

Painless Particles[®]

Quarterly Global Newsletter
Volume 17, #1, March, 2004



A DIVISION OF POLYSCIENCES, INC.

B E A D S • A B O V E T H E R E S T[™]

● In This Issue

| | |
|---|---|
| NIST-Traceable Fluorescence | |
| Quantitation | 1 |
| <i>The Latex Course[™] 2004</i> | 1 |
| <i>"On the Road Again!"</i> | 1 |
| Flow By The Numbers | 1 |
| BioMag [®] Solutions | 2 |
| P(articles) ₂ = Particles Articles | 2 |
| Ask <i>"The Particle Doctor[®]"</i> | 3 |
| Mail Bonding (<i>"do the 'write' thing!"</i>) .. | 3 |
| Technical References & TechNotes | 4 |

"On the Road Again!"

Tradeshaw Schedule: Stop by and see us!

❖ AACR 95th Annual Meeting. March 27-31, 2004. Orlando, FL. Booth 839.
<http://www.aacr.org>

❖ ISAC XXII International Congress. May 22-27, 2004. Montpellier, France. Booth 15.
<http://www.isac-net.org>

❖ ASM 104th General Meeting. May 23-27, 2004. New Orleans, LA. Booth 1651.
<http://www.asm.org>

❖ AACC Clinical Lab Expo. July 27-29, 2004. Los Angeles, CA. Booth: to be assigned.
<http://www.aacc.org>

Flow By The Numbers

Practical Quantitative Flow Cytometry Workshop

This workshop is being held in conjunction with the ISAC XXII International Congress-**May 25, 2004** from 6:00 - 9:00p.m. in Sully 2 Room of the Le Corum Convention Center in **Montpellier, France**.

With emphasis on:

- performing Quantitative Flow Cytometry
- theory into practice
- specific examples
- protocols

Look for the link on our website (www.bangslabs.com).

NIST-Traceable Fluorescence Quantitation

Adoption of MESF Unit Allows for Accurate, Consistent Flow Cytometry Results

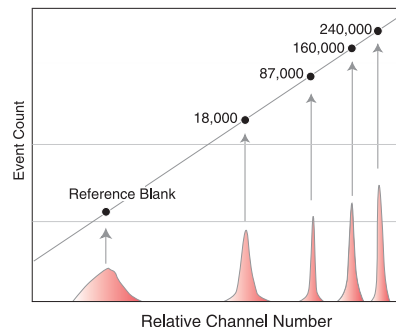
The National Institute of Standards and Technology (NIST) supports accurate and compatible measurements by certifying and providing over 1300 Standard Reference Materials with well-characterized properties. These materials are used to perform instrument calibrations, to verify the accuracy of specific measurements and to support the development of new measurement methods.

NIST recently adopted Bangs Laboratories' MESF (Molecules of Equivalent Soluble Fluorochrome) unit as the standard of fluorescence intensity. NIST assigned MESF values to FITC-labeled microspheres by comparison to a fluorescein reference solution, SRM 1932.

Bangs to offer NIST-Traceable Quantum[™] MESF Kits

An NTRM (NIST Traceable Reference Material) is a commercially-produced reference material with well-defined traceability to an existing NIST standard. Traceability is established via criteria and protocols defined by NIST. Commercial manufacturers of reference materials may affix the NTRM mark to materials produced in accordance with these criteria and protocols.

In the coming weeks, Bangs' **Quantum FITC MESF** kits will undergo NTRM certification. Look for the NTRM mark of NIST-traceability to ensure the highest degree of accuracy in quantitative flow cytometry.



SCHOOL IS IN SESSION

The Latex Course[™] 2004

"Designing Microsphere-Based Tests and Assays"

The Latex Course is a three day education extravaganza! Experts in various fields will share their knowledge with attendees through lecture and discussion. Topics will include: microsphere applications, assay development, and reagent manufacture. All attendees will also receive a copy of The Latex Course 2004 Book.

We are currently planning our next annual (almost) course for **September 26-29, 2004** at **Loews Coronado Bay Resort** - on beautiful Coronado Island (across the bay from San Diego, CA). Please mark your calendars, and budget for your travel and educational expenses.

As more details about speakers and pricing become available, they will be posted on our website. Please also look forward to the June issue of Painless Particles and a Latex Course brochure for updates. We hope to see you there!

BioMag® Solutions for Applications in Molecular Biology

Bangs Laboratories has a variety of BioMag products that can help you with your applications in molecular biology, including nuclease-free streptavidin and oligo (dT)₂₀ particles, and mRNA purification kits. BioMag are fully encapsulated in a functionalized silane coating, so you need not worry about exposed iron oxide interfering with reactions or causing nonspecific binding. BioMag's tremendous surface area (and our specialized linker technology for BioMag oligo dT) enable us to achieve high binding capacities, and will enable you to achieve high capture efficiencies.

