

Painless Particles®

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**NEW ISO
9001:2000
Certification**
Sept. 2002

If You Need Help "by the book," just Buy the Book!

"Designing Microsphere-Based Tests and Assays"

OK, you missed the course (June 2002, Indianapolis), but you don't need to miss out on the information. You can still order the >500 page course book that all attendees received while the extra copies last (\$395 + shipping). Hurry – the 2001 book sold out! (For speakers, topics, biographies, and order information, see our website.)

BLI's Trade Show Schedule: If you go, stop by and see us!

❖ **AACR (Cancer Research).** July 11-13, 2003. Washington, DC. Booth #1304. www.aaacr.org/2003am/2003am.asp

❖ **AACC Clinical Lab Expo.** July 22-24, 2003. Philadelphia, PA. Booth #650. www.aacc.org/2003am/

Product Spotlight: Quantum™ Simply Cellular®

A Novel Approach to Quantitation of Cell Surface Antigens

But, What is It?

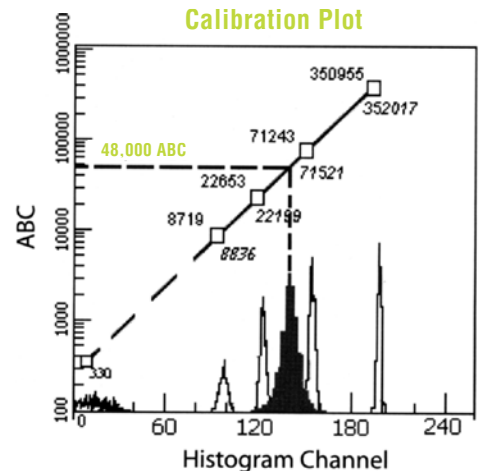
The ABC (Antibody Binding Capacity) value of a sample represents the number of monoclonal antibodies a sample will bind, and correlates to the number of antigens expressed on the cell surface. Quantum Simply Cellular (QSC) offers a straightforward approach to determining the ABC values of stained cell samples.

How Do They Work?

Each Quantum Simply Cellular kit contains a series of four microsphere populations labeled with varying amounts of anti-Mouse or anti-Human IgG (Fc-specific). In addition to the four binding standards, each kit includes a Certified Blank™. These unlabeled microspheres are used to determine the minimum detection threshold, or background noise level, of the instrument.

Each microsphere population in the Quantum Simply Cellular kit is assigned a different ABC value, reflecting its ability to bind more or less antibody. For example, a Quantum Simply Cellular microsphere with an ABC value of 48,000 has the ability to bind 48,000 mouse monoclonal antibodies.

The Quantum Simply Cellular microspheres are stained just like your cell samples, and with the same antibody. By plotting each population's fluorescence intensity versus its



assigned ABC value, a standard ABC curve is generated, and the ABC value of stained cell samples may be easily determined.

Are these Standards Spectral Matching or Environmentally Responsive?

The same antibody is used to stain both the Quantum Simply Cellular microspheres and your cell samples. The result is a standard that exhibits the same excitation and emission properties as your stained samples, under a variety of different conditions. We call this being "environmentally responsive." The environmentally responsive nature of the stained microspheres compensates for minor changes in your system, such as slight variations in pH, and produces consistent results each time.

Quantum Simply Cellular antibody binding standards are available in versions intended for use with mouse or human monoclonal IgG antibodies.

Blue Light Specials Now Include Silica Beads (Bangs Bargain Beads)

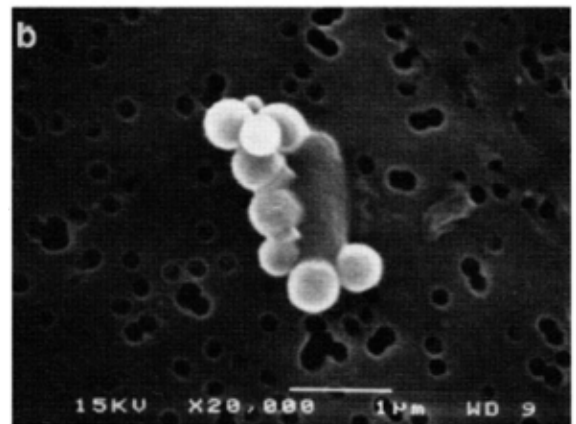
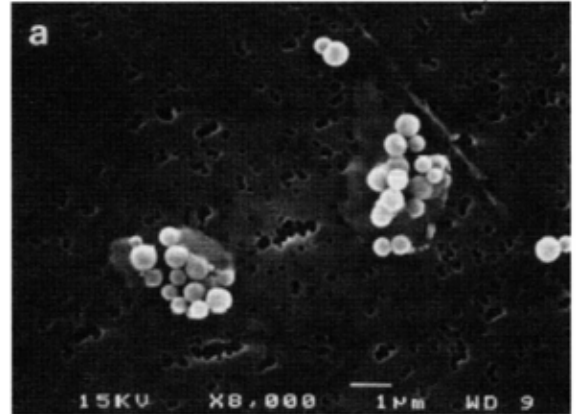
For special prices on small quantities of end-of-run, "close-outs," or left-over lots of our microspheres and flow products – all sizes, colors, and "flavors" – including our silica beads, see our online Selection Guide (at www.bangslabs.com/products/bangs/guide.php) and click on "Search for this week's Bargain Beads."

