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BOOK OF BEADS

FLOW CYTOMETRY SUPPLEMENT



Bangs Laboratories, Inc.

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FLOW CYTOMETRY

Flow cytometers are versatile instruments that allow rapid, multiparametric analysis of cells and other particles. They are commonly used to support diagnostic and predictive medicine programs in clinical settings, and a broad range of research applications in life sciences laboratories.

Regardless of application, researchers and clinicians must be able to have confidence in the results of the test or assay. To this end, it is important that the flow cytometry facility establish a comprehensive quality assurance program that encompasses employee training and routine instrument maintenance and QC, and that studies, particularly those involving quantitative fluorescence measurements, are conducted with appropriate standardization. The sections that follow outline a basic program of quality control and standardization that includes suggestions regarding specific products and their frequency and method of use.

I. INSTRUMENT 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.

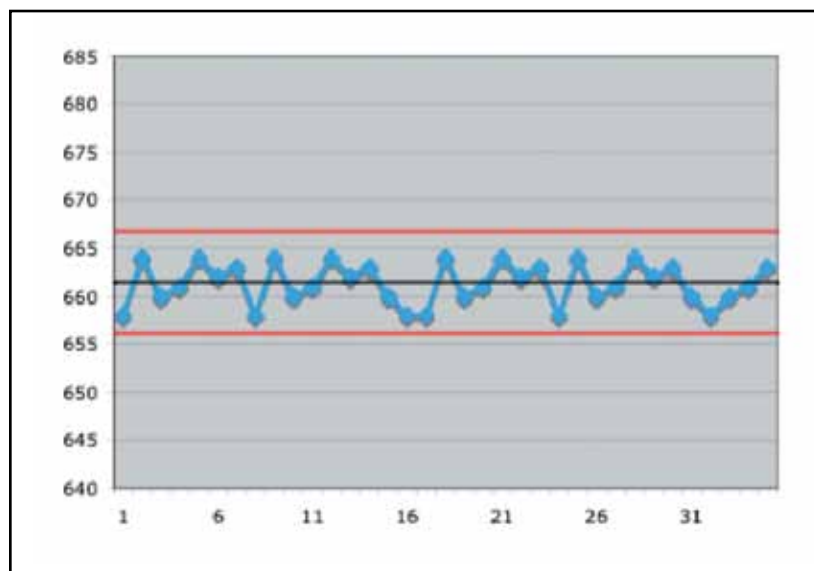
Microsphere standards aid in defining the instrument's capabilities and limitations in terms of sensitivity, precision and accuracy, and provide a means for ensuring that the instrument is stable and suitable for use. They are also helpful in understanding the effects of extraneous factors, such as temperature, 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.

GENERAL STABILITY / STATUS

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

Figure 1



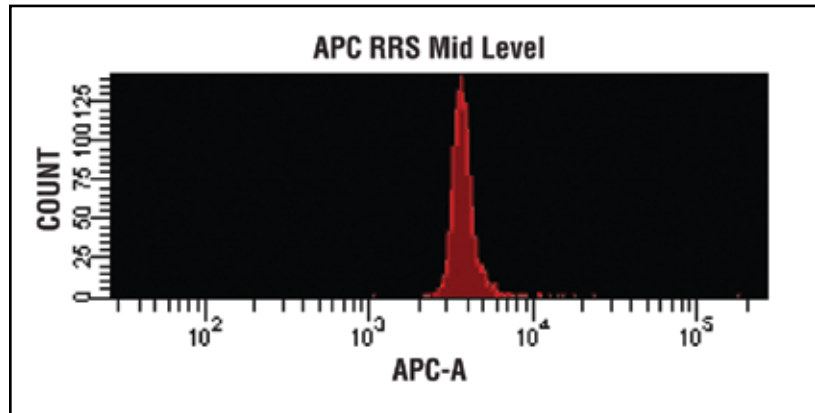
Suggested Products	Full Spectrum™ (3-6 color instruments)	885
	Ultra Rainbow Fluorescent Particles (3-9 color instruments)	610, 611
	Fluorescence Reference Standards (single color)	889-910, 914-915
	Right Reference Standard™ (single color, multiple intensities available)	510-521
Recommended Use	Initial daily check	

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.

Figure 2



Suggested Products Right Reference Standard™ (single color, multiple intensities available) 510-521

Recommended Use Weekly for instruments with fixed alignment (verification)
Daily for instruments with manual alignment (e.g. stream-in-air sorters) or for specific applications (e.g. DNA content analysis)

Catalog Code	Description	Product Data Sheet	Price (\$US)
			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

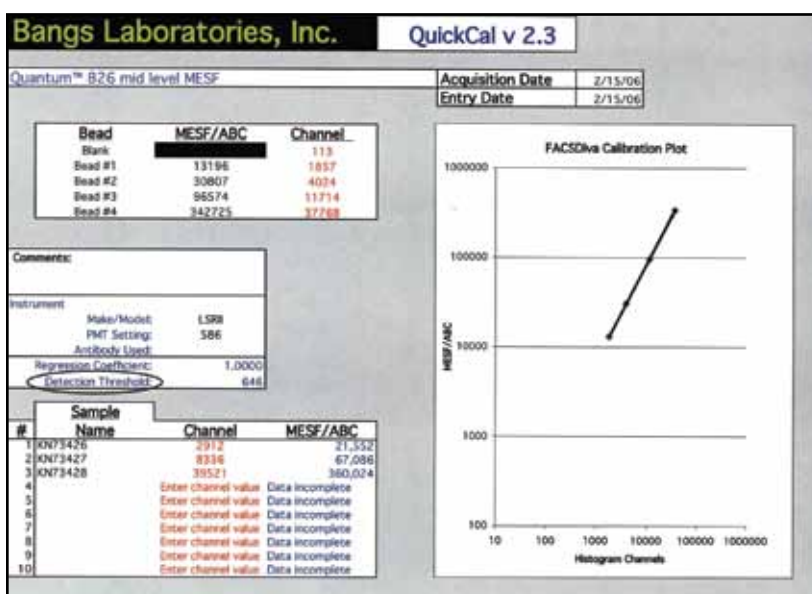
520	Right Reference Standard™ APC Medium Level	510	255
521	Right Reference Standard™ APC High Level	510	255

