

Painless Particles®

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A DIVISION OF POLYSCIENCES, INC.

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.

The Tie Story

(From Chicago AACC Meeting, 2001)

In the hotel elevator one morning, Dr. A.C. van der Graaf, or Groningen, NL, noticed and commented on LBB's tie – a replica of the periodic table of elements. LBB said, "I don't wear this tie all the time; I only wear it periodically," and Dr. vdG was greatly amused. Then, LBB noticed and commented on Dr. vdG's black tie with colored balls as being a nice one with "beads" on it.

Later that same day, they met again accidentally at the show and again admired each other's ties. Dr. vdG readily agreed to LBB's suggestion of a tie swap on the spot. People in the vicinity were then amazed and amused to see two men tear off and exchange ties – in the middle of the show floor!

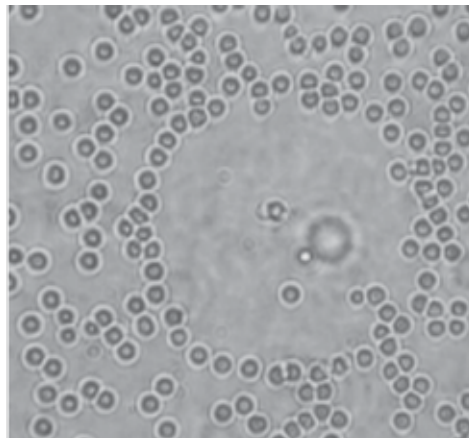
LBB is still wearing his "new" bead tie – but not all the time!

QuantumPlex™ SP

We've received a number of requests from researchers for a single population of "QuantumPlex-like" beads. These researchers either wanted to create a flow assay for a single analyte, or were looking for a bead that could be used to inexpensively optimize their conjugation protocols before moving to the full QuantumPlex kits. To that end, we're pleased to release our new product, QuantumPlex SP (SP for "single population").

Our QuantumPlex SP beads are great for researchers who are interested in exploring the benefits and power of flow cytometry. The single population of beads is available with goat anti-Mouse IgG (GAM), streptavidin or carboxyl surface, just like their big-brother QuantumPlex kits.

QuantumPlex SP is ideal for applications that do not require, or simply are not ready for, a multiplexed platform. Because the microspheres are internally labeled with the same Starfire Red™ dye used in the QuantumPlex multiplexing kits, the beads provide the researcher with an inexpensive means of optimizing surface conjugations, which may be then readily transferred to the multiplexed format.



Bead Corral

In a dense collection of beads, the BioRyx™ 200 system creates a corral of beads that fences in an area for study of a small number of beads. ARRYX, Inc. employs holographic optical trapping (HOT) technology in their BioRyx 200 system. This platform tool for using beads in research and diagnostics can create 1-200 steerable traps or microscopic manipulators to control beads in 3D. <www.arryx.com>

The Latex Course™ 2002: June 10-12

"Designing Microsphere-Based Tests and Assays"

What can you do if you missed it? Order the >500 page course book (that all attendees received) for \$395. We'll sell any extra copies while they last. (Details and order information at website.)

We assembled 11 experts in various aspects of microsphere or latex particle manufacture, reagent preparation, and applications, including 3 new speakers this year. We had 12 lectures covering the latest uses and handling techniques for beads. (See speaker bios and lecture topics at the website.)

"On the Road Again!"

Below is the BLI meeting/show schedule for the rest of 2002. If you will be attending any of these shows, please stop by or booth and meet our fabulous folks, a.k.a., *The Bangs Gang!*

- ❖ AACC Clinical Lab Expo: July 30-August 1, Orlando, FL. Meet us at Booth 4923 in the OEM section. www.aacc.org/2002am/
- ❖ Drug Discovery Technology 2002: August 4-9, Boston, MA. Meet us at Booth 3003. www.drugdisc.com/us/
- ❖ Society for Biomolecular Scening: September 23-26, The Hague, Netherlands. Booth to be assigned. www.sbsonline.com
- ❖ GLIIFCA – Great Lakes International Imaging and Flow Cytometry Association: October 4-6, Detroit, MI. www.cyto.purdue.edu/flowcyt/glifca/gliifca.htm
- ❖ Clinical Applications of Cytometry: October 13-16, Keystone, CO. Booth to be assigned.
- ❖ American Society for Human Genetics: October 15-19, Baltimore, MD. Meet us at Booth 324. www.ashg.org/genetics/ashg/meet-2002/2002-info.htm
- ❖ NIH Research Festival 2002: October 17-18. Meet us at Booth 853.
- ❖ MEDICA: November 20-23, Düsseldorf, Germany.
- ❖ American Society for Hematology: December 6-10, Philadelphia, PA
- ❖ American Society for Cell Biology: December 14-18, San Francisco, CA



"OF COURSE YOU CAN'T PUT YOUR FINGER ON IT. IT'S A HYPOTHETICAL PARTICLE."

