled with FITC, PE, and FITC/PE. The Autofluor™ population is dyed with a low level of fluorophore to approximate the autofluorescence of an unstained resting lymphocyte. This kit is useful for establishing quadrant boundaries and compensation settings for analyses utilizing FITC and PE fluorochromes.

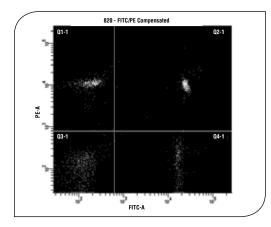


Figure 6: Quadrants 1-4 illustrate the expected performance of the FITC / PE Compensation Standard. Once compensation circuits have been correctly adjusted using the product, the effect of spectral overlap into secondary fluorescence channels is eliminated. Thus, single-labeled cell populations will appear in quadrants 1 and 4, and will be separated from dual-labeled populations in quadrant 2.

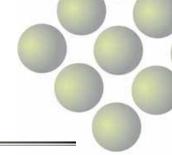
Catalog	Product Data	Price (\$US)			
Code	Description	Sheet	A 20 tests	B 100 tests	C 280 tests
820	FITC / PE Compensation Standard (Autofluor™ / FITC-PE / FITC / PE)	820	207	517	911

Compensation, continued

SIMPLY CELLULAR® COMPENSATION STANDARD

The Simply Cellular® Compensation Standard includes a mixed population of low- and high-binding antibody-coated beads. Users label aliquots with antibody conjugates of interest to establish suitable compensation settings for a specific analysis.

Catalog Code	Description	Product Data Sheet	Price (\$US) B – 100 tests
550	Simply Cellular® Compensation Standard (anti-Mouse IgG)	850	255
551	Simply Cellular® Compensation Standard (anti-Rat IgG)	851	255
552	Simply Cellular® Compensation Standard (anti-Human IgG)	852	255



Compensation, continued

SIMPLY CELLULAR® ANTI-MOUSE FOR VIOLET LASER

The Simply Cellular® anti-Mouse for Violet Laser standard features one microsphere population comprised of a proprietary matrix that exhibits low autofluorescence with violet excitation and one blank population. 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 with other fluorochromes and lasers / detectors, e.g. 488nm, 633nm.

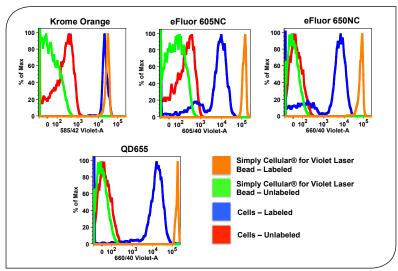


Figure 7: Simply Cellular® anti-Mouse for Violet Laser can be labeled with many different fluorochromes.

Catalog	atalog Product Data		Price	(\$US)
Code	Description	Sheet	A – 20 tests	B – 100 tests
835	Simply Cellular® anti-Mouse for Violet Laser	835	266	448

Compensation, continued

FLOW CYTOMETRY PROTEIN A OR PROTEIN G ANTIBODY BINDING BEADS

Single population Protein A or Protein G microspheres are suitable for labeling with conjugated antibodies from a range of hosts. Labeled microspheres may be used as single-population reference standards or in conjunction with an unlabeled population for compensation purposes.

Catalog		Product Data	Price (\$US)		
Code	Description	Sheet	Α	В	С
Out		Oncot	20 tests	100 tests	280 tests
553	Flow Cytometry Protein A Antibody Binding Beads	854	253	538	962
554	Flow Cytometry Protein G Antibody Binding Beads	854	253	538	962

VIABILITY DYE COMPENSATION STANDARD

Viability Dye Compensation Standards are suitable for labeling with LIVE / DEAD® stains or similarly-reactive dyes to generate compensation standards for flow cytometric analyses.

Catalog	Description	Product Data	Price (\$US)
Code	2000	Sheet	3mL
450	Viability Dye Compensation Standard, 4µm	853	326
451	Viability Dye Compensation Standard, 8µm	853	326

Bangs' tools for quantitative flow cytometry provide the means to standardize fluorescence intensity measurements, thereby permitting truly quantitative analyses. Our products include **Quantum™ MESF** (Molecules of Equivalent Soluble Fluorochrome) and **Quantum™ Simply Cellular®** (ABC, Antibody Binding Capacity) kits.

QUANTUM™ MESF	21
QUANTUM™ SIMPLY CELLULAR®	
SIMPLY CELLULAR®	
QUICKCAL® ANALYSIS TEMPLATE	
QUICKCAL® ANALYSIS TEMPLATE INSTRUCTIONS	

Fluorescence Quantitation

Fluorescence cytometry is an important tool for investigations in cell and molecular biology. This technology is routinely used for immunophenotyping and an expansive array of research applications, such as the study of protein phosphorylation and the determination of telomere length.