BioMag can help you to:

Purify mRNA from total RNA

BioMag oligo (dT)₂₀ particles and the BioMag mRNA purification kit offer a convenient method for the efficient isolation of mRNA from total RNA.

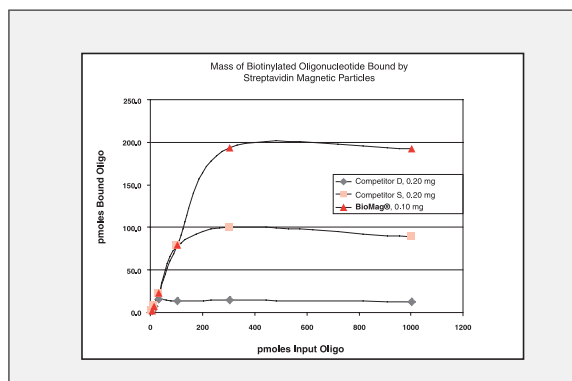
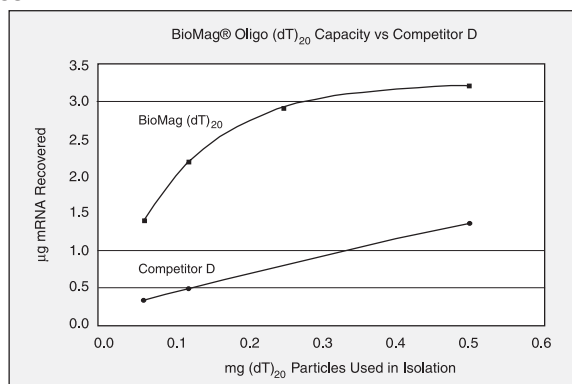
Capture or immobilize biotinylated DNA or RNA

If you're looking for a simplified method to immobilize a specific nucleic acid sequence, or you want to capture biotinylated PCR products or constructs, try our nuclease-free streptavidin BioMag.

Perform particle-based PCR

You may also thermocycle away with our nuclease-free streptavidin BioMag! Our studies demonstrate that BioMag particles do not inhibit PCR reactions.

- | | |
|--------------------|-----------------|
| ❖ High-performance | ❖ Encapsulated |
| ❖ Convenient | ❖ Nuclease-free |
| ❖ Cost-effective | ❖ PCR-Safe |



Order these products by Catalog Codes: BM568 BioMag Streptavidin, Nuclease-free; BM529 BioMag Oligo (dT)₂₀; BM569 BioMag mRNA Purification Kit. If we don't have an ideal product for your application, our **immobilization starter kits** will allow you to coat BioMag particles with a sequence of choice. Please contact us or visit our website, www.bangslabs.com, for **Technical Data Sheets**, and full product availability, including **magnetic separators**.

P(articles)₂ = Particles Articles

❖ mRNA Purification for Northern Blot Analysis

Kameya S, et. al. (2002) *Mfrp*, a gene encoding a frizzled related protein, is mutated in the mouse retinal degeneration 6. *Human Mol Ger*; 11(16):1879-86. Poly(A)⁺ RNA was purified from total RNA using **BioMag® Oligo (dT)** particles.

❖ Affinity Capture of Duplex DNA

Bukanov NO, et. al. (1998). PD-loop: A complex of duplex DNA with an oligonucleotide. *PNAS*; 95:5516-20. **BioMag® Streptavidin** particles were used to capture biotinylated plasmid DNA.



← **Whether a biotechnologist or a chemist, we are always happy to assist!**
Please contact us or visit us online for more information. **BEADS • ABOVE THE REST™**
(Cartoon reprinted with special permission from Sidney Harris <SHarris777@aol.com> and www.sciencecartoonsplus.com)

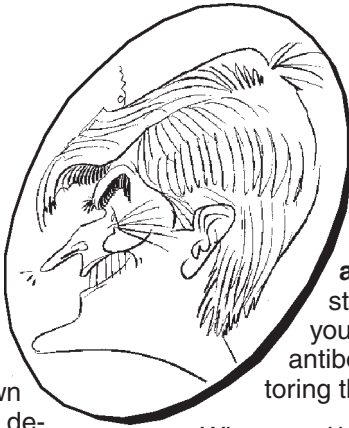
Ask "The Particle Doctor®":

Q: I need to quantify the number of white cells in a sample or a study I am doing. My study is currently qualitative only, but I need to ensure that the changes I am seeing are not just relative but quantitatively different.

I thought that using beads and adding a known concentration to my sample would allow me to determine the absolute number of white cells that I have. Basically I want to take a blood sample, add the beads, lyse the Red blood cells, and then use the flow cytometer to quantify the WBC concentration.

Could you recommend beads that I could use for this process. The less expensive the better.

