ABOUT BANGS LABORATORIES, INC.

At Bangs Labs, we’ve been in the business of microspheres for more than 25 years. Along the way, we had the pleasure of joining the flow cytometry community with our acquisition of Flow Cytometry Standards Corporation (FCSC).

We’re proud to continue a tradition of innovative products for instrument validation, QC and standardization, and to contribute our expertise for the development and manufacture of specialty standards for instrument manufacturers and assay developers.

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.

**Bangs Laboratories, Inc.**
9025 Technology Drive
Fishers, IN 46038-2886
info@bangslabs.com

**Polysciences, Inc.**
400 Valley Road
Warrington, PA 18976
info@polysciences.com

**Polysciences Europe GmbH**
Badener Str. 13
69493 Hirschberg an der Bergstrasse, Germany
info@polysciences.de

**Polysciences Asia Pacific, Inc.**
2F-1, 207 DunHua N. Rd.
10595 Taipei, Taiwan
info@polysciences.tw

ISO CERTIFICATIONS

Bangs Laboratories has been certified by American Management Technology, Inc. as having demonstrated that our Quality Management System complies with **ISO 13485:2016** for the manufacture, processing, and distribution of microspheres and related products.
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www.BangsLabs.com
www.stratech.co.uk/bangs_labs
INTRODUCTION

Flow cytometry is a complex but highly informative technology that permits evaluation of cells, subcellular compartments / organelles and microparticles.

The instruments that make these analyses possible house a complex architecture of lasers, detectors and fluidics that work in concert to provide detailed information about the samples that are analyzed. Information regarding every particle that passes the flow cell is collected, including relative size (forward scatter - FSC), internal complexity (side scatter - SSC) and fluorescence. Instruments are often equipped with 2 or more lasers and 2+ detectors per laser, much like the configuration described in Table 1.

<table>
<thead>
<tr>
<th>Laser</th>
<th>Detector</th>
<th>Dichoric mirror</th>
<th>Bandpass filter</th>
</tr>
</thead>
<tbody>
<tr>
<td>405nm (50mW)</td>
<td>VIO 450</td>
<td>-</td>
<td>450/50</td>
</tr>
<tr>
<td></td>
<td>VIO 525</td>
<td>505 LP</td>
<td>525/50</td>
</tr>
<tr>
<td>488 nm (25mW)</td>
<td>SSC</td>
<td>-</td>
<td>488/10</td>
</tr>
<tr>
<td></td>
<td>FITC</td>
<td>505 LP</td>
<td>530/30</td>
</tr>
<tr>
<td></td>
<td>PE</td>
<td>550 LP</td>
<td>575/26</td>
</tr>
<tr>
<td></td>
<td>PE-TR</td>
<td>595 LP</td>
<td>610/20</td>
</tr>
<tr>
<td></td>
<td>PerCP-Cy™5.5</td>
<td>685 LP</td>
<td>695/40</td>
</tr>
<tr>
<td></td>
<td>PE-Cy™5</td>
<td>655 LP</td>
<td>660/20</td>
</tr>
<tr>
<td></td>
<td>PE-Cy™7</td>
<td>735 LP</td>
<td>780/60</td>
</tr>
<tr>
<td>633 (20mW)</td>
<td>APC</td>
<td>-</td>
<td>660/20</td>
</tr>
<tr>
<td></td>
<td>APC-Cy™7</td>
<td>735 LP</td>
<td>780/60</td>
</tr>
</tbody>
</table>

Table 1: Sample configuration for a BD LSRII cytometer, including violet (405nm) laser / detector add-on.

Figure 1: Alignment of particles with laser beam in the flow cell.

Figure 2: Concept of 488nm optical array.

Data may be displayed in single- or multiparametric format with associated statistics, per the typical dual parameter dot plot (FSC / SSC) and single parameter fluorescence histogram in Figure 3.

**Figure 3:**

3a. FSC / SSC dot plot with gated singlets population and associated statistics:

3b. Fluorescence (FITC) histogram of singlets from the FSC/SSC gate shown in 3a.