Although fluorescence cytometry has proven to be a very powerful and versatile technology, it is not without limitations. Notably, without a standardized measure of fluorescence intensity, results of analyses can be described only in relative terms, such as "negative," "dim," "intermediate," and "bright," or in arbitrary fluorescence intensity units. The interpretation of fluorescence intensity measurements can be further complicated by factors such as daily instrument variation, differences in hardware (laser power, filter sets), PMT settings, software, environmental factors such as buffer pH, and fluorochrome labeling density of antibodies (F/P ratio). Quantitative fluorescence analyses demand the highest level of standardization. However, cytometers lack internal calibrators for fluorescence intensity, and are limited to reporting results in relative terms.

Our Quantum™ MESF and Quantum™ Simply Cellular® microspheres are external standards that enable the standardization of fluorescence intensity units irrespective of instrument and software. Moreover, they are labeled with the actual fluorochromes used to label cells, for synchronous response to the environment (consider the pH-responsive fluorescence intensity of fluorescein, Figure 8). The beads are run on the same day and at the same settings as samples to establish a calibration curve relating instrument channel values and standardized fluorescence intensity units. Unknowns may then be read against the curve for determination of expression (i.e. quantitation of the signal from each cell population).

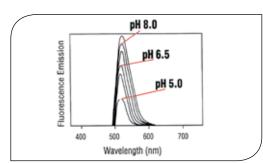
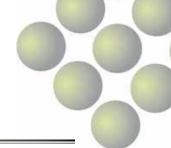


Figure 8: pH-dependent intensity of fluorescein.



Fluorescence Quantitation, continued

QUANTUM™ MESF

Each Quantum™ MESF kit contains one blank population and a series of either four fluorescent microsphere

populations labeled with varying amounts of Alexa Fluor® 488, FITC, R-PE, PE-Cy™5, Cy™5, Alexa Fluor® 647, or APC, or five fluorescent microsphere populations labeled with varying amounts of FITC. The assignment of fluorescence intensity in Molecules of Equivalent Soluble Fluorochrome (MESF) units is performed through direct comparison of fluorescence measurements from solutions of the pure fluorochrome with those from microspheres surface-labeled with the same fluorochrome. A free QuickCal® analysis template (see pages 23 − 24) is provided with each kit to aid in determining expression levels of cells, and for evaluating instrument linearity and detection threshold.



Figure 9: Quantum™ APC MESF Kit.

Catalog		Product Data	Price (\$US)		
Code	Description	Sheet	A 20 tests	B 100 tests	C 280 tests
488	Quantum™ Alexa Fluor® 488 MESF	888	358	693	1123
555	Quantum™ FITC-5 MESF	855	358	693	1123
555p	Quantum™ FITC-5 MESF (Premix)	856	358	693	1123
827	Quantum™ R-PE MESF	827	358	693	1123
828	Quantum™ PE-Cy™5 MESF	828	358	693	1123
822	Quantum™ Cy™5 MESF	822	358	693	1123
647	Quantum™ Alexa Fluor® 647 MESF	889	358	693	1123
823	Quantum™ APC MESF	823	358	693	1123

Fluorescence Quantitation, continued

QUANTUM™ SIMPLY CELLULAR®

Quantum™ Simply Cellular® (QSC) kits are comprised of five microsphere populations: one blank and four labeled with increasing amounts of antibody. Each coated population binds a specific number of monoclonal antibodies of the noted species, which is equal to its Antibody Binding Capacity (ABC) value. The investigator labels QSC beads using the same antibodies used to stain cells. In addition to quantitative fluorescence cytometry, QSC kits may be used for compensation. A free QuickCal® analysis template is provided with each kit to aid in determining expression levels of cells, and for evaluating instrument linearity and detection threshold.

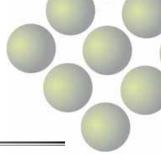
Catalog		Product Data Sheet	Price (\$US)			
Code	Description		A 20 tests	B 100 tests	C 280 tests	
815	Quantum™ Simply Cellular® anti-Mouse IgG	814	453	754	1277	
816	Quantum™ Simply Cellular® anti-Human IgG	814	453	754	1277	
817	Quantum™ Simply Cellular® anti-Rat IgG	814	453	754	1277	

SIMPLY CELLULAR®

Our Simply Cellular® standard consists of a single population of antibody-coated microspheres of known Antibody Binding Capacity (ABC). The population is stained with the user's primary antibody and used in conjunction with a suitable Quantum™ MESF kit for determination of the antibody's effective Fluorophore / Protein (F/P) ratio. This standard may also be used to QC the fluorescence intensity of different antibody lots or clones.

Catalog	atalog Product Data	F	rice (\$US	S)	
Code	Description	Sheet	А	В	С
Couo			20 tests	100 tests	280 tests
810	Simply Cellular® anti-Mouse IgG	810	253	538	962
812	Simply Cellular® anti-Human IgG	812	253	538	962
813	Simply Cellular® anti-Rat IgG	813	253	538	962

QSC and SC standards are available for use with mouse (Cat. #815 and #810), rat (Cat. #817 and #813), and human (Cat. #816 and #812) monoclonal antibodies. For example, the anti-Mouse kit is intended to bind mouse mAbs, not for the analysis of mouse cells.