SENSITIVITY: DETECTION THRESHOLD

Determining an instrument's threshold sensitivity at test-specific settings is important for both qualitative and quantitative fluorescence analyses. Detection threshold may be determined by using fluorochrome-labeled Quantum™ microspheres and the QuickCal® analysis template. QuickCal® constructs a calibration curve associating fluorescence channel values to standardized fluorescence intensity units. The channel value corresponding to the kit's blank bead is converted to the appropriate standardized fluorescence intensity unit (MESF or ABC); this is the instrument's detection threshold for the specific reporter and detector. (More on MESF and ABC units, p. 11, 10, and 20.)

As a QC tool, a drifting detection threshold can alert the user to problems such as contaminated fluids (e.g. free label or debris adhering to the unstained bead), or a shortcoming within the optical system.

Figure 3



Suggested Products Quantum™ MESF 488, 555, 555p, 822-828
 QuickCal® Analysis Template (provided free with purchase of Quantum™ MESF) N/A

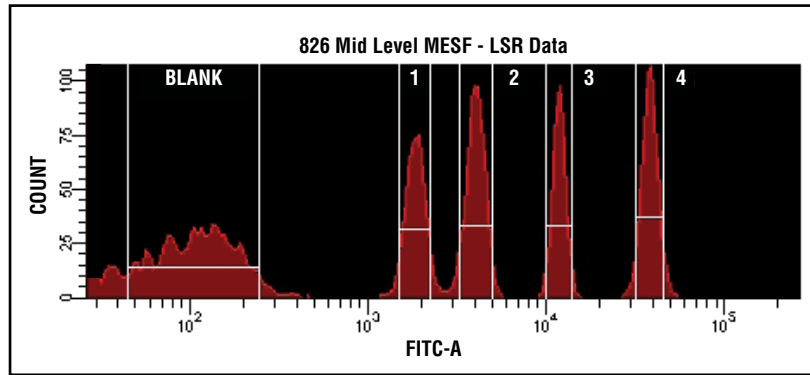
Recommended Use Weekly; or
 Daily for quantitative analyses (at test-specific settings)

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

SENSITIVITY: RESOLUTION

An instrument's sensitivity is also dependent upon its ability to distinguish unlabeled (autofluorescent) from dimly stained samples. Acceptable resolution may be verified using bead kits that contain multiple populations spanning a range of fluorescence intensities. Poor resolution may indicate a problem within the optical or fluidics systems, or suboptimal instrument settings (e.g. use of Linear Mode for fluorescence analyses, or an excessive flow rate).

Figure 4

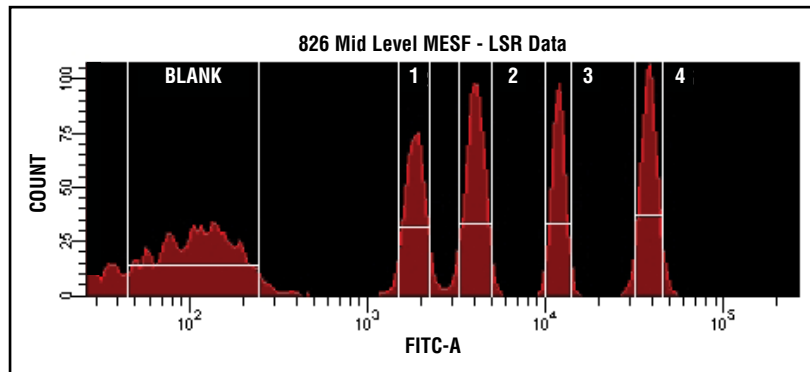


Suggested Products Quantum™ MESF 488, 555, 555p, 822-828
Recommended Use Weekly; or
 Daily for quantitative analyses (at test-specific settings)

LINEARITY

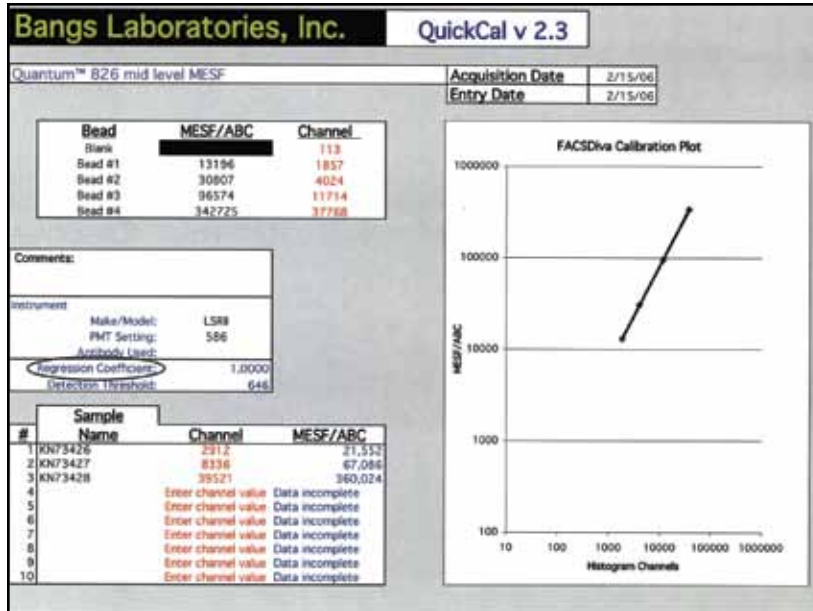
The accurate measurement of fluorescence signal is imperative for applications in quantitative fluorescence cytometry, such as surface marker expression or telomere length determination. To this end, the linear response of the PMTs should be assessed regularly.