P(articles)₂ = Particle Articles

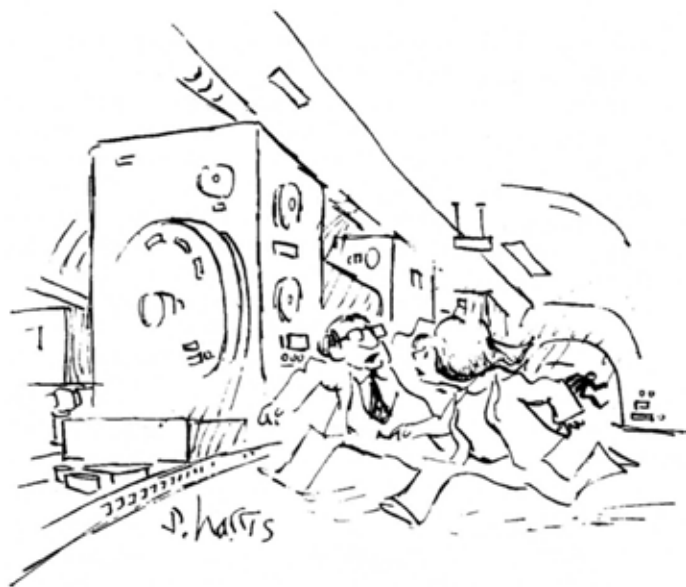
❖ Immunomagnetic Separation and Detection of Bacteria

Ensuring the quality of food and water sources is a critical issue, and the development and improvement of rapid detection methods for pathogenic organisms has become an ongoing imperative of public health organizations.

Barry Pyle and colleagues in the Department of Microbiology at Montana State University, Bozeman, developed a rapid, direct method for the assessment of *Escherichia coli* O157:H7 in food and water samples. The researchers coated superparamagnetic microspheres (~0.6µm) from Bangs Laboratories using anti-O157 serum, and incubated the microspheres with inoculated food and water samples. Isolated cells were incubated with a respiratory fluorochrome to permit viability assessment, and directly labeled with FITC-conjugated anti-*E. coli* O157:H7 antibody for confirmation. *E. coli* O157:H7 cells were counted via microscopy or solid-phase laser cytometry.



Images of immunomagnetic microspheres attached to *E. coli* O157:H7 cells. Images reprinted with permissions from the American Society of Microbiology and Montana State University. [Images first published in: Pyle BH, Broadaway SC, McFeters GA. "Sensitive detection of *Escherichia coli* O157:H7 in food and water by immunomagnetic separation and solid-phase laser cytometry." *Appl. Environ. Microbiol.*, **65**, #5, 1966-72 (1999)]



"IT STARTED WITH JUST THE PARTICLES BEING ACCELERATED, BUT NOW EVERYTHING AROUND HERE HAS SPEEDED UP."

❖ QuantumPlex™ beads

Lawry J, Clayton R, Kenmore M, Naylor M, Harris S. "The Design and Development of Cytokine Multiplex Assays for Flow Cytometers," poster at the XXI Congress of The International Society for Analytical Cytology, May 2002. San Diego, CA.

❖ Fluorescent magnetic beads

Hinds KA, Hill JM, Shapiro EM, Laukkanen MO, Silva AC, Combs CA, Varney TR, Balaban RS, Koretsky AP, Dunbar CE. "Highly efficient endosomal labeling of progenitor and stem cells with large magnetic particles allows magnetic resonance imaging of single cells." *Blood First Edition Paper*, prepublished online April 3, 2003; DOI 10.1182/blood-2002-12-3669.

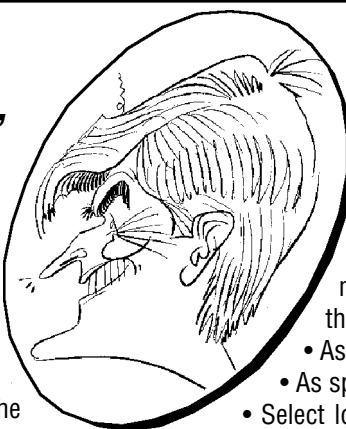


We think that we are doing things pretty swiftly, but please ask if you want any "accelerated" particles or service. Just call or write and ask for help with BEADS • ABOVE THE REST™
(Cartoon reprinted with special permission from Sidney Harris <SHarris777@aol.com> and www.sciencecartoonsplus.com.)