QuickCal® Analysis Template

QUICKCAL® ANALYSIS TEMPLATE

Bangs' tools for quantitative flow cytometry provide the means to standardize fluorescence intensity measurements, thereby permitting truly quantitative analyses. Our products include Quantum™ MESF (Molecules of Equivalent Soluble Fluorochrome) and Quantum™ Simply Cellular® (ABC, Antibody Binding Capacity) kits. Fluorochrome-labeled microspheres are used to generate a standard curve relating fluorescence intensity to standardized MESF or ABC values from Quantum™ MESF or Quantum™ Simply Cellular® beads. The MESF or ABC values of labeled cell samples may be determined by measuring their fluorescence intensities, and "reading" the corresponding MESF or ABC values from the standard curve using the QuickCal® analysis template that is provided with the kit.

Bangs' Quantum™ kits are uniquely qualified for applications in quantitative fluorescence cytometry:

- Precise MESF or ABC values are assigned to bead populations through meticulous primary calibrations.
- MESF and ABC values provide standardized units of fluorescence intensity. The MESF unit has been formally adopted by NIST and NCCLS as a standardized measure of fluorescence intensity.
- Quantum[™] microspheres are labeled with the actual fluorochromes used in flow cytometry, ensuring that quantitative assignments are truly relevant.
- Surface-labeled microspheres are environmentally-responsive; the fluorochrome on the bead responds
 to the buffer (pH, ionic strength) in the same manner as the fluorochrome on the labeled cell. The
 fluorescence intensity of beads thus mirrors that of cells, preserving the calibration when quantitative
 assignments are made.

QuickCal® Analysis Template, continued

QuickCal® Analysis Template Instructions

- 1. Run Quantum™ MESF or Quantum™ Simply Cellular® microspheres on the same day, same instrument, and at the same instrument settings (PMT and compensation) as labeled cell samples.
- 2. Gate on each peak within the fluorescence histogram.
- 3. Enter the median channel value of each fluorescence peak against its calibrated MESF or ABC value that appears within the QuickCal® analysis template. A calibration curve will be drawn automatically.
- 4. Enter median channel values of labeled samples for the assignment of MESF or ABC values.

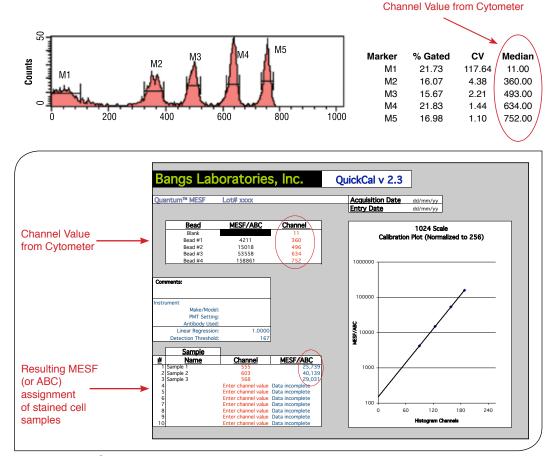


Figure 10: QuickCal® v 2.3 Analysis Template.

Current applications in flow cytometry extend far beyond traditional lymphocyte immunophenotyping. Flow cytometry is used for such varied applications as cell cycle analysis, telomere length determination, microparticle analysis, and in phagocytosis and many other studies.

CELL CYCLE ANALYSIS	
FLUORESCENCE REFERENCE STANDARDS	
VIABILITY DYE COMPENSATION STANDARD	
VIACHECK™ VIABILITY AND CONCENTRATION CONTROLS	
CELL SIZE ESTIMATION. SIZE CALIBRATION STANDARDS KIT	28
SIZE CALIBRATION STANDARDS KIT	28
SMALL BEAD CALIBRATION KITS	
CELL COUNTING	
FLOW CYTOMETRY ABSOLUTE COUNT STANDARD™	29
SURECOUNT™ PARTICLE COUNT STANDARDS	29
SUSPENSION ARRAYS	
QUANTUMPLEX™ .	
QUANTUMPLEX TM SP	
QUANTUMPLEX TM M	
QUANTUMPLEX TM M SP	

Cell Cycle Analysis

Bangs Laboratories offers a range of microspheres that may be used as standards to support assays in cell proliferation and apoptosis. Beads may be used for routine instrument set-up and QC, or as test-specific standards. Standards are available for use with flow cytometers, fluorescence microscopes, and cell viability analyzers. Custom products or preparations can also be manufactured to meet the requirements of specific assays or analyzers.

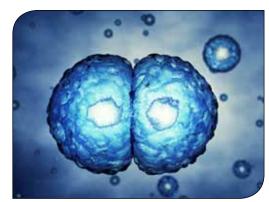
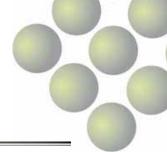


Figure 11: Cell mitosis.