<table>
<thead>
<tr>
<th>Population</th>
<th>%Parent</th>
<th>FSC-A Median</th>
<th>FSC-A CV</th>
<th>SSC-A Median</th>
<th>SSC-A CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>86.0</td>
<td>134,829</td>
<td>5.5</td>
<td>43,436</td>
<td>4.4</td>
</tr>
</tbody>
</table>

Though unstained cells will yield characteristic scatter patterns that can be readily identified in a FSC / SSC dot plot (Figure 4a), fluorescent reporters and stains are used individually or in combination to provide specific information about the expression of various surface or intracellular markers, metabolic state, membrane integrity, etc. In a classic immunophenotyping example, Figure 4b demonstrates the exclusion of granulocytes and monocytes, and the analysis of CD45 expressing lymphocytes stained with an anti-CD45-APC-Cy™7 Ab.

**Figure 4a:** FSC / SSC dot plot of unstained leukocytes (lysed RBC whole blood)

**Figure 4b:** SSC / APC-Cy™7 fluor dot plot of stained CD45+ lymphocytes.
Within the life sciences, there is a heavy reliance on analytical instruments to make decisions related to research, manufacturing and, for clinical applications, patient care. As this is important work that demands accurate, reliable and relevant data, instruments must be thoughtfully selected, thoroughly qualified, and have capabilities verified throughout their active lives. Qualification is a comprehensive process that is undertaken to ensure that each instrument meets expected capabilities, and is suited to its intended use. It features thorough performance tests, which upon completion, will serve as a foundation for ongoing instrument QC and proficiency programs.

Following qualification, the instrument QC program is intended to provide an accurate picture of instrument status, and provide confidence in resulting data. Specific QC tests should be relevant in type and frequency to the work being performed, and the maintenance and service history should also be considered. If certain components or subsystems have been shown to be less stable, these may warrant more rigorous surveillance.

Each day should begin with a general system check that provides an indication that subsystems and components are functioning. Additional tests should then be performed to address the specific use of the instrument. In particular, more stringent QC is required for quantitative assays.

Figure 5: QC process

Run microsphere standards

Record and Track QC data

PASS: Approve for use

FAIL: Troubleshoot & Correct Service Visit

Figure 6: Example of a basic QC program for a 2 laser cytometer

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Product &amp; Catalog Code</th>
<th>Purpose</th>
<th>Coverage</th>
<th>Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily</td>
<td>Full Spectrum (#885) or Quantum QC (#725)</td>
<td>Basic check of system; Laser alignment check</td>
<td>All lasers / detectors</td>
<td>Chart channel values; Record CVs</td>
</tr>
<tr>
<td>Daily</td>
<td>Quantum MESF</td>
<td>Run at specific PMTs for quantitative expression analyses: Linearity, resolution, detection threshold, alignment specific detector</td>
<td></td>
<td>Confirm resolution; Record linearity; Chart detection threshold and CV</td>
</tr>
<tr>
<td>Daily</td>
<td>or Weekly Quantum QC (#725)</td>
<td>For qualitative analyses; Linearity, resolution, detection threshold, alignment</td>
<td>All lasers / detectors</td>
<td>Confirm resolution; Record Linearity; Chart detection threshold and CV</td>
</tr>
<tr>
<td>Weekly</td>
<td>Time Delay Standard (#830)</td>
<td>Time delay check</td>
<td>Delay between laser 1 (488nm) and laser 2 (635nm)</td>
<td>Confirm time delay</td>
</tr>
</tbody>
</table>

Figure 7: Sample Levey Jennings chart for a single fluorescence channel

INSTRUMENT QUALIFICATION & QC

Within the life sciences, there is a heavy reliance on analytical instruments to make decisions related to research, manufacturing and, for clinical applications, patient care. As this is important work that demands accurate, reliable and relevant data, instruments must be thoughtfully selected, thoroughly qualified, and have capabilities verified throughout their active lives. Qualification is a comprehensive process that is undertaken to ensure that each instrument meets expected capabilities, and is suited to its intended use. It features thorough performance tests, which upon completion, will serve as a foundation for ongoing instrument QC and proficiency programs.