Figure 5



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 (r^2), 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 6



Suggested Products Quantum™ MESF 488, 555, 555p, 822-828

QuickCal® Analysis Template (provided free with purchase of Quantum™ MESF) N/A

Recommended Use Weekly; or

Daily for quantitative analyses (at test-specific settings)

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

II. STANDARDIZATION

In flow cytometry, standardization is 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.

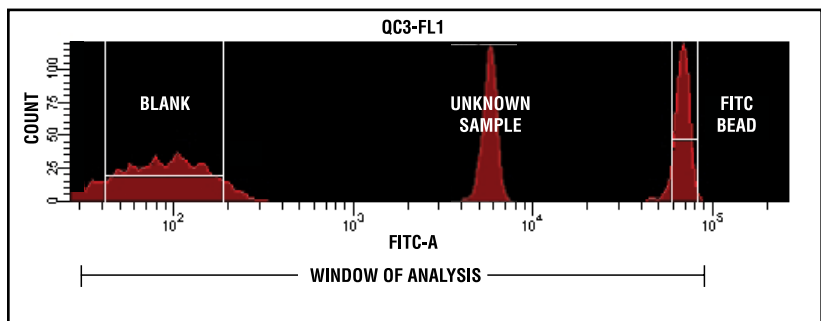
Our microsphere-based tools for standardization can serve a significant role in supporting flow cytometry programs, and establishing a framework for the comparison of data from different instruments, laboratories, and over time.

Instrument	
Instrument configuration	PMT settings
Assay	
Protocols	Sample collection <ul style="list-style-type: none"> • Anticoagulant Sample storage conditions <ul style="list-style-type: none"> • Time • Temperature Sample preparation <ul style="list-style-type: none"> • Lysis • Fixation • Cooling / warming • Staining
Reagents	Antibodies <ul style="list-style-type: none"> • Clone • Purity • Concentration • Labeling density, i.e. Fluorophore:Protein (F/P) ratio Fixatives Lysing agents Anticoagulants
Scale	Window of Analysis Fluorescence Intensity Units

DAILY SET-UP

Flow cytometers are highly configurable, and results can vary dramatically 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 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.

Figure 7



Suggested Products	QC Windows®	845-848
	QC3™	841-844
Recommended Use	Daily instrument set-up, general set-up and at test-specific settings	

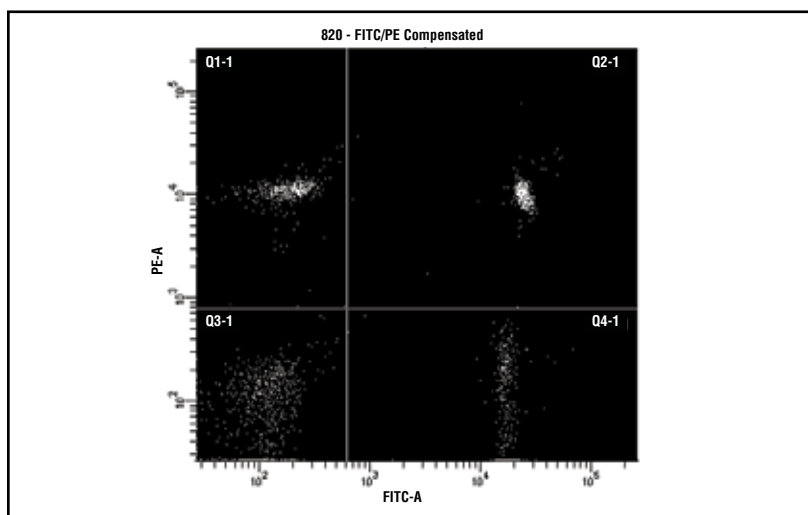
Catalog Code	Description	Product Data Sheet	Price (\$US)		
			A 20 tests	B 100 tests	C 280 tests
845	QC Windows® (FITC/PE)	845	288	484	994
846	QC Windows® (FITC/PE/PE-TR)	845	379	641	1139
847	QC Windows® (FITC/PE/PE-Cy™5)	845	379	641	1139
848	QC Windows® (FITC/PE/PE-Cy™5, APC)	845	453	754	1277

Catalog Code	Description	Product Data Sheet	Price (\$US)		
			A 20 tests	B 100 tests	C 280 tests
841	QC3™ (FITC/PE)	841	263	425	778
842	QC3™ (FITC/PE/PE-TR)	841	310	547	913
843	QC3™ (FITC/PE/PE-Cy™5)	841	310	547	913
844	QC3™ (FITC/PE/PE-Cy™5, APC)	841	310	547	913

COMPENSATION

Standards offer a convenient means for establishing compensation settings in multicolor flow cytometry. Our FITC/PE 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.

Figure 8



EXPECTED VALUES

Quadrants 1-4 illustrate the expected performance of the FITC / R-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.