FLUORESCENCE REFERENCE STANDARDS

Products include beads that may be used as single-color fluorescent reference standards, or mixed at specific ratios to achieve different levels of surrogate viable, apoptotic, or dead populations. Several of the actual dyes that are commonly used in cell cycle analysis are represented within our line of Fluorescence Reference Standards, and we additionally offer many fluorescent beads that may be used as spectral surrogates for other stains and indicators. A full listing of Fluorescence Reference Standards can be found on pages 6-7.

Catalog	talog Product Data	F	Price (\$US	S)	
Code	Description	Sheet	A 20 tests	B 100 tests	C 280 tests
897	Acridine Orange	890	150	300	580
906	DAPI	890	150	300	580
891	Fluorescein	890	150	300	580
851	Fluorescein, 2µm	890	150	300	580
894	Hoechst 33342	890	150	300	580
892	Propidium Iodide	890	150	300	580
907	Rhodamine 123	890	150	300	580



Cell Cycle Analysis, continued

VIABILITY DYE COMPENSATION STANDARD

Viability Dye Compensation Standards are suitable for labeling with LIVE / DEAD® stains or other amine-reactive dyes to generate compensation or general reference standards for flow cytometric analyses. Beads are available in two diameters that approximate the sizes of common cell populations.

Catalog	Description	Product Data	Price (\$US)
Code	Description	Sheet	3mL
450	Viability Dye Compensation Standard, 4µm	853	326
451	Viability Dye Compensation Standard, 8µm	853	326

VIACHECK™ VIABILITY AND CONCENTRATION CONTROLS

Our ViaCheckTM microsphere standards mimic the optical characteristics of live (undyed) and dead (blue dyed) cells in the trypan blue dye exclusion method. Non-biological surrogates remove the need for significant sample preparation, and offer exceptional stability and reproducibility. ViaCheckTM microsphere standards are prestained, and need only be loaded into the analyzer for confirmation of live / dead ratios and counts.

Catalog Code	Description	Product Data Sheet	Price (\$US) 20mL
VC10B	ViaCheck™ 0% Viability Control	706	379
VC20B	ViaCheck™ 50% Viability Control	707	379
VC30B	ViaCheck™ 75% Viability Control	708	379
VC40B	ViaCheck™ 90% Viability Control	709	379
VC50B	ViaCheck™ 100% Viability Control	710	379
VC60N	ViaCheck™ Concentration Control (1 x 10 ⁶)	711	163
VC70N	ViaCheck [™] Concentration Control (4 x 10 ⁶)	712	238
VC80N	ViaCheck™ Concentration Control (8 x 10 ⁶)	713	301

Cell Size Estimation

Flow cytometry can be a useful tool for the estimation of cell size, as forward angle light scatter (FSC) measurements are proportional to the cross-sectional area of cells. FSC data from microspheres of known size may be compared to unknown samples to estimate cell size, and can also be used to generate calibration curves for quality control purposes.

SIZE CALIBRATION STANDARDS KIT

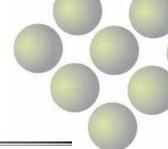
The Size Calibration Standards Kit is comprised of five populations of undyed microspheres in the range of \sim 4 – 12µm. Forward scatter channel (FSC) values may be plotted against reported bead diameter to generate a curve that may be used for estimation of the sizes of cells run at the same instrument settings.

Catalog	Description	Product Data Sheet	Price	(\$US)
Code	Description		B – 5mL	C – 14mL
829	Size Calibration Standards Kit	829	372	660

SMALL BEAD CALIBRATION KITS

Some current applications in flow cytometry extend far beyond traditional lymphocyte immunophenotyping, with some involving the analysis of very small particles such as platelet- and endothelial-derived microparticles or microbial species. The Small Bead Calibration Kits allow operators to verify the resolution capabilities of the flow cytometer, and to establish appropriate instrument settings for the analyses.

Catalog Code	Description	Product Data Sheet	Price (\$US) 3mL
832	Submicron Bead Calibration Kit • 0.2μm, 0.5μm, 0.8μm	832	224
833	Micron Bead Calibration Kit • 1.0μm, 3.0μm, 6.0μm	832	224



Cell Counting

Enumeration of cells in a sample may be achieved through concurrent use of a microsphere count standard. Cell count may be calculated by determining the ratio of fluorescent count beads to unlabeled cells.

FLOW CYTOMETRY ABSOLUTE COUNT STANDARD™

Our Flow Cytometry Absolute Count Standard™ is a precisely-counted population of microspheres for estimating counts of unlabeled cells via flow cytometry. Beads are internally labeled with multiple fluorophores for excitation with common lasers (e.g. 488nm, 633nm) and discrimination from the cell population. By evaluating the ratio of microspheres to cells, the volumetric number of cells may be determined.