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**Figure 5:** QC process

- Run microsphere standards
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- Service Visit

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<th>Coverage</th>
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<tbody>
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<td>All lasers/detectors</td>
<td>Chart channel values; Record CVs</td>
</tr>
<tr>
<td>Daily for quantitative</td>
<td>Quantum MESF (See page 14)</td>
<td>Run at specific PMTs for quantitative expression analyses: Linearity, resolution, detection threshold, alignment</td>
<td>specific detector</td>
<td>Confirm resolution; Record linearity; Chart detection threshold and CV</td>
</tr>
<tr>
<td>Daily for quantitative; or Weekly</td>
<td>Quantum QC (#725)</td>
<td>For qualitative analyses; Linearity, resolution, detection threshold, alignment</td>
<td>All lasers/detectors</td>
<td>Confirm resolution; Record linearity; Chart detection threshold and CV</td>
</tr>
<tr>
<td>Weekly</td>
<td>Time Delay Standard (#830)</td>
<td>Time delay check</td>
<td>Delay between laser 1 (488nm) and laser 2 (635nm)</td>
<td>Confirm time delay</td>
</tr>
</tbody>
</table>

A basic program like the example in **Figure 7** ensures surveillance of the complete system, i.e. the optics (lasers, detectors, flow cell alignment), fluidics (observation of flow rates, time delay confirmation), and associated computing. Recording values for certain parameters in Levey Jennings charts can readily confirm satisfactory performance, or aid in identifying both random errors (electronic noise, air bubbles, etc.) and systemic errors (bias, shifts and trends due to temperature fluctuation, laser deterioration, misalignment, etc.) so that corrective action may be taken. Thresholds may be developed for watchful monitoring (A) or intervention (B), **Figure 7**.

**Figure 7:** Sample Levey Jennings chart for a single fluorescence channel

---


While the extremely sensitive nature of flow cytometers permits the analysis of micron-scale (or smaller) and dimly fluorescent particles, it also makes them sensitive to even the most subtle changes in cell samples, instrument operation, and the laboratory environment. For these reasons, it is imperative that instrument configuration and operating conditions be standardized as much as possible, and that suitable reference materials are used for tests and assays.

The use of reference beads can ameliorate differences in range, relative scale and reporting units, as well as daily fluctuation due to electronic noise, and ambient temperature and humidity. As one example, Quantum™ QC may be used to set up all detectors by positioning a specific peak at a relevant target channel value.

Figure 8: Use Quantum™ QC to define window of analysis i.e. upper & lower fluorescence limits
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### Figure 8: Use Quantum™ QC to define window of analysis i.e. upper & lower fluorescence limits

<table>
<thead>
<tr>
<th>Catalog #</th>
<th>PDS</th>
<th>Name</th>
<th># beads</th>
<th>Fluorescence</th>
</tr>
</thead>
<tbody>
<tr>
<td>725</td>
<td>725</td>
<td>Quantum™ QC</td>
<td>8</td>
<td>Full spectrum + Blank</td>
</tr>
<tr>
<td>885</td>
<td>885</td>
<td>Full Spectrum™</td>
<td>1</td>
<td>Full spectrum</td>
</tr>
<tr>
<td>512, 515, 518, 521</td>
<td>510</td>
<td>Right Reference Standards</td>
<td>1-3</td>
<td>FITC, PE, PE-Cy™5, or APC</td>
</tr>
</tbody>
</table>

See Table 5

<table>
<thead>
<tr>
<th>Catalog #</th>
<th>PDS</th>
<th>Name</th>
<th># beads</th>
<th>Fluorescence</th>
</tr>
</thead>
<tbody>
<tr>
<td>890</td>
<td></td>
<td>Fluorescence Reference Standards</td>
<td>1</td>
<td>See Page 13</td>
</tr>
</tbody>
</table>