Suggested Products	FITC/PE Compensation Standard (includes Autofluor™ Reference Standard)	820
	Simply Cellular® Compensation Standards (for Mouse, Rat, or Human primary monoclonal antibodies)	550-552
	Quantum™ Simply Cellular® anti-Mouse, anti-Rat, or anti-Human (for Mouse, Rat, or Human primary monoclonal antibodies)	815-817
Recommended Use	Color compensation for multicolor analyses (at test-specific settings)	

Catalog Code	Description	Product Data Sheet	Price (\$US)		
			A 20 tests	B 100 tests	C 280 tests
820	FITC/PE Compensation Standard	820	198	493	867

Catalog Code	Description	Product Data Sheet	Price (\$US)
			5mL
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

Catalog Code	Description	Product Data Sheet	Price (\$US)		
			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

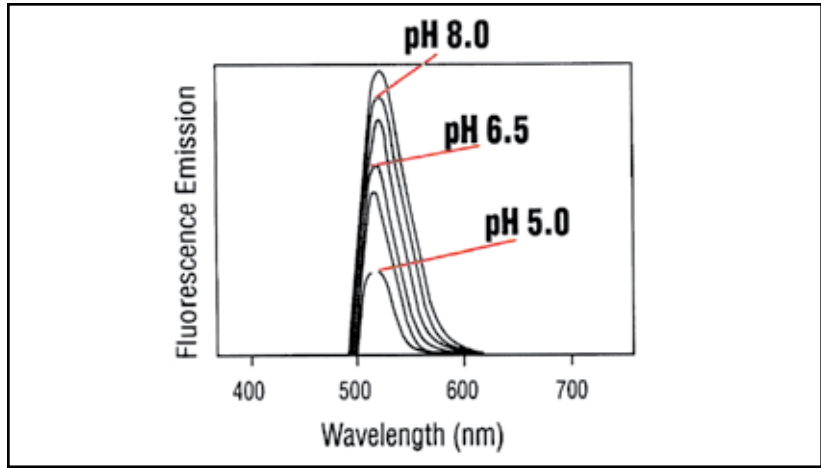
STANDARDIZED FLUORESCENCE INTENSITY UNITS

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 9). 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). See Quantitative Fluorescence Cytometry, p. 11 and 12.

Suggested Products	Quantum™ MESF	488, 555, 555p, 822-828
	Quantum™ Simply Cellular®	815-817
	QuickCal® Analysis Template (provided free with purchase of Quantum™ MESF and Quantum™ Simply Cellular® products)	N/A
Recommended Use	Daily or with each run if instrument settings (detector voltages/gains) are changed	

Figure 9



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

Catalog Code	Description	Product Data Sheet	Price (\$US)		
			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

III. APPLICATIONS

QUANTITATIVE FLUORESCENCE CYTOMETRY

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).

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

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

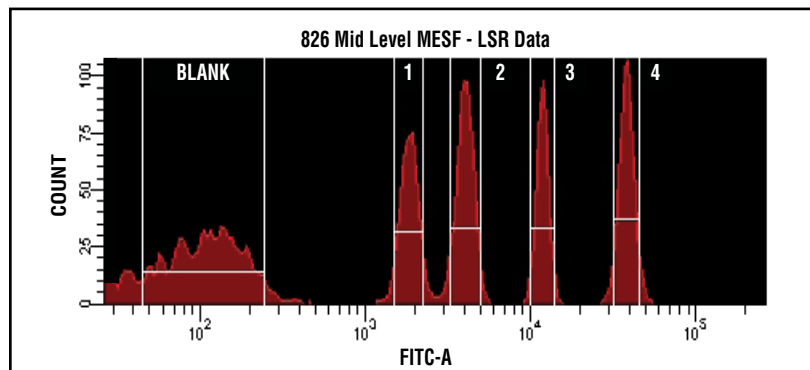
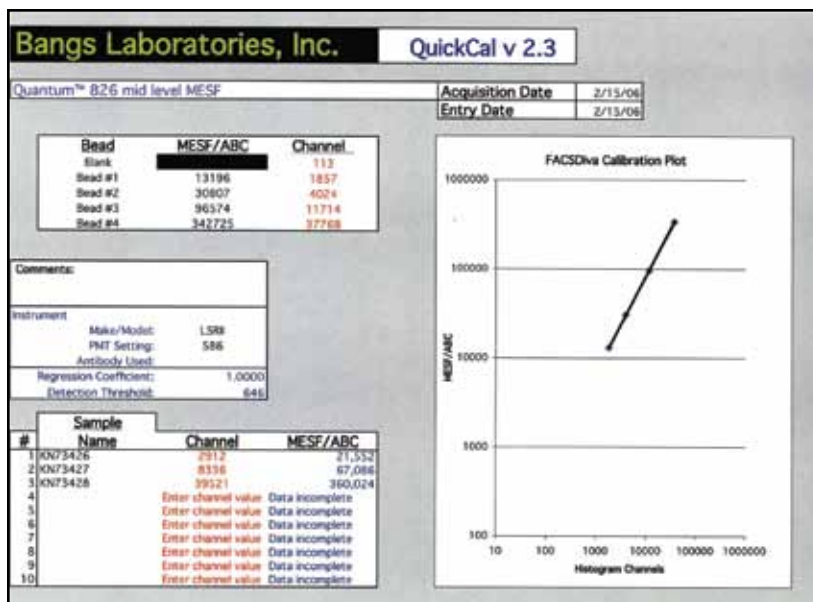


Figure 11

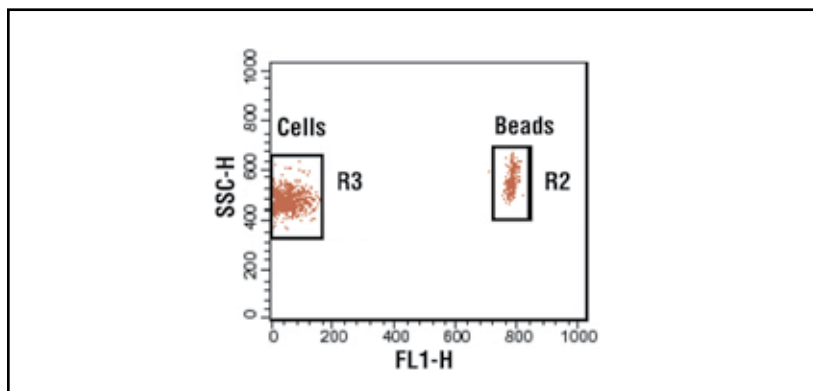


Suggested Products	Quantum™ MESF	488, 555, 555p, 822-828
	Quantum™ Simply Cellular®	815-817
	QuickCal® Analysis Template (provided free with purchase of Quantum™ MESF and Quantum™ Simply Cellular® products)	N/A
Recommended Use	Weekly; or Daily for quantitative analyses (at test-specific settings)	

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.