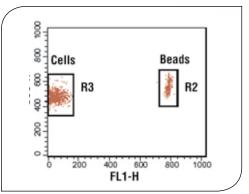


Figure 12: Flow Cytometry Absolute Count Standard™ used with cell samples to determine cell counts.

Catalog	Description	Product Data	Price (\$US)
Code	Description	Sheet	10mL
580	Flow Cytometry Absolute Count Standard™	880	255

SURECOUNT™ PARTICLE COUNT STANDARDS

SureCount™ beads are suspensions of polymer microspheres intended to aid in particle counting and support sample preparation processes. They complement our other count and concentration controls and may be used in flow cytometry when an undyed bead is required. SureCount™ standards are available in four sizes (3µm, 5µm, 10µm, or 15µm), with diameters traceable to NIST Standard Reference Materials. The standards are supplied as ~1 x 106 microspheres/mL aqueous suspensions in 10mL volumes.



Figure 13: SureCount™ Particle Count Standards.

Catalog	Description	Diameter	Price (\$US)
Code	Description	(μm)	10mL
CC03N	SureCount™ Particle Count Standard	3	255
CC05N	SureCount™ Particle Count Standard	5	255
CC10N	SureCount™ Particle Count Standard	10	255
CC15N	SureCount™ Particle Count Standard	15	255

Suspension Arrays

Suspension arrays for the flow cytometer feature populations of microspheres coated with different ligands for the interrogation of multiple targets within a given sample. Ligand-specific microsphere populations are encoded so that results for corresponding individual targets may be discerned. Encoding may be in the form of microsphere size and / or fluorescence signature, as with our QuantumPlex™ and QuantumPlex™M platforms.

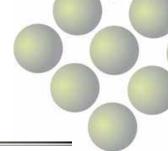
Bangs' QuantumPlex[™] platform is an innovative bead platform specifically designed for suspension array applications. The beads may be analyzed using any standard flow cytometer (488nm or 633nm excitation), and do not require the use of specialized software.

QUANTUMPLEXTM

The microsphere populations in the QuantumPlex™ five-bead kits are encoded with different intensities of Starfire Red™, and microspheres in our ten-bead kits are distinguished by both fluorescence intensity and size. Starfire Red™ is a fluorescent dye with unique characteristics that make it ideal for multiplexing applications. The dye's broad excitation band allows it to be excited at a number of wavelengths, and it emits in the red channel (e.g., PE-Cy™5, APC) with very little carry-over into lower wavelengths, leaving other detectors available for determination of positive binding events via common reporters such as FITC and PE.

QuantumPlexTM kits are available with three different surfaces to accommodate the coating strategy of choice: carboxyl (COOH), streptavidin (SA), or anti-Mouse IgG functional groups.

			P	rice (\$U	S)
Catalog Code	Description	Product Data Sheet	A 1mL/pop 5 x 100 data points	B 5mL/pop 5 x 500 data points	C 10mL/pop 5 x 1000 data points
235	QuantumPlex™ COOH 4.4μm, 5 populations	235	899	3708	5853
238	QuantumPlex™ COOH 5.5μm, 5 populations	235	899	3708	5853
239	QuantumPlex™ COOH 4.4μm and 5.5μm, 10 populations	235	1641	6828	10,534
215	QuantumPlex™ SA 4.4μm, 5 populations	215	899	3708	5853
218	QuantumPlex™ SA 5.5μm, 5 populations	215	899	3708	5853
219	QuantumPlex™ SA 4.4µm and 5.5µm, 10 populations	215	1641	6828	10,534
205	QuantumPlex™ anti-Mouse IgG 4.4µm, 5 populations	209	899	3708	5853
208	QuantumPlex™ anti-Mouse IgG 5.5µm, 5 populations	209	899	3708	5853
209	QuantumPlex™ anti-Mouse IgG 4.4µm and 5.5µm, 10 populations	209	1641	6828	10,534



Suspension Arrays, continued

QUANTUMPLEX™ SP

QuantumPlex[™] Single Population (SP) is useful for the development of simplex flow cytometric assays or for the optimization of attachment chemistry and assay parameters before transitioning to a multiplexed format. Like QuantumPlex[™], they are dyed with Starfire Red[™], and are suitable for use on standard flow cytometers.

Description			Price (\$US)	
	Product Data Sheet	A – 1mL 100 data points	B – 3mL 300 data points	
234	QuantumPlex™ SP COOH 4.4µm	234	213	526
237	QuantumPlex™ SP COOH 5.5µm	234	213	526
214	QuantumPlex™ SP SA 4.4µm	214	213	526
217	QuantumPlex™ SP SA 5.5µm	214	213	526
204	QuantumPlex™ SP anti-Mouse 4.4µm	207	213	526
207	QuantumPlex™ SP anti-Mouse 5.5µm	207	213	526

QUANTUMPLEX™M

QuantumPlex[™] Magnetic (M) kits consist of five populations of ~6µm highly uniform superparamagnetic microspheres encoded with different intensities of Starfire Red[™]. Like QuantumPlex[™], they are suitable for use on standard flow cytometers. Highly efficient separations may be performed using rare earth magnetic separators.