Ask "The Particle Doctor[®]"

Switching (Antibody) Sources in Midstream?

Q : I use your Quantum™ Simply Cellular® kits to determine CD61 expression of platelets and their precursors. The manufacturer of the anti-CD61-PE I had been using is no longer offering that antibody. If I switch to another antibody source, will my results still compare to the work I've already done? I'm worried about the difference in F/P ratios between the two antibodies.



A : While the F/P ratio (fluorochrome/protein ratio - the number of fluorochromes per antibody) will differ between the two antibodies, that difference will not be reflected in the Antibody Binding Capacity (ABC) results obtained from the QSC kit. This is also due to the fact that each of the beads in the Quantum Simply Cellular kit is calibrated to bind a specific number of monoclonal antibodies, regardless of the F/P ratio of the antibody. You may notice a difference in the fluorescence intensity of both your samples and the QSC beads with the new antibody, but as long as your samples and the QSC beads are stained with the same antibody, the results should correlate very well with your previous work.

Applications for Plain Jane Silica?

Q : I am familiar with the use of plain polystyrene for adsorption of protein (specifically, antibody). I have always heard that silica does not bind protein. If this is the case, what is its use?

A : Silica has become the substrate of choice for many applications, due to its low nonspecific protein binding properties. It is hydrophilic and is less likely to adsorb high amounts of protein nonspecifically than hydrophobic polymer, such as polystyrene. Additionally, its negative charge will cause it to resist nonspecific binding of negatively-charged molecules, such as nucleic acids. However, nothing is absolute - adsorption is dependent upon a number of factors, including characteristics of both the biomolecule (concentration, solubility, charge, pl, etc.) and the solution (pH, salt content, presence of competing molecules, etc.). In fact, our silica microspheres have been utilized for the study of protein adsorption, as reported in:

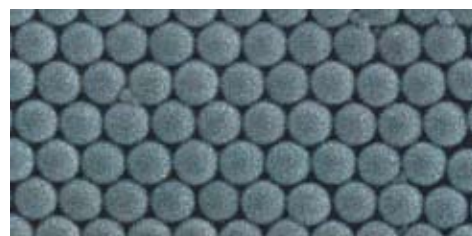
Docoslis, A., L.A. Ruskinski, R.F. Giese, C.J van Oss. 2001. Kinetics and interaction constants of protein adsorption onto mineral microparticles - measurement of the constants at the onset of hysteresis. *Colloids and Surfaces B: Biointerfaces*, 22: 267-283.

van Oss, C.J., A. Docoslis, R.F. Giese. 2001. Free energies of protein adsorption onto mineral particles - from the initial encounter to the onset of hysteresis. *Colloids and Surfaces B: Biointerfaces*, 22: 285-300.

Our silica microspheres have been proven to be useful for many applications, including the following:

- In solid-phase diagnostics (after functionalization of the surface for coupling of biomolecules).
- For nonspecific immobilization or purification of nucleic acids (in the presence of divalent cations or in the presence of a chaotropic agent and high salt).
- As substrates for the self-assembly of lipid bilayers.
- As spacers for flat panel displays.
- Select lots of highly uniform silica have also been used for microstructure assembly.

Figure 1: Electron micrograph of 0.97µm uniform silica beads.



Mail Bonding (Subscribers "do the 'write' thing"!)

- ❖ "I've been using your 0.3 to 0.4µm diameter plain microspheres in my assay for _____, and they work brilliantly." (KH, New Zealand) Thank you! We need the feedback – good or bad.
- ❖ "I offer my compliments on your website. It is much better than most larger companies' websites. I can find information very easily, including specific contacts for different departments." (Major Customer). Thank you! We really appreciate your kind comments.
- ❖ "I have very good success with your microspheres. They have performed much better than those from _____. Do you have a method of coating cholesterol or fatty substance on a 2µm microsphere?" (MR, MN). We're happy you found a reliable source here. See our website for general coating procedures and call or email us anytime for more specific technical advice.
- ❖ "I would like to thank you for the rapid response in supplying the technical notes I requested previously. I only wish that our technical service was as efficient and helpful to our customers as you are to yours!" (Anonymous to protect source) Many thanks.
- ❖ "We need TechNotes ___ & ___." (RB, New Orleans). You can instantly download and print most of our TechNotes (see the list on page 4) directly from our website. We hope this gives you the information at your fingertips. Please let us know if you have any difficulty.

