

Painless Particles®

Quarterly Global Newsletter
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Now Including Flow Cytometry Standards Corp..

B E A D S ● A B O V E T H E R E S T™

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ISO-9002 Certification



BLI has been certified by TRA Certification as having a Registered Quality System—ISO 9002.

Bargain

Bangs ^ Beads & OverFlow™ Beads

See our online lists for BLI beads (at www.bangslabs.com) and for FCSC (at www.fcstd.com) for special prices on small quantities of end-of-run, "close-outs", or left-over lots of our microspheres – all sizes, colors, and "flavors". These lists are updated at least monthly, so check back regularly for special deals.

We're Teenagers Now!

On April 1, 2001, we will celebrate our 13th birthday as a corporation. We have steadily grown in annual sales, number of people at BLI, and in the number of friends that we serve. Many thanks for helping us stay alive and grow as we have. We know that we owe it all to you!

See Newly Redesigned Website

On February 19th, we unveiled the *new* version of our website: www.bangslabs.com. Check it out if you haven't visited recently.

QuantumPlex™: Streptavidin follows GAM

(I asked Kathy Turner and Nathan Foushee to write this section about QuantumPlex. That might have been a mistake, but here goes anyway! LBB)

Now, our most popular protein coating is available on QuantumPlex beads, allowing users to enjoy the many benefits conferred by the streptavidin-biotin system. And, it doesn't even cost more. (Hey, why aren't we charging more for these?) Biotinylated ligands are readily available, and may be easily conjugated to a streptavidin-coated surface with little or no optimization.

We utilize our proven methods to coat the beads with streptavidin, yielding a stable and highly reactive surface. Furthermore, the biotin/streptavidin bond is able to withstand a range of temperature and pH, and is very strong, approaching that of a covalent bond ($K = 10^{13}$).

These kits are available in the same sizes as our goat anti-mouse (GAM) QuantumPlex beads, 4.4µm and 5.5µm. Each five-bead kit is composed of a blank bead population and four others, dyed with one of four different intensities of our proprietary fluorophore, Starfire Red™.

Now, with two binding proteins to choose from, users may enjoy the increased flexibility in assay development. And, just in time for summer, we will have naked beads. (Actually, they are "clothed" with COOH groups for covalent coupling, if you wish.)

We're trying to help you get your assay out the door, and you can bet your assay on that! (Okay, the naked joke was better, this is one of LBB's, so we were compelled to use it.)

Come on, give us a call. Let's multiplex! (Check out our new website, www.quantumplex.com)

(Now you see the abuse I get around here. LBB)

QuantumPlex - maximizing flow cytometry!



Last Call for The Latex Course™ for 2001

April 30-May 2 in San Diego

"Designing Microsphere-Based Tests and Assays" – This is your last chance to sign up for the 14th version of our popular course. We have assembled 10 internationally respected experts to help you learn all about making and using latex particles, microspheres, and polymer beads for diagnostic, flow cytometric, molecular biological, and other assay technologies. For details, see our printed course brochure sent earlier or download the brochure at www.bangslabs.com/2001latexcourse. Please enroll by March 31 (by mail or online) and save money if you pay by March 19.

Bring all your "particular" questions and problems and we'll try to answer them. Note that since the printed brochure was sent out, one of our speakers encountered a scheduling conflict. Dr. Wesley Tamashiro from Beckman-Coulter has graciously agreed to fill in for us. (The online brochure has the correct information.)

New Product Developments

NEW Esptapor® Magnetic Beads (ask if interested)

- ❖ Hydrophobic magnetic beads (0.7-1.3µm diameter, 35-40% ferrite) – designed for protein adsorption.
- ❖ Fluorescent magnetic beads which can act as both separable solid phase and label.
- ❖ Classical type magnetic beads with thiol (-SH) surface groups for an alternative binding chemistry.
- ❖ Large, polystyrene magnetic beads (4-8µm diameters) provide very strong response to the magnet.
- ❖ Classical magnetic beads with tertiary amine surface groups.

P(articles)₂ = Particle Articles

"Solubilization and Display of G-Protein-Coupled Receptors on Beads for Real-Time Fluorescence and Flow Cytometric Analysis," Sklar LA, Viven J, Lynam E, Neldon D, Bennett TA, Prossnitz E, *Biotechniques*, **28**(5), 967-985 (2000). Cites use of our (FCSC) Quantum beads for standardization and use of non-spherical (*ugh!*) non-uniform (*double ugh!*) silica particles. But you can get "way better" silica beads from us. (*We don't make "ugh" beads.*)



"I GUESS I WAS ATTRACTED TO PARTICLE PHYSICS FOR THE USUAL REASON. I LIKE TO WORK AT HOME, AND I HAVE A VERY SMALL APARTMENT."