			Price (\$US)		
Catalog Code	Description	Product Data Sheet	A 1mL/pop 5 x 100 data points	B 5mL/pop 5 x 500 data points	C 10mL/pop 5 x 1000 data points
250	QuantumPlex™M COOH, 5 populations	250	899	3708	5853
252	QuantumPlex™M SA, 5 populations	252	899	3708	5853

Suspension Arrays, continued

QUANTUMPLEX™M SP

QuantumPlex[™]M SP (Single Population) is useful for the development of simplex flow cytometric assays, or for the optimization of attachment chemistry and assay parameters before transitioning to a multiplexed format. Like QuantumPlex[™]M, they are ~6µm highly uniform superparamagnetic microspheres dyed with Starfire Red[™] and are suitable for use on standard flow cytometers.

	Description		Price (\$US)	
Catalog Code		Product Data Sheet	A – 1mL 100 data points	B – 3mL 300 data points
251	QuantumPlex™M SP COOH	251	213	526
253	QuantumPlex™M SP Streptavidin	253	213	526

Our full line of ViaCheck™ Viability and Concentration Controls can be found on page 34.

STANDARDS

We manufacture a comprehensive range of standards for analytical instruments such as cell analyzers, particle sizers, flow cytometers, and fluorescence microscopes. Our catalog includes NIST Traceable Particle Size Standards, SureCount™ Particle Count Standards, ViaCheck™ Cell Viability and Concentration Controls, and a broad range of fluorescent and antibody capture beads for flow cytometric and imaging applications.

VIABILITY STANDARDS	
SIZE STANDARDS	
SURECOUNT™ PARTICLE COUNT STANDARDS	37
FLUORESCENCE INTENSITY STANDARDS DRAGON GREEN INTENSITY STANDARD	
STARLIGHT™ CALIBRATION SLIDES	

STANDARDS

Viability Standards

Instrumental methods for cell viability analysis provide significant advantages over manual determinations, offering high accuracy, precision, and throughput. However, as with any analytical instrument, it is important to implement a QC program to ensure confidence in results.

VIACHECK™ VIABILITY AND CONCENTRATION INSTRUMENT STANDARDS

Trypan blue exclusion is a common method for the determination of cell viability. It is used extensively

in cell and tissue culture programs, and for a range of research studies including apoptosis, cytopathic effects of viral infection, and effects of sample processing methods on cell viability and concentration.

Our ViaCheck™ Viability Instrument Standards complement our extensive line of microsphere standards for instrument QC. ViaCheck™ standards mimic the light scattering characteristics of live and dead cells in the trypan blue dye exclusion method, and may be used to confirm the capabilities and verify the performance of image-based cell viability instruments. The standards are available in a range of common concentrations and live / dead ratios.

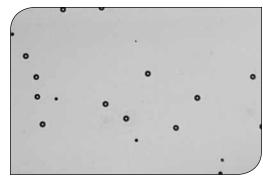


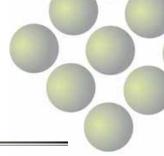
Figure 14: ViaCheck™ microspheres viewed through microscope.

Catalog	talog Product Data	Product Data	Price (\$US)	
Code	de Description Sheet		20mL	
VC10B	ViaCheck™ 0% Viability Control	706	379	
VC20B	ViaCheck™ 50% Viability Control	707	379	
VC30B	ViaCheck™ 75% Viability Control	708	379	
VC40B	ViaCheck™ 90% Viability Control	709	379	
VC50B	ViaCheck™ 100% Viability Control	710	379	
VC60N	ViaCheck™ Concentration Control (1 x 10 ⁶)	711	163	
VC70N	ViaCheck™ Concentration Control (4 x 10 ⁶)	712	238	
VC80N	ViaCheck™ Concentration Control (8 x 10 ⁶)	713	301	

VIABILITY DYE COMPENSATION STANDARDS

Viability Dye Compensation Standards are suitable for labeling with LIVE / DEAD® stains or similarly-reactive dyes to generate compensation standards for flow cytometric analyses.

Catalog	Description Product Data Sheet	Product Data	Price (\$US)	
Code		Sheet	3mL	
450	Viability Dye Compensation Standard, 4µm	853	326	
451	Viability Dye Compensation Standard, 8µm	853	326	



Size Standards

Particle size standards may be used to validate sizing instruments across their dynamic ranges. They are suitable for use in the performance of routine instrument calibration checks and corrections, and in the support of practice standards, such as those published by ISO, ASTM International, CEN, and other organizations. Additionally, the use of a reference material permits the standardization of results between runs, instruments, laboratories, and over time.

NIST TRACEABLE PARTICLE SIZE STANDARDS

Our NIST Traceable Particle Size Standards are monodisperse polystyrene spheres available in diameters

ranging from $40\text{nm} - 175\mu\text{m}$. Suspensions are conveniently packaged in dropper bottles at 1% solids, and each bottle is provided with a Certificate of Traceability. See TechNote 105, *Microsphere Size Standards*.