Well, at Bangs Labs, we have no hypothetical, imaginary, or phantom particles – only real ones! Please ask us for help supplying all your "particular" needs – for service or for BEADS • ABOVE THE REST™ (Cartoon reprinted with special permission from Sidney Harris <SHarris777@aol.com> and www.sciencecartoonsplus.com.)

The Bangs Gang

More News of our Employees

Kate Edwards has moved from Customer Service to a newly created job of Product Manager/Marketing Coordinator. She just got back from a "road show" trip with Chad and they are still speaking to one another, so I guess she passed the highway test.

Andrew Cox came aboard in April as our QC Technician – responsible for all product testing, as well as operational checks on our equipment. With his strong background in biology and previous work experiences, Andrew brings a great perspective to product quality. He discovered what "the track" and "the race" means in Indy.

Mark Schemmel filled the role of shipping technician in January 2002. His careful attention to detail helps our packages go out the door flawlessly. His strong work ethic and positive attitude make Mark a perfect fit for the Bangs team. His experience as a volunteer fireman should help us "putting out fires" around here, too!

Amanda Jones Good news; bad news! The good news is that Mandy is getting married on June 8th. The bad news is that she insists on living with her new husband (what a quaint idea!) -- in Grand Rapids. So, we are losing her. Please join us all in wishing her happiness!



P(articles)₂ = Particle Articles

❖ Dederich DA, *et al.*, "Glass Bead Purification of Plasmid Template DNA for High Throughput Sequencing of Mammalian Genomes," *Nucleic Acids Res.*, **30/7**, paper #e32 (2002). A new (high-throughput) method for the purification of plasmid template DNA. Acid-washed glass beads (and other glass [silicate] supports) were used with success. Authors assert that this method has advantages over other methods, including Hawkins/SPRI and Engelstein/high salt + PEG. Perfect for silica beads.

❖ Spiro A, Lowe M, Brown D, "A Bead-Based Method for Multiplexed Identification and Quantitation of DNA Sequences Using Flow Cytometry," *Appl. Environ. Microbiol.* **66/10**, 4258-4265 (2000). Using BLI's 5.6µm COOH-modified beads.

Ask "The Particle Doctor"®

Flow Question

Q : I'm looking for some beads to calibrate my cytometer in terms of GFP molecules. I heard that you made such a kit, but I can't find it on your website. (*This looks like a question for Nathan Foushee.*)

A : The kit is called "FACS EGFP Calibration Beads." Similar to the Quantum™ MESF kits, they may be used to construct a standard curve based on the fluorescence intensity of EGFP molecules. The kits are available exclusively through BD Biosciences Clontech, and may be ordered by calling 1-877-232-8995. (*Thanks, Nathan.*)

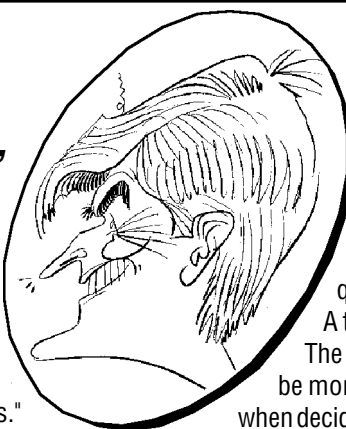
Oligonucleotide Immobilization

Q : After oligo coupling, how can I determine the efficiency of the oligo immobilization?