We can help you work with microspheres (particles) in your very small lab. Check out all our TechNotes (p. 4) and consider attending "The Latex Course"™ - April 30-May 2 in San Diego. (Cartoon reprinted with special permission from Sidney Harris.)

Idea for QuantumPlex™ Beads

We received the following e-mail on January 10, 2001:

"Hello Painless Particles People:

Your QuantumPlex mailing [beads for multiplex assays on flow cytometers] prompted this.

Wouldn't it be great to have some tests using Bangs Beads for the metals that are known to have an increased concentration in the blood of Alzheimer's Diseased people? Mercury and lead have been documented to have an increased concentration in the blood and aluminum in brain tissue. [Since] enzymes function on the basis of an active site in which a metal ion is located, perhaps it is proper to expect malfunctioning enzymes within the brain when odd or excessive metal ions are present. [Since there are] two types of observed entities (tangles & ?) found in the brain and associated with AD, [then, perhaps] the odd structures observed [can be linked to] enzymes that process materials for those structures; that is, with enzymes whose product molecules are affected by odd metal ions. Thus, removal of those possibly counter-productive metal ions may be possible by blood chelation techniques, [with attendant help] for AD individuals.

[Currently] no attempts at trying such specifically directed blood chelation process can be test[ed] for major change in AD outcome, [because] of the inconvenience and expense of many separate tests, for several metals in the blood, at regular intervals. Bead-based tests are needed to [confirm] the validity of odd metal concentrations in those diagnosed or suspected, and to [assay the metal] concentrations after chelation trials.

Chelation works or it doesn't, for positive change for real AD people! And reasonable trials depend on having cheap and easy tests for the metals! The AD problem is multi-faceted, with blood flow, anti-oxidant levels, and genetic aspects, but even with perfect genetics, etc., and plenty of odd metals, I'll bet the odd metals win.

Further, a nanoparticles approach for grabbing the metal atoms or odd-metal containing compound molecules [might] be worked out over time, by-passing blood chelation as such. But the immediate need – here and now – is for a crude but functional chelation technique for changing some metals in the inner-brain surroundings, and [this treatment] lacks bead [assays] in order to be tested.

Perhaps [some] of your regular customers can bring such odd metal bead tests into being.

*Best to you,
Erich M. Helbig,
Jefferson City, TN"*

Many thanks for your thoughtful suggestion. We hope that someone who might want to make such assays will read this and follow-up for more information, help, and products to do the job.

Ask "The Particle Doctor[®]"

Parking Area Calculation and Use

Q : We're using some of your COOH-modified microspheres and I just encountered the term "parking area". What's that? Are you guys running a parking lot?

A : Yes, a parking lot for molecules! Actually, the "parking area" permits one to compare particles with different titration values (meq/g or $\mu\text{eq/g}$) and different diameters for their surface charge density, which relates to their relative stabilities and binding capacities for proteins. Calculated parking areas ($\text{\AA}^2/\text{charge group}$) are the reciprocal of the surface charge density (groups/ \AA^2 or groups/ nm^2), and are calculated from the diameter and titration of surface charge of clean microspheres.

If the parking area for any lot of microspheres is $\sim 20 \text{\AA}^2/\text{COOH group}$, then the microspheres are assumed to be covered with a monolayer of COOH groups. This number comes from the packing density for a close-packed monolayer of fatty acids at an air-water interface. The number for sulfate groups would be about the same; primary amino groups might be smaller.

One envisions a model where all the microspheres are the same diameter and the charged groups are neatly arranged closely-packed on the surface of the microspheres. This is strictly true only for sulfate-modified microspheres and for certain COOH-modified microspheres which have charged groups only at the ends of the polymer chains. Most of our COOH microspheres are made by copolymerizing a small portion of acrylic acid with styrene (S/AA). In this case, the COOH groups are on random polymer chains which will tend to arrange themselves with the hydrophilic COOH groups in the aqueous phase and at the microsphere surfaces. The COOH groups are certainly not arranged as a neat monolayer here, but probably exist as hydrophilic chains attached at one or both ends and extending out into the aqueous phase, rather like tennis ball fuzz. The $\sim 20 \text{\AA}^2/\text{COOH group}$ "rule" can very easily be violated here, of course.