Composition:	Polystyrene	
Refractive Index (589nm):	1.59*	
Density (g/cm³):	1.05*	
* Reported value for bulk polymer.		

Table 4: Properties of NIST Traceable Particle Size Standards.



Figure 15: NIST Traceable Particle Size Standards.

Catalog	Naminal Maan Diameter (um)	Price (\$US)
Code	Nominal Mean Diameter (µm)	15mL
NT02N	0.04	244
NT03N	0.06	244
NT04N	0.08	244
NT05N	0.10	244
NT06N	0.15	244
NT07N	0.20	244
NT08N	0.30	244
NT09N	040	244
NT10N	0.50	244
NT11N	0.60	244
NT12N	0.70	244
NT13N	0.80	244
NT14N	0.90	244
NT15N	1.00	285
NT16N	1.50	285

continued

Size Standards, continued

Catalog	Naminal Maan Diameter (um)	Price (\$US)
Code	Nominal Mean Diameter (µm) —	15mL
NT17N	2.00	285
NT18N	2.50	285
NT19N	3.00	285
NT20N	3.50	285
NT21N	4.00	285
NT22N	5.00	285
NT23N	6.00	285
NT24N	7.00	285
NT25N	8.00	285
NT26N	9.00	285
NT27N	10.00	285
NT28N	12.00	285
NT29N	15.00	285
NT30N	20.00	285
NT31N	25.00	285
NT32N	30.00	285
NT33N	40.00	285
NT34N	50.00	285
NT35N	60.00	285
NT36N	80.00	285
NT37N	100.00	285
NT38N	125.00	285
NT39N	150.00	285
NT40N	175.00	285

SureCount™ Particle Count Standards

Particle counting instruments are employed in many research fields and commercial industries. This technology is used to assess the effectiveness of laboratory processes such as water filtration, and to determine particulate levels in environmental water samples. Automated particle counters are used to support industrial contamination control programs and also in the evaluation of finished products such as ultrapure chemicals or pharmaceutical parenterals.

Though the application of particle counting technology is diverse, there is a common need for instrument validation and ongoing QC. Microsphere-



Figure 16: SureCount™ Particle Count Standards.

based particle count standards may be used to validate liquid counters across their dynamic ranges and to ensure continued capability through the performance of daily QC checks. The use of a reference material permits the standardization of results between runs, instruments, and laboratories, and over time.

SureCount™ standards are suspensions of polymer microspheres intended for the validation and monitoring of particle counters and supporting sample preparation processes. SureCount™ standards are available in four sizes (3um, 5um, 10um, or 15um), with diameters traceable to NIST Standard Reference Materials. The standards are supplied as ~1 x 106 microspheres/mL aqueous suspensions in 10mL volumes.

SureCount™ microspheres complement our existing catalog of standards for analytical instruments, including viability analyzers, particle sizers, flow cytometers and microscopes.

Catalog	alog Description Diameter		Price (\$US)
Code	Description	(µm)	10mL
CC03N	SureCount™ Particle Count Standard	3	255
CC05N	SureCount™ Particle Count Standard	5	255
CC10N	SureCount™ Particle Count Standard	10	255
CC15N	SureCount™ Particle Count Standard	15	255

For all your cell counting needs, check out our ViaCheck™ Viability and Concentration Controls (Page 34) and our Flow Cytometry Absolute Count Standard™ (Page 29).

Fluorescence Intensity Standards

Fluorescence intensity standards have many applications both in instrument QC programs and in biological analyses.

We offer two intensity standards for these applications. Both the Dragon Green and Flash Red kits consist of five populations of ~8µm polystyrene-based microspheres dyed with increasing amounts of their respective fluorophore.

The different intensity populations may serve as relative intensity standards for applications in fluorescence microscopy, where, as internally-dyed beads, they will stand up to the rigors of imaging. The beads may also serve as bright relative intensity

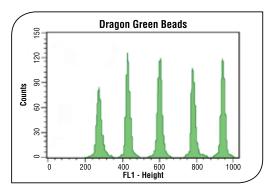
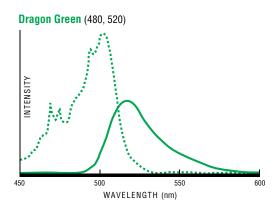


Figure 17: Histogram for the Dragon Green Intensity Standard Kit.

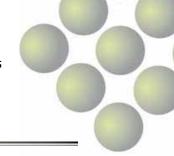
or linearity standards for flow cytometry; ask about our QuickCal® Linearity Template if this is your interest.

DRAGON GREEN INTENSITY STANDARD

The Dragon Green Intensity Standard is a kit consisting of five populations of ~8µm polystyrene-based microspheres dyed with increasing amounts of Dragon Green fluorophore. Dragon Green is an excellent spectral surrogate for fluorescein and is suitable for use with fluorescein filter sets.