Figure 12



Suggested Products	Flow Cytometry Absolute Count Standard™	580
Recommended Use	Use with cell samples to determine cell counts	

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

REFERENCES

Chase ES, Hoffman, RA. (1988) Resolution of dimly fluorescent particles: a practical measure of fluorescence sensitivity. *Cytometry*; 33: 267-279.

Davis KA, Abrams B, Iyer SB, Hoffman RA, Bishop JE. (1988) Determination of CD4 antigen density on cells: role of antibody valency, avidity, clones and conjugation. *Cytometry*; 33: 197-205.

Gaigalas AK, Wang L, Schwartz A, Vogt Jr., RF. (2005) Quantitating fluorescence intensity from fluorophore: assignment of MESF values. *J Res Natl Inst Stand Technol*; 110: 101-114.

National Committee for Clinical Laboratory Standards (2004) I/LA24-A, Fluorescence Calibration and Quantitative Measurement of Fluorescence Intensity; Approved Guideline, 24(26).

Purvis N, Stelzer G. (1998) Multi-platform, multi-site instrumentation and reagent standardization. *Cytometry*; 33: 156-165.

Shapiro HM. (2003). *Practical Flow Cytometry, Fourth Edition*. John Wiley & Sons: Hoboken. (ISBN: 0-471-41125-6)

Schwartz A, Fernández-Repollet E, Vogt R, Gratama JW. (1996) Standardizing flow cytometry: construction of a standardized fluorescence calibration plot using matching spectral calibrators. *Cytometry*; 26(1): 22-31.

Schwartz A, Gaigalas AK, Wang L, Marti GE, Vogt RF, Fernández-Repollet E. (2004) Formalization of the MESF unit of fluorescence intensity. *Cytometry B Clin Cytom*; 57(1): 1-6.

Schwartz A, Marti GE, Poon R, Gratama JW, Fernández-Repollet E. (1998) Standardizing flow cytometry: a classification system of fluorescence standards used for flow cytometry. *Cytometry*; 33: 106-114.

Watson JW. (1991) *Introduction to Flow Cytometry*. Cambridge University Press: New York. (ISBN: 0-521-38061-8)

Wood JCS, Hoffman RA. (1998) Evaluating fluorescence sensitivity on flow cytometers: an overview. *Cytometry*; 33: 2566-2569.

Zenger VE, Vogt R, Mandy F, Schwartz A, Marti GE. (1998) Quantitative flow cytometry: inter-laboratory variation. *Cytometry*; 33: 138-145.

VERIFYING PERFORMANCE OF CELL VIABILITY INSTRUMENTS

Trypan blue dye 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.

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 Instrument Standards mimic the light scattering characteristics of live and dead cells in the trypan blue exclusion method, and may be used to confirm the capabilities and verify the performance of image-based cell viability instruments.

Suggested Products ViaCheck™ Viability Instrument Standards 580

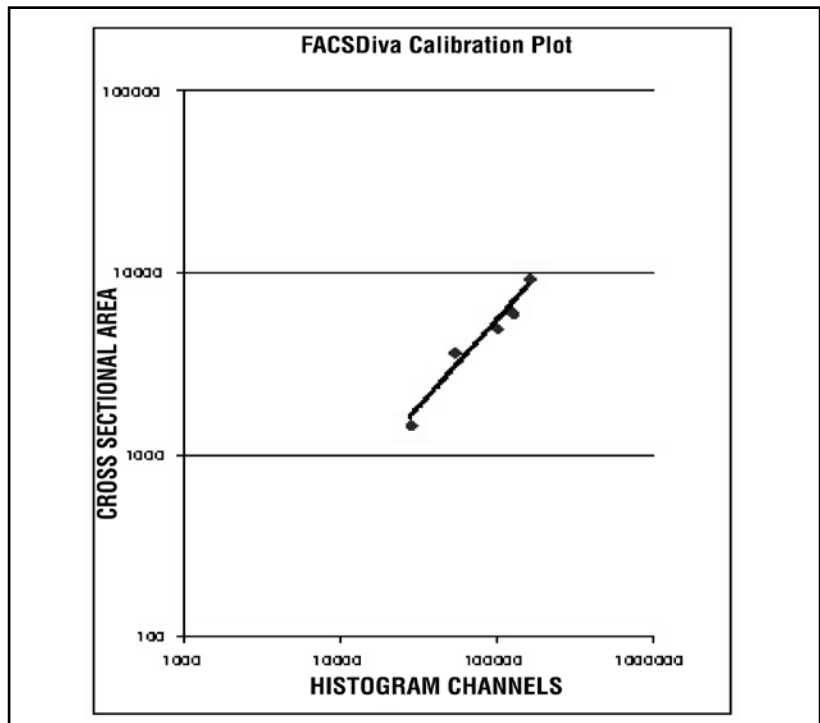
Recommended Use Use to confirm the capabilities and verify the performance of image-based cell viability instruments

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

CELL SIZE ESTIMATION

Forward angle light scatter analysis of microsphere size standards may be performed to construct a calibration plot for the estimation of cell size.