If particles with non-uniform size (like our magnetic particles) are considered, then the model, based upon a calculation on spheres of one diameter will fail again, due to errors in estimating surface area per gram.

With S/AA microspheres, often only about half of the acid which is added actually appears on the particles; the rest ends up as water-soluble polymer (WSP, or polymer with so much acid that the chains are fully water-soluble and they completely escape the particle surface). WSP is removed by ion exchange cleaning of the particles before they are titrated. Of course, some acid may also be buried in the interior of the particles and not titrated.

We report titration values (μeq of COOH/g) and then calculate **apparent** parking area and report it for most lots of these beads. (For more on this topic, see TechNotes 201 and 206).



Hydrophilic Bead Coating

Q : We could deposit our special hydrophobic coating on your beads from isopropanol. Would that be okay for polystyrene? If so, are there other ways to get the microspheres out of aqueous solution, besides centrifuging? And finally, since I can work with large sized spheres, what would be an ideal size to remove water and then deposit our coating?

A : 1) Isopropanol would be fine for the beads. And, you could add it to the beads directly because it mixes with water.

2) Cross-flow filtration is a good way to remove some (but not all) solvent - water or isopropanol.

3) If you used $>1 \mu\text{m}$ beads, they would be very easy to spin down and to resuspend. Larger beads will also settle easily and rather quickly without centrifuging (the larger the bead, the faster they will settle, of course.) They will also settle faster in isopropanol than in water. You can do "dead-end" or bed filtration of large beads more easily, too. (Flow through a filter cake is much easier with $1 \mu\text{m}$ beads than with $0.1 \mu\text{m}$ beads.) But, since they have a lower specific surface area, you might need to use more beads. I would suggest choosing the size by what surface area you need and by how easy it will be to clean them before and after coating. (For more information, see our TechNotes- especially 201, 203, and 206.)



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

- ❖ "I liked your ideas about boulders in the stream and Third Wave. What's your latest idea?". (Anon.) Thanks for writing in, MOM. Anyway, how about using ultrasonics to promote adsorption or covalent binding or agglutination. (Note: for other ideas see our earlier newsletters and TechNotes – all downloadable at our website.)
- ❖ "I love the technical literature." (TR, Germany) Glad you like it.
- ❖ "Keep up the good work on the newsletter." (JS, Detroit) Will do.
- ❖ "I have used your products, technotes, and expertise in the past and found you extremely helpful." (EC, Dublin) Many thanks.
- ❖ Recently a good customer called to speak to one of our managers. Amanda Jones answered the phone and passed him on to the appropriate person. His first comment to the manager was, "You have a wonderful person answering the phone this morning. She just makes you want to smile." (GB, USA) Mandy is one of our treasures and we're happy for others to discover why we smile, too.
- ❖ "Dear Kathy: Thank you so much for your assistance! I really appreciate it." (LA, Tampa) Now we'll have to be nice to Kathy, too!

"If we knew what we were doing, it wouldn't be called research, would it?" – Albert Einstein

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. Includes confocal microscopy standards.
104. **Silica Microspheres** – For immunoassays, nucleic acid capture, velocimetry (LDV, PIV), flat panel display spacers, and others.
105. **Microsphere Size Standards** – Data for 9 sizes (0.2-20µm), available singly or in kits, with certificates of analysis.
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.
207. **ProActive® Streptavidin Coated Microspheres and Their Binding Capacity for Biotin and Biotinylated Oligonucleotides** – K. Turner, 2000 AACC OEM Lecture Slides
208. **Microsphere Sizing** – Various manual and automated methods are described and discussed, with references and supplier list.

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:

401. **estapor® "Microspheres" booklet** – 1995 revision: Information on fluorescents, encapsulated and narrow magnetics, nanoparticles (<50nm), NIST-traceable standards; many handling tips; >60 references.
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).
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 Janski, *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).

Flow Standards? See the FCSC website (www.fcstd.com) for lots of technical information about flow cytometry standardization in general and our new flow cytometry standards products in particular.

Free Literature for you! What information do *you* need? We freely share our library: >1000 papers about microspheres, cross referenced, so we can search for types of particles, coupling methods, uses, author, etc. New papers are added as we get them.
Help from you? Please tell *us* about good papers which we should have as you find them. And please send us any good bead art that you find – photos, drawings, etc. showing microspheres or their applications.