"I'd a whole lot rather explain the price than apologize for the quality." – Sausage Maker Jimmy Dean

Technical References – See our website (www.bangslabs.com) for "downloadable" TechNotes and Product Data Sheets or ask for copies by mail or fax. We continually update and add new TechNotes and Product Data Sheets to our website.

Product-Specific TechNotes:

101. **ProActive® Microspheres** – Handling tips plus protocols for streptavidin, Protein A, and goat anti-Mouse coated microspheres.
102. **Magnetic Microparticles** – Characteristics, handling tips, and applications for superparamagnetic particles.
103. **Fluorescent/Dyed Microspheres** – Applications, fluorescence spectra, and product descriptions. Plus QuantumPlex™ microspheres for multiplexing, flow cytometry, and confocal microscope standards.
104. **Silica Microspheres** – For immunoassays, nucleic acid capture, velocimetry (LDV, PIV), flat panel display spacers, and others.
105. **Microsphere Size Standards** – Beads for cell size estimation, filter challenge, and instrument checks and calibrations. NIST-traceable standards from 0.27µm to 25µm.
106. **Confocal Standards** – Using our three, bright, single-label 60nm fluorescent beads in confocal microscopy.

Handling-Specific TechNotes:

201. **Working with Microspheres** – Choosing, cleaning, characterizing, coating beads, etc.
202. **Microsphere Aggregation** – Preventing, detecting, and reversing aggregation. Chemicals and equipment sources.
203. **Washing Microspheres** – Variety of methods for cleaning microspheres; advantages/disadvantages of methods; suppliers of equipment.
204. **Adsorption to Microspheres** – Adsorbing protein onto particles; use of "surface diluents" (blockers); recipes and references.
205. **Covalent Coupling** – Chemical attachment of proteins, nucleic acids, etc. to various types of surface-functionalized microspheres; recipes for buffers, blockers; miscellaneous coupling ideas, vendor information, and references.
206. **Equations** – For calculating particles/mL, area/g, "parking area", settling velocity @ 1G and in centrifuge, etc.
208. **Microsphere Sizing** – Various manual and automated methods are described and discussed, with references and supplier list.

Flow Cytometry Standards? See the "flow" portion of our website for lots of technical information about flow cytometry standardization in general and our expanding line of flow cytometry standards products in particular.

Application-Specific TechNotes:

301. **Immunological Applications** – Review of commercial applications of microspheres.
302. **Molecular Biology** – Overview of purification and solid phase separation methods.
303. **Lateral Flow Tests** – Putting dyed particles on membranes so they will move properly.
304. **Light-Scattering Assays** – Turbidimetric and nephelometric applications of microspheres.

Reprints:

402. **Microspheres, part 1: Selection, cleaning, and characterization, and part 2: Ligand attachment and test formulation** – LB Bangs & Mary Meza, *IVD Technology (in Medical Device & Diagnostic Industry)*, **17**, #3, 18-26, March, and #4, 20-26, April, 1995. (Note that you can download these papers at the IVDT website: www.devicelink.com/ivdt/archive/95/03/009.html and [.../95/04/006.html](http://www.devicelink.com/ivdt/archive/95/04/006.html)).
403. **New Developments in Particle-Based Immunoassays** – Leigh B. Bangs, *Pure & Appl. Chem.*, **68**, #10, 1873-1879 (1996). Review of 40 years of diagnostic uses of microspheres – from LATs to biosensors.
405. **Applications of Magnetic Particles in Immunoassays** – Mary Meza, Ch. 22 (pp. 303-309) in *Scientific and Clinical Applications of Magnetic Carriers*, U. Häfeli, *et al*, Eds., Plenum Press, New York, 1997.
406. **Measuring Microsphere Binding Capacity** – JM Duffy, JV Wall, MB Meza, LJ Jenki, *IVD Technology*, **4**, #7, 28-34 (1988). (No reprints are available; you can download from our website.)
407. **Bead-based HTS Applications in Drug Discovery** – MB Meza, *Drug Discovery Today: HTS Supplement*, **1**, #1, 38-41 (2000).

BLI Presentations and References See our website for copies of the latest public presentations by the technical people at BLI and for publications that cite use of Bangs Beads or were authored by BLI personnel.

Visit our website for the recently updated and expanded FAQs. See the "Hot Links" page at our website for help in locating equipment, instruments, etc.

If you aren't able to locate answers to your microsphere application or handling/use questions (within our TechNotes, Product Data Sheets, FAQs, References, or Product Brochures), we invite you to call us directly, or to contact "The Particle Doctor®" through our website.