Figure 13



Catalog Code	Description	Product Data Sheet	Price (\$US)	
			B 100 tests	C 280 tests
829	Size Calibration Standards Kit	829	354	629

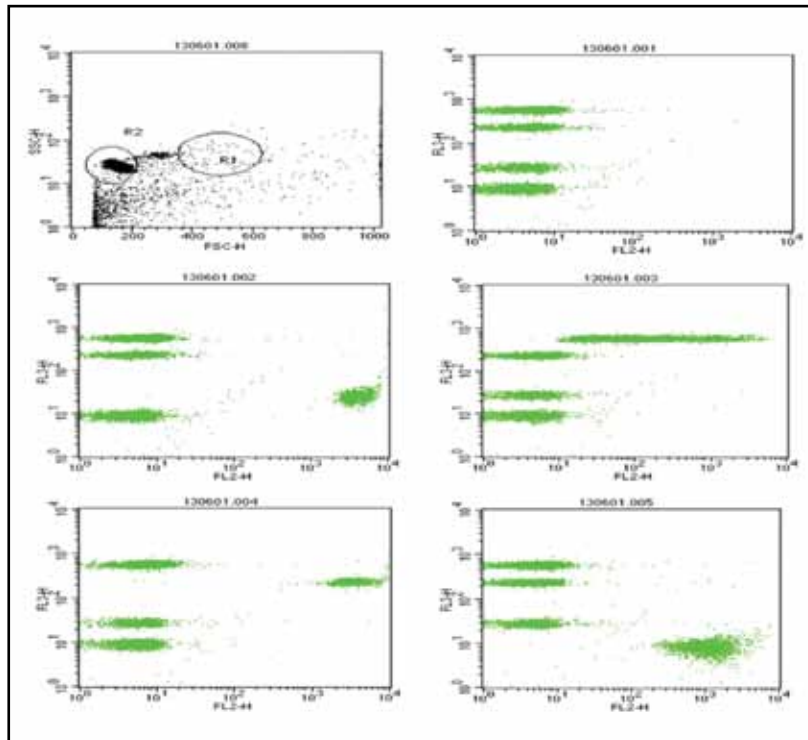
SUSPENSION ARRAYS

Applications in flow cytometry now extend far beyond traditional cellular analyses. The flow cytometer's ability to perform multiparametric detection of virtually any cell-sized particle has enabled the proliferation of simplex and multiplexed bead-based assays. Suspension arrays are statistically robust and additionally offer the benefits of economy, convenience, flexibility, and exceptional binding kinetics.

Our QuantumPlex™ products offer a fully customizable platform for the development of assays that may be run on any standard cytometer. Beads are encoded with varying intensities of our Starfire Red™ fluorophore for the identification of bead populations corresponding to specific capture molecules or sequences. Starfire Red™ has very little carryover into lower wavelengths, leaving other detectors available for determination of positive binding events via common reporters such as FITC and PE.

Figure 14

Multiplexed cytokine assay developed using QuantumPlex™. Courtesy of Dr. John Lawry, The University of Sheffield, UK, 2001.



Suggested Products	QuantumPlex™	204-239
	QuantumPlex™™, superparamagnetic	250-253
Recommended Use	For development of simplex or multiplexed suspension arrays	

Catalog Code	Description	Product Data Sheet	Price (\$US)		
			A 1mL/pop 5x100 data points	B 5mL/pop 5x500 data points	C 10mL/pop 5x1000 data points
235	QuantumPlex™ COOH 4.4µm, 5 populations	235	856	3532	5575
238	QuantumPlex™ COOH 5.5µm, 5 populations	235	856	3532	5575
239	QuantumPlex™ COOH 4.4µm and 5.5µm, 10 populations	235	1563	6503	10,032
215	QuantumPlex™ SA 4.4µm, 5 populations	215	856	3532	5575
218	QuantumPlex™ SA 5.5µm, 5 populations	215	856	3532	5575
219	QuantumPlex™ SA 4.4µm and 5.5µm, 10 populations	215	1563	6503	10,032
205	QuantumPlex™ anti-Mouse IgG 4.4µm, 5 populations	209	856	3532	5575
208	QuantumPlex™ anti-Mouse IgG 5.5µm, 5 populations	209	856	3532	5575
209	QuantumPlex™ anti-Mouse IgG 4.4µm and 5.5µm, 10 populations	209	1563	6503	10,032

Catalog Code	Description	Product Data Sheet	Price (\$US)	
			A 1mL 100 data points	B 3mL 300 data points
234	QuantumPlex™ SP COOH 4.4µm	234	202	501
237	QuantumPlex™ SP COOH 5.5µm	234	202	501
214	QuantumPlex™ SP SA 4.4µm	214	202	501
217	QuantumPlex™ SP SA 5.5µm	214	202	501
204	QuantumPlex™ SP anti-Mouse IgG 4.4µm	207	202	501
207	QuantumPlex™ SP anti-Mouse IgG 5.5µm	207	202	501

Catalog Code	Description	Product Data Sheet	Price (\$US)		
			A 1mL/pop 5x100 data points	B 5mL/pop 5x500 data points	C 10mL/pop 5x1000 data points
250	QuantumPlex™™ COOH, 5 populations	250	856	3532	5575
252	QuantumPlex™™ Streptavidin, 5 populations	252	856	3532	5575

Catalog Code	Description	Product Data Sheet	Price (\$US)	
			A 1mL 100 data points	B 3mL 300 data points
251	QuantumPlex™™ SP COOH	251	202	501
253	QuantumPlex™™ SP Streptavidin	253	202	501

IV. FLOW CYTOMETRY PRODUCTS

Full Spectrum™

Full Spectrum™ microspheres provide a convenient means for performing the initial daily QC check on 3-6 color instruments. The microspheres are internally labeled with multiple fluorophores for use with common excitation and detection wavelengths within the visible spectrum.