### Table 2: Products for Instrument Set Up

<table>
<thead>
<tr>
<th>Catalog #</th>
<th>PDS</th>
<th>Name</th>
<th># beads</th>
<th>Fluorescence</th>
</tr>
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<tbody>
<tr>
<td>725</td>
<td>725</td>
<td>Quantum™ QC</td>
<td>8</td>
<td>Full spectrum + Blank</td>
</tr>
<tr>
<td>885</td>
<td>885</td>
<td>Full Spectrum™</td>
<td>1</td>
<td>Full spectrum</td>
</tr>
<tr>
<td>512, 515, 518, 521</td>
<td>510</td>
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See Table 5

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<th>Catalog #</th>
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<th>Name</th>
<th># beads</th>
<th>Fluorescence</th>
</tr>
</thead>
<tbody>
<tr>
<td>890</td>
<td></td>
<td>Fluorescence Reference Standards</td>
<td>1</td>
<td>See Page 13</td>
</tr>
</tbody>
</table>

### Figure 9: Quantum™ QC Histograms


See Compensation Section (Pg 10) for set-up related to compensation.
INSTRUMENT SET-UP: SMALL PARTICLES

Current applications in flow cytometry extend beyond the analysis of lymphocytes, and push cytometers to their limits of detection for particle size and fluorescence. Small particle analyses, including platelet and endothelial-derived microparticles, microvesicles or microbial species, require modified processes and specialized instrument set-up. Our fluorescent small bead calibration kits can aid in:

- determining an instrument’s limit of size detection;
- assessing background particulates and developing modified preparatory processes (e.g. fluid filtration)
- small particle size calibration
- refining instrument settings (threshold, PMT, windows extension)

**Figure 10:** Micron Bead Calibration Kit - LSRII settings
FSC log 536 - Threshold 200
SSC log 247 - Threshold 200
FITC log 346 - Threshold 200

**Figure 11:** Submicron Bead Calibration Kit - LSRII settings
FSC log 500 - Threshold 200
SSC log 494 - Threshold 200
FITC log 587 - Threshold 200

<table>
<thead>
<tr>
<th>Catalog #</th>
<th>Name</th>
<th>Nominal Diameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>833</td>
<td>Micron Bead Calibration Kit</td>
<td>1.0μm, 3.0μm, 6.0μm</td>
</tr>
<tr>
<td>832</td>
<td>Submicron Bead Calibration Kit</td>
<td>0.2μm, 0.5μm, 0.8μm</td>
</tr>
<tr>
<td>834</td>
<td>Nanobead Calibration Kit</td>
<td>50 nm, 100 nm</td>
</tr>
</tbody>
</table>

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FSC log 500 - Threshold 200
SSC log 494 - Threshold 200
FITC log 587 - Threshold 200

Table 3: Small Bead Calibration Kits

<table>
<thead>
<tr>
<th>Catalog #</th>
<th>PDS</th>
<th>Name</th>
<th>Nominal Diameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>833</td>
<td>832</td>
<td>Micron Bead Calibration Kit</td>
<td>1.0μm, 3.0μm, 6.0μm</td>
</tr>
<tr>
<td>832</td>
<td>832</td>
<td>Submicron Bead Calibration Kit</td>
<td>0.2μm, 0.5μm, 0.8μm</td>
</tr>
<tr>
<td>834</td>
<td>834</td>
<td>Nanobead Calibration Kit</td>
<td>50 nm, 100 nm</td>
</tr>
</tbody>
</table>


Due to the nature of the cytometer (sensitive detection, specific filter sets) and the fluorophores themselves (broad emission bands), fluorescence typically spills over into regions beyond that covered by the intended detector. The most pronounced carryover tends to be into longer wavelengths (i.e. is red-shifted), though it can often be observed to a lesser extent at shorter wavelengths.

Multicolor analyses necessitate the correction of spectral overlap for each fluorochrome and detector. Compensation is performed by electronically subtracting the percentage of fluorescence signal that is equivalent to the carryover.

Proper compensation requires reference materials that represent the actual fluorophore combinations of stained cells. Bangs offers both fluorophore - matched microspheres and microspheres with capture Abs or functional groups for labeling with reactive fluorophores or fluorescent antibody conjugates. Figure 14 illustrates the use of microsphere standards to develop a compensation matrix.