A: We have just the product for you. It is called the **Flow Cytometry Absolute Count Standard™, Cat# 580**. The beads are roughly 7-8 micron in size, and exhibit broad-spectrum fluorescence. Just as you mentioned, beads are added to your cells prior to acquiring them on the cytometer, and, using a simple equation provided in the product insert, allow you to calculate the concentration and absolute number of cells in the sample.



A: QC of fluorescently-conjugated antibodies may be performed with our **Simply Cellular®** microspheres. The single population of beads has an **anti-mouse- (Cat # 810)** or **anti-human- (Cat # 812) IgG** surface. When stained, the beads will bind a known number of your fluorescently-conjugated mouse or human IgG antibodies. QC of the antibody is as simple as monitoring the fluorescence intensity of the stained beads.

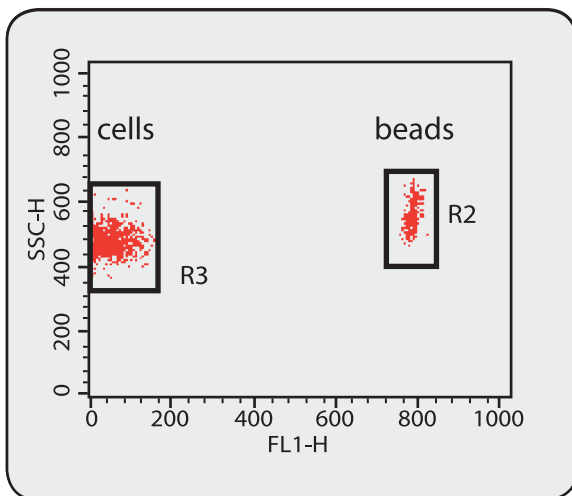
When used in conjunction with one of our **Quantum™ MESF** kits, the **Simply Cellular** beads also allow you to determine the effective fluorochrome/protein (F/P) ratio of your antibody.

Q: I would be interested in using biotin-coated beads. Do you have them available?

A: We certainly do! We offer **BioMag® biotin-coated** superparamagnetic particles as a standard product. We also have **custom coating services**, and would be pleased to coat a base bead selected from our many polymer, magnetic, fluorescent, or dyed microsphere product lines.

Q: I would like to bind a peptide to microspheres. What type of microsphere do you recommend?

A: For peptides and other small molecules, you may wish to employ the use of a spacing molecule to ameliorate steric effects, or a crosslinker to target a specific residue and optimally orient the molecule. Crosslinking agents are available with a variety of reactive groups for use with **functionalized microspheres** (covalent coupling), or with a biotin molecule for affinity binding to **streptavidin-coated microspheres**. Depending on reactive groups that are present on the peptide, you may wish to first modify the microspheres (to avoid peptide crosslinking). If a homobifunctional linker is used (like glutaraldehyde), you will want to use it in excess to prevent crosslinking or "hairpin" binding.



Q: I perform standard WBC immunophenotyping, and use your fluorescent beads as controls. I'm worried that differences in my conjugated antibodies either lot-to-lot or over time may be contributing variability to my results. Is there any way to QC my antibodies using the bead controls?

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

❖ "I was excited about the products you have, and especially I was pleased with the very helpful advice from the technical support staff." (AS, AL)

❖ "I am amazed how well you guys respond." (FLJ, Norway)

❖ "Thanks to technical service emails I was able to order exactly what I needed!" (AG, MA)

❖ "I have been extremely impressed overall." (DF, PA)

Please remember BLI for... **BEADS • ABOVE THE REST.™**

"I think that a particle must have a separate reality independent of the measurements. That is an electron has spin, location and so forth even when it is not being measured. I like to think that the moon is there even if I am not looking at it." - Albert Einstein

Technical References – See our website (www.bangslabs.com) for “downloadable” TechNotes, papers and BioMag® Technical Data Sheets, or ask for copies by mail or fax. We continually update and add newest TechNotes and Data Sheets to our website.

Product-Specific TechNotes:

- 101. ProActive® Microspheres** – Handling tips + protocols for streptavidin, Protein A, and goat anti-mouse coated microspheres.
- 102. Magnetic Microparticles** – Characteristics, handling tips and applications for superparamagnetic particles. **NEW**
- 103. Fluorescent/Dyed Microspheres** – Applications, fluorescence spectra, & 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, 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 60 nm 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 proteins 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; misc. coupling ideas, vendor info., and refs.
- 206. Useful 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.

NEWEST TECHNICAL INFO AVAILABLE ON WEBSITE:

We now have more than 45 Technical Data Sheets available in support of our more than 50 new **BioMag®** products. Feel free to browse!!

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.deviceink.com/ivdt/archive/95/03/009.html and [.../95/04/006.html](http://www.deviceink.com/ivdt/archive/95/04/006.html)).
- 403. New Developments in Particle-Based Immunoassays: Introduction** – 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 (1998). (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 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.

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.

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