Catalog Code	Description	Product Data Sheet	Price (\$US)		
			A 20 tests	B 100 tests	C 280 tests
885	Full Spectrum™	885	143	285	552

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.

Catalog Code	Description	Product Data Sheet	Price (\$US)		
			A 20 tests	B 100 tests	C 280 tests
890	Certified Blank™ Undyed for determination of threshold detection	890	143	285	552
910	Autofluor™ Dyed with a low level of fluorophore to approximate the autofluorescence of an unstained resting lymphocyte	890	143	285	552
897	Acridine Orange	890	143	285	552
886	Alexa Fluor® 488	892	143	285	552
887	Alexa Fluor® 647	892	143	285	552
901	Allophycocyanine (APC)	890	143	285	552
914	APC-Cy™7	890	143	285	552
898	Chlorophyll	890	143	285	552
896	Dansyl Chloride	890	143	285	552
906	DAPI	890	143	285	552
913	Far-Out Red	890	143	285	552
891	Fluorescein	890	143	285	552
851	Fluorescein 2µm	890	143	285	552
903	Fura 2	890	143	285	552
894	Hoechst 33342	890	143	285	552
902	Indo 1	890	143	285	552
900	Oxazine 1	890	143	285	552
908	PE-Cy™5 Tandem	890	143	285	552
889	PE-Cy™7 Tandem	890	143	285	552
909	PE-TR Tandem	890	143	285	552
892	Propidium Iodide	890	143	285	552
899	R-Phycoerythrin	890	143	285	552

Catalog Code	Description	Product Data Sheet	Price (\$US)		
			A 20 tests	B 100 tests	C 280 tests
907	Rhodamine 123	890	143	285	552
904	Rhodamine B	890	143	285	552
905	T.M. Rhodamine	890	143	285	552
893	Texas Red®	890	143	285	552
915	Violet Laser	890	143	285	552

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 Code	Description	Product Data Sheet	Price (\$US)
			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

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 Code	Description	Product Data Sheet	Price (\$US)		
			A 20 tests	B 100 tests	C 280 tests
610	Ultra Rainbow Fluorescent Particles, ~3.8µm (3.61 – 3.99µm)	610	143	285	552
611	Ultra Rainbow Fluorescent Particles, ~10.2µm (8.1 – 12.0µm)	611	143	285	552

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 Sheet	Price (\$US)		
			A 20 tests	B 100 tests	C 280 tests
845	QC Windows® (FITC/PE)	845	288	484	994
846	QC Windows® (FITC/PE/PE-TR)	845	379	641	1139
847	QC Windows® (FITC/PE/PE-Cy™5)	845	379	641	1139
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	Product Data Sheet	Price (\$US)		
			A 20 tests	B 100 tests	C 280 tests
841	QC3™ (FITC/PE)	841	263	425	778
842	QC3™ (FITC/PE/PE-TR)	841	310	547	913
843	QC3™ (FITC/PE/PE-Cy™5)	841	310	547	913
844	QC3™ (FITC/PE/PE-Cy™5, APC)	841	310	547	913

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.

Catalog Code	Description	Product Data Sheet	Price (\$US)		
			A 20 tests	B 100 tests	C 280 tests
820	FITC/PE Compensation Standard (Autofluor™/FITC-PE/FITC/PE)	820	198	493	867

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)
			5mL
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

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 Code	Description	Product Data Sheet	Price (\$US)
			5mL
450	Viability Dye Compensation Standard	853	311
451	Viability Dye Compensation Standard	853	311

Flow Cytometry 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 Code	Description	Product Data Sheet	Price (\$US)		
			A 20 tests	B 100 tests	C 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

Quantum™ MESF

Our Quantum™ MESF kits include five microsphere populations: one blank and four labeled with increasing amounts of fluorochrome. 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. In addition to quantitative fluorescence cytometry, Quantum™ MESF kits may be used to assess the detection threshold, resolution, and linearity of the instrument (p. 4, 5, and 6). 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 Code	Description	Product Data Sheet	Price (\$US)		
			A 20 tests	B 100 tests	C 280 tests
823	Quantum™ APC MESF	823	358	693	1123
488	Quantum™ Alexa Fluor® 488 MESF	888	358	693	1123
822	Quantum™ Cy™5 MESF	822	358	693	1123
555	Quantum™ FITC-5 MESF	855	358	693	1123
555p	Quantum™ FITC-5 MESF (Premix)	856	358	693	1123
828	Quantum™ PE-Cy™5 MESF	828	358	693	1123
827	Quantum™ R-PE MESF	827	358	693	1123

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 (p. 9). 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 Code	Description	Product Data Sheet	Price (\$US)		
			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 Code	Description	Product Data Sheet	Price (\$US)		
			A 20 tests	B 100 tests	C 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

Simply Cellular® anti-Mouse for Violet Laser

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

Catalog Code	Description	Product Data Sheet	Price (\$US)	
			A 1mL	B 5mL
835	Simply Cellular® anti-Mouse for Violet Laser	835	266	448

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 excitation, and exhibits red / far-red emission.

Catalog Code	Description	Product Data Sheet	Price (\$US)		
			A 1mL	B 5mL	C 14mL
830	Time Delay Calibration Standard	831	132	262	510

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.

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

Size Calibration Standards Kit

The Size Calibration Standards Kit is comprised of five populations of undyed microspheres in the range of ~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 Code	Description	Product Data Sheet	Price (\$US)	
			B 100 tests	C 280 tests
829	Size Calibration Standards Kit	829	354	629

QuantumPlex™

QuantumPlex™ kits provide a platform for the design of multiplexed suspension arrays that may be run on standard flow cytometers (488nm or 633nm excitation). Microsphere populations in 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™ exhibits very little carryover into lower wavelengths, leaving other detectors available for determination of positive binding events via common reporters such as FITC and PE. Carboxyl, streptavidin (SA), and anti-Mouse IgG surfaces are available.