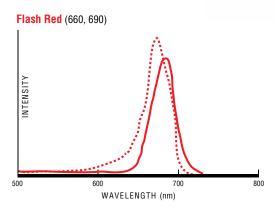
Catalog Code	Description	Product Data Sheet	Price (\$US) 1 Kit
DG06M	Dragon Green Intensity Standard (5 intensities)	704	282



Fluorescence Intensity Standards, continued

FLASH RED INTENSITY STANDARD

The Flash Red Intensity Standard is a kit consisting of five populations of ~8 μ m polystyrene-based microspheres dyed with increasing amounts of Flash Red fluorophore. As Flash Red is spectrally similar to CyTM5, traditional red fluorophore filter sets (e.g. CyTM5 for the microscope; PE-CyTM5 or APC on the cytometer) may be used with this standard.



Catalog	Description Product Da Sheet	Product Data	Price (\$US)
Code		Sheet	1 Kit
FR06M	Flash Red Intensity Standard (5 intensities)	729	282

StarLight™ Calibration Slides

Our new StarLight[™] Calibration Slides feature vibrant ~6µm fluorescent microspheres dyed with a single fluorophore for basic imaging checks and calibrations. The four standard versions are appropriate for use with common microscope filter sets: Glacial Blue (360, 450), Dragon Green (480, 520), Envy Green (525, 565), Flash Red (660, 690), and are available individually or as the full 4-slide collection.

Catalog	Description	Product Data	Price (\$US)
Code	Description	Sheet	1 each
SL1GB	StarLight [™] Calibration Slide – Glacial Blue (1 slide)	857	99
SL1DG	StarLight [™] Calibration Slide – Dragon Green (1 slide)	857	99
SL1EG	StarLight [™] Calibration Slide – Envy Green (1 slide)	857	99
SL1FR	StarLight [™] Calibration Slide – Flash Red (1 slide)	857	99
SL04K	StarLight [™] Collection – 4-Slide Pack (1 slide of each color)	857	295

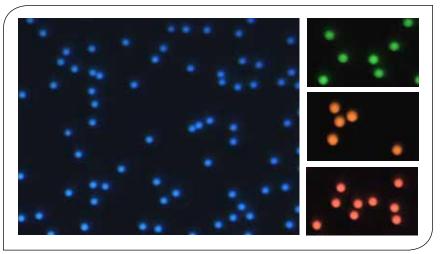
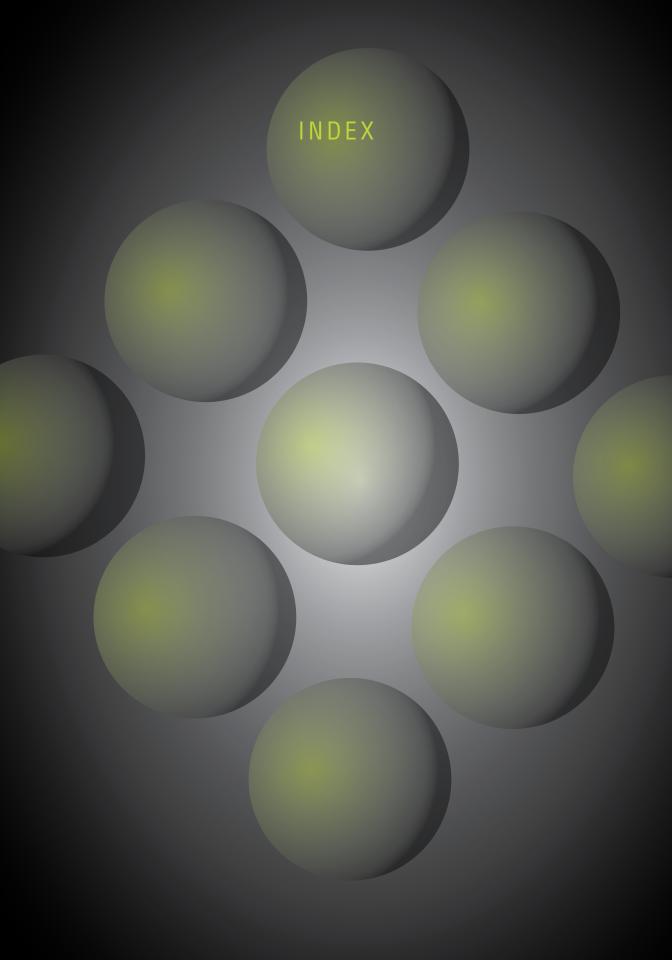
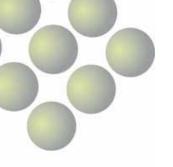


Figure 18: Images of StarLight™ Calibration Slides. The left image is Glacial Blue. The right images are (from top to bottom) Dragon Green, Envy Green, and Flash Red.

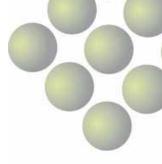


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