**Figure 13:** Fluorescence Carryover

Using compensation, carryover fluorescence is electronically “subtracted” from unintended detectors so that the measured signal is as pure as possible. This figure illustrates the carryover of Fluorophore A into the Fluorophore B detector, as well as the carryover from Fluor B into the Fluor A detector. A compensation matrix might be:

- Fluor A – 2% Fluor B
- Fluor B – 1% Fluor A
**Figure 13:** Fluorescence Carryover

**INSTRUMENT SET-UP: COMPENSATION**

The use of microsphere standards to develop a compensation matrix. With reactive fluorophores or fluorescent antibody conjugates, illustrations microspheres and microspheres with capture Abs or functional groups for labeling actual. Proper compensation requires reference materials that represent the percentage of fluorescence signal that is equivalent to the carryover.

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<table>
<thead>
<tr>
<th>Catalog #</th>
<th>PDS</th>
<th>Product</th>
<th>Binds</th>
</tr>
</thead>
<tbody>
<tr>
<td>820</td>
<td>820</td>
<td>FITC/PE Compensation Standard</td>
<td>Pre-labeled with FITC/PE</td>
</tr>
<tr>
<td>See pg. 13</td>
<td>890</td>
<td>Fluorescence Reference Standards</td>
<td>Pre-labeled with designated fluor pg.13</td>
</tr>
<tr>
<td>550-552, 556</td>
<td>850</td>
<td>Simply Cellular® Compensation Standards</td>
<td>IgG from Mouse, Rat or Human, as noted</td>
</tr>
<tr>
<td>835</td>
<td>835, 850</td>
<td>Simply Cellular® anti-Mouse for Violet Laser</td>
<td>IgG from Mouse</td>
</tr>
<tr>
<td>553-554</td>
<td>854</td>
<td>Protein A, Protein G Antibody Binding Beads</td>
<td>See PDS 854 for IgG affinities</td>
</tr>
<tr>
<td>450-451</td>
<td>853</td>
<td>Viability Dye Compensation Standards</td>
<td>Amine-reactive dyes</td>
</tr>
</tbody>
</table>

**Figure 14:** Compensation Matrix - Simply Cellular® anti-Mouse IgG Bead for Violet Laser produces comparable data when compared to cells.

Compensated with: Stained Violet Beads

<table>
<thead>
<tr>
<th>Detectors</th>
<th>Value (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>515/20 Violet - 450/50 Violet</td>
<td>32.6</td>
</tr>
<tr>
<td>605/40 Violet - 450/50 Violet</td>
<td>3.06</td>
</tr>
<tr>
<td>450/50 Violet - 515/20 Violet</td>
<td>7.42</td>
</tr>
<tr>
<td>605/40 Violet - 515/20 Violet</td>
<td>33.51</td>
</tr>
<tr>
<td>450/50 Violet - 605/40 Violet</td>
<td>0.05</td>
</tr>
<tr>
<td>515/20 Violet - 605/40 Violet</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Compensated with: Stained Cells

<table>
<thead>
<tr>
<th>Detectors</th>
<th>Value (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>515/20 Violet - 450/50 Violet</td>
<td>31.93</td>
</tr>
<tr>
<td>605/40 Violet - 450/50 Violet</td>
<td>0.00</td>
</tr>
<tr>
<td>450/50 Violet - 515/20 Violet</td>
<td>6.86</td>
</tr>
<tr>
<td>605/40 Violet - 515/20 Violet</td>
<td>32.06</td>
</tr>
<tr>
<td>450/50 Violet - 605/40 Violet</td>
<td>0.16</td>
</tr>
<tr>
<td>515/20 Violet - 605/40 Violet</td>
<td>0.09</td>
</tr>
</tbody>
</table>


Classic immunophenotyping involves fairly straightforward sample preparation. Following collection of the blood sample, there may be a depletion or enrichment step (e.g. via density centrifugation, RBC lysis, antibody-coated magnetic particles [e.g. BioMag® anti-leukocyte particles]), in addition to fixation and staining. Though the specific steps may be routine, sample preparation should be thoughtfully designed and standardized as cellular processes, expression of certain markers, cell viability, microvesicle counts and size distribution may be sensitive to temperature, fixatives, lysing agents, etc. Changes in reagents, handling or storage conditions may result in alterations in samples and resulting data.