A : There are a number of methods for assessing binding efficiency. 1) Binding capacity of oligo dT beads may be determined by binding mRNA and measuring the amount of eluted RNA by wavelength scanning (see our TechNote 302). 2) The amount of bound oligonucleotide may also be estimated by hybridizing it to its biotinylated complement, with detection via a streptavidin-labeled fluorophore. [A similar approach is detailed in: Kumar, A., et al. 2000. *Nucleic Acids Res*, 28(14):e71. Available for free download from the journal website.] 3) Use radiolabeled probes. [Lund, V., et al. 1988. *Nucleic Acids Res*, 16(22):10861-10880; Day, P.J.R., et al. 1991. *Biochem J*. 278:735-740.] 4) Nucleic acid dyes/stains may also be used to determine the amount of DNA attached to beads (or unbound in solution). Walsh, M.K., et al. 2001. *J Biochem Biophys Methods*, 47: 221-231 describes use of Molecular Probe's OliGreen for DNA quantitation (suitable for ssDNA and oligonucleotides). (*Thanks to Kathy Turner for our answer.*)

CML Binding

Q : 1) When coupling to a -COOH surface in a two step carbodiimide (EDAC) reaction (where step 1 = EDAC activation of beads in MES pH 4.5 and step 2 = Protein coupling in Carb/bicarb pH 9.6, with excess protein), is there really a need to block the unreacted sites with ethanolamine? 2) Can a similar two step EDAC reaction scheme be used with the encapsulated magnetic particles? I have a protocol that shows diethanolamine at pH 10.5. Is diethanolamine pH 10.5 preferred? 3) What is the surfactant used with the -COOH beads? Will this interfere with the coupling reaction? Is pre-washing recommended? I know, there are really seven questions, not a couple, as I bunched a few together. But, I figured if I worded it 1-7, then by the time you got to #7, you might have inadvertently hit the "delete" button. Thanks for your help. (*Kathy, please answer this one too.*)



A : 1) Some protocols call for blocking of unreacted sites; others omit it since the reactive intermediary will hydrolyze back to COOH anyway. You may wish to determine empirically the need for quenching (e.g., the impact on nonspecific binding). 2) A two-step protocol may be used for magnetic particles. The reactive group, particle size and MW of biomolecule will be more important than the composition of the base particle when deciding between one- and two-step protocols. For example, particle size will in part dictate the wash method that is utilized, and whether washes can be accomplished relatively quickly, before the reactive intermediary hydrolyzes. 3) Our TechNote 205 has a more typical (but generic) COOH bead/EDAC/MES buffer procedure. You might have better luck this way (with some optimization), as the optimal pH for an EDAC reaction is considerably lower, i.e. 4.5-7.5. Also, an amine buffer (ethanolamine) might interfere with the reaction between bead/COOH and ligand/NH₂. 4) The surfactant utilized for the synthesis of COOH-functionalized microspheres may vary by lot. As this is something that is generally held as a trade secret by manufacturers, we can't advise with certainty on the specific surfactant utilized, or its concentration. 5) We highly recommend the 'pre-washing' of microspheres. If use of surfactant is indicated (i.e., if beads are aggregating or clinging to pipette tips and tubes), we generally recommend using the lowest possible concentration of a non-ionic surfactant such as Tween® 20 (0.01%).

Mom, Make the Streptavidin Let Go!

Q : OK, I bound SA to your beads and it really grabs the biotinylated thingamabob that I wanted to bind, but enough already! I want to recover my Biotin-Thing conjugate. Make the SA let go! It's hanging on like a 3-year old kid! (*Another hot one for Kathy!*)

A : I am glad to learn that you got good binding. Also, I located a reference that cited use of biotin to competitively detach biotinylated molecules - from modified avidin. [Morag, E., E.A. Bayer, M. Wilchek. 1996. Reversibility of biotin-binding by selective modification of tyrosine in avidin. *Biochem J*, 316 (Pt 1): 193-199. PubMed ID: 8645205] Also, the prebinding of biotin before binding of biotinylated ligand has been cited much more extensively, i.e., it is the basis of several competitive assays. This may help control the level of loading when using streptavidin-coated beads. (*Kathy's real-life experience with a 3 year old helps here.*)

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

❖ *If I inject a bunch of these dyed beads into mice, will I still be able to excite them at 488nm?* (Researcher, CDC) If you inject them with a bunch of beads, I think the mice will get pretty excited!

**"If you can stay calm, while all around you is chaos...
then you may not have understood the seriousness of the situation."** – Anonymous

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.
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.

Ask us for help to find... cleaning equipment, big beads, slides, membranes for strip tests, etc. (Or see the "Hot Links" page at our website.)

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.