Catalog Code	Description	Product Data Sheet	Price (\$US)		
			A 1mL/pop 5x100 data points	B 5mL/pop 5x500 data points	C 10mL/pop 5x1000 data points
235	QuantumPlex™ COOH 4.4µm, 5 populations	235	856	3532	5575
238	QuantumPlex™ COOH 5.5µm, 5 populations	235	856	3532	5575
239	QuantumPlex™ COOH 4.4µm and 5.5µm, 10 populations	235	1563	6503	10,032
215	QuantumPlex™ SA 4.4µm, 5 populations	215	856	3532	5575
218	QuantumPlex™ SA 5.5µm, 5 populations	215	856	3532	5575
219	QuantumPlex™ SA 4.4µm and 5.5µm, 10 populations	215	1563	6503	10,032
205	QuantumPlex™ anti-Mouse IgG 4.4µm, 5 populations	209	856	3532	5575
208	QuantumPlex™ anti-Mouse IgG 5.5µm, 5 populations	209	856	3532	5575
209	QuantumPlex™ anti-Mouse IgG 4.4µm and 5.5µm, 10 populations	209	1563	6503	10,032

QuantumPlex™ SP

QuantumPlex™ 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™, they are dyed with Starfire Red™ and are suitable for use on standard flow cytometers.

Catalog Code	Description	Product Data Sheet	Price (\$US)	
			A 1mL 100 data points	B 3mL 300 data points
234	QuantumPlex™ SP COOH 4.4µm	234	202	501
237	QuantumPlex™ SP COOH 5.5µm	234	202	501
214	QuantumPlex™ SP SA 4.4µm	214	202	501
217	QuantumPlex™ SP SA 5.5µm	214	202	501
204	QuantumPlex™ SP anti-Mouse IgG 4.4µm	207	202	501
207	QuantumPlex™ SP anti-Mouse IgG 5.5µm	207	202	501

QuantumPlex™ M

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

Catalog Code	Description	Product Data Sheet	Price (\$US)		
			A 1mL/pop 5x100 data points	B 5mL/pop 5x500 data points	C 10mL/pop 5x1000 data points
250	QuantumPlex™™ COOH, 5 populations	250	856	3532	5575
252	QuantumPlex™™ Streptavidin, 5 populations	252	856	3532	5575

QuantumPlex™™ SP

QuantumPlex™™ 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™™, they are ~6µm highly uniform superparamagnetic microspheres dyed with Starfire Red™ and are suitable for use on standard flow cytometers.

Catalog Code	Description	Product Data Sheet	Price (\$US)	
			A 1mL 100 data points	B 3mL 300 data points
251	QuantumPlex™™ SP COOH	251	202	501
253	QuantumPlex™™ SP Streptavidin	253	202	501

ViaCheck™ Viability Instrument Standards SP

ViaCheck™ Viability Instrument Standards mimic the light scattering characteristics of live and dead cells in the trypan blue 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.

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

TRADEMARKS AND REGISTERED TRADEMARKS

To the best of our knowledge, the trademarks and registered trademarks listed here are accurate.

Bangs Laboratories, Inc. – Absolute Count Standard™, Autofluor™, Bangs Laboratories, Inc.™, Certified Blank™, COMPEL™, Full Spectrum™, ProActive®, QC3™, QC Windows®, Quantum™, QuantumPlex™, QuickCal®, Right Reference Standard™, Simply Cellular®, Starfire Red™

GE Healthcare Limited – Cy™, including Cy5 and Cy7, are trademarks 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.

Life Technologies Corporation – Alexa Fluor®, Texas Red®

Polysciences, Inc. – ViaCheck™

Spherotech – Spherotech™

FLOW CYTOMETRY CHART

Category	Purpose	Frequency	Products
Daily QC	General check of instrument stability/status	Daily	Full Spectrum™ (multi) Ultra Rainbow Fluorescent Particles (multi) Fluorescent Reference Standards (single)
Daily QC	General check of instrument optical system	Daily	Full Spectrum™ (multi) Ultra Rainbow Fluorescent Particles (multi) Fluorescent Reference Standards (single)
Daily QC	Optical Alignment	Daily	Right Reference Standard™
Daily QC	Fluidics check	Daily	Surface-labeled fluorescent microspheres, e.g. QC Windows® QC3™ Fluorescent Reference Standards Quantum™ MESF
Weekly QC	Optical System Sensitivity, Resolution for Linearity (for specific lasers/PMTs)	Weekly	Quantum™ MESF
Daily Set-Up	Standardized instrument set-up	Daily, or Between runs if settings are changed	QC Windows® QC3™
Daily Set-Up	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®
Application	Fluorescence quantitation in cellular expression studies or bead-based assays	Daily when quantitative analyses are performed, or Between different applications if fluorescence PMT settings are changed	Quantum™ MESF Quantum™ Simply Cellular®
Application	F:P Ratio determination for quantitative fluorescence analyses	As needed, i.e. with each new Lot of fluorochrome-conjugated antibody	Simply Cellular® (used in conjunction with Quantum™ MESF)
Application	Compensation for multicolor flow cytometry	Daily, or Between different applications if fluorescence PMT settings are changed	FITC/PE Compensation Standard Simply Cellular® Compensation Standard Quantum™ Simply Cellular®
Application	Cell Counting	As needed	Flow Cytometry Absolute Count Standard™
Application	Cell Size Estimation	As needed	Size Calibration Standards Kit
Application	Suspension Array	Platform for development of bead-based flow cytometric assays	QuantumPlex™ QuantumPlex™M



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