As an additional note on sample preparation, fluorophore selection is an important factor, where markers with low expression are labeled with bright fluorochromes, and those that express at high levels are labeled with dimmer reporters. Consideration should also be given to the size of the fluorescent reporter in the context of potential steric effects (e.g. PE MW 260,000; FITC MW 389), stability, nonspecific binding and spectral overlap. (See Table 5.)


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(See Table 5.)

---

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

### Visible Spectrum

---

**Table 5: Fluorescence Reference Spectrum Products**

<table>
<thead>
<tr>
<th>Catalog #</th>
<th>Description</th>
<th>MW</th>
<th>Excitation (nm)</th>
<th>Emission (nm)</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>890</td>
<td>Certified Blank™</td>
<td>265</td>
<td>500</td>
<td>526</td>
<td>reference</td>
</tr>
<tr>
<td>897</td>
<td>Acridine Orange</td>
<td>643</td>
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<td>519</td>
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<td>360</td>
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QUALITATIVE & QUANTITATIVE ASSAYS

Many immunophenotyping assays are qualitative in nature. For these types of studies, cells are stained for a certain marker, and the shift over an unstained population is used to determine relative expression (low, mid, high) or presence of the marker in general (positivity). In these types of studies, bead standards can be used to define the window of analysis, and to serve as reference points for a comparison of results. (see pg. 6-7).

Some applications require true quantitation of cell surface markers, intracellular proteins, etc., as with pharmaceutical trials that determine changes in cellular marker expression levels or distribution in response to administration of a particular drug. For these types of expression studies, kits such as Quantum™ MESF and Quantum™ Simply Cellular® (QSC) permit the quantitation of fluorescence signal, and by extension, determination of antibody binding to the surface marker or expressed protein. Read more about these systems in our literature on Quantitative Fluorescence Cytometry.

Figure 15: Quantum MESF histogram and QuickCal analysis template
QUALITATIVE & QUANTITATIVE ASSAYS

Quantum™ Simply Cellular® (QSC) permit the quantitation of fluorescence signal, drug. For these types of expression studies, kits such as Quantum™ MESF and marker expression levels or distribution in response to administration of a particular proteins, etc., as with pharmaceutical trials that determine changes in cellular Some applications require true quantitation of cell surface markers, intracellular comparison of results. (see pg. 6-7) be used to define the window of analysis, and to serve as reference points for a of the marker in general (positivity). In these types of studies, bead standards can population is used to determine relative expression (low, mid, high) or presence studies, cells are stained for a certain marker, and the shift over an unstained Many immunophenotyping assays are qualitative in nature. For these types of

Table 6: Quantitative Cytometry Products

<table>
<thead>
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<th>Catalog #</th>
<th>Description</th>
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<th>MW</th>
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<td>488</td>
<td>Quantum MESF Alexa Fluor® 488</td>
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<tr>
<td>823</td>
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<td>anti-Mouse IgG (Fc-specific)</td>
<td>Mouse mAb (Fc)</td>
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<td>816</td>
<td>QSC anti-Rat IgG (Fc)</td>
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<td>Rat mAb (Fc)</td>
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<td>817</td>
<td>QSC anti-Human IgG (Fc)</td>
<td>anti-Human IgG (Fc-specific)</td>
<td>Human mAb (Fc)</td>
</tr>
</tbody>
</table>


www.stratech.co.uk/bangs_labs
Orders may be placed via phone (317-570-7020 or 800-387-0672), fax (317-570-7034), website, or email (info@bangslabs.com). If you’d prefer, you can also place orders directly with one of our distributors. In terms of payment methods, orders may be placed using a purchase order or credit card (Visa or MasterCard). Payment must be in U.S. dollars by check (cheque) drawn on a U.S. bank, or by wire transfer.

Some other products that may be of interest:

- Cell Cycle Analysis
- Microparticle Analysis
- Size Estimation
- Imaging Standards
- Cell Viability Standards
- Concentration Standards

We are here for you; let us know how we can help.

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