

Painless Particles®

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B E A D S ● A B O V E T H E R E S T™

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MESF Product Spotlight

Fluorescence Intensity: MESF Units and Quantitative Bead Standards

The MESF unit (Molecules of Equivalent Soluble Fluorochrome) is widely accepted among researchers as the benchmark against which fluorescence intensity measurements are made. With several quantitative bead standards available, it is important to consider the following when choosing a standard:

Is this a spectral-matching standard?

In order to make quantitative fluorescence determinations independent of the instrument, it is necessary to use standards that match both the excitation and emission characteristics of your samples (known as "spectral-matching"). Some kits utilize dyes that "approximate" the spectra of stained samples, while others use combinations of dyes in order to cover a broad range of emission wavelengths. The best method, however, is to use standards incorporating the actual fluorochromes that are used to label your samples. Our patented QuantumPlex™ MESF beads are labeled with actual FITC, R-PE, or PE-Cy5 fluorochromes in order to provide you with a spectral-matching standard.

Is this standard environmentally responsive?

The fluorescence intensity of a fluorochrome is often affected by its surrounding conditions. The intensity of FITC, for example, will vary in solutions of different pH values. A fluorochrome with the ability to change fluorescence intensity in response to environmental changes is said to be "environmentally responsive". When performing quantitative fluorescence determinations, it is paramount that the standards be environmentally responsive. (Imaging trying to quantitate fluorescence using standards that maintain a constant intensity in conditions that cause your samples to alter theirs!) Quantum™ MESF kits are comprised on surface-labeled beads (not internally dyed). Surface-labeling leaves the fluorochromes free to interact with their surroundings, just as your samples will.

By asking these two questions, you'll be sure to select the most accurate and reliable fluorescent intensity standards available. For more information on QuantumPlex™ MESF kits, see page Flow-4 of the Product Selection Guide, or visit www.bangslabs.com.

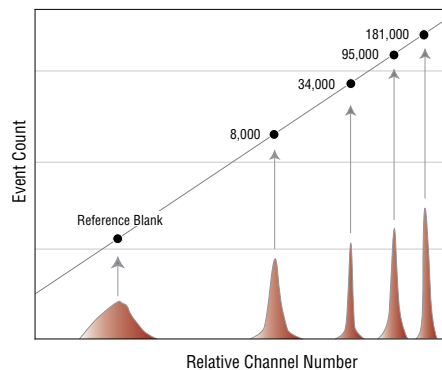


**NEW ISO
9001:2000
Certification**
Sept. 2002

BLI Celebrates 15 Yrs on April Fool's Day

(Big Fanfare, with cymbals, please!) On April 1st, 1988, we opened our doors. It seemed like a good day to start a new business. It has been a fun and sometimes scary ride, with exciting moments, like that one day >10 years ago when we shipped one order which doubled our sales for that year. In the past 15 years, we have grown from 2 of us to more than 20 employees, and our sales have grown many-fold. We have many happy memories to sustain us, and much experience, which we hope helps us to serve you better. We always try to remember that you are the reason we stay in business. If we aren't serving you, then we cannot prosper or even continue to exist. Many thanks for 15 happy years from all of us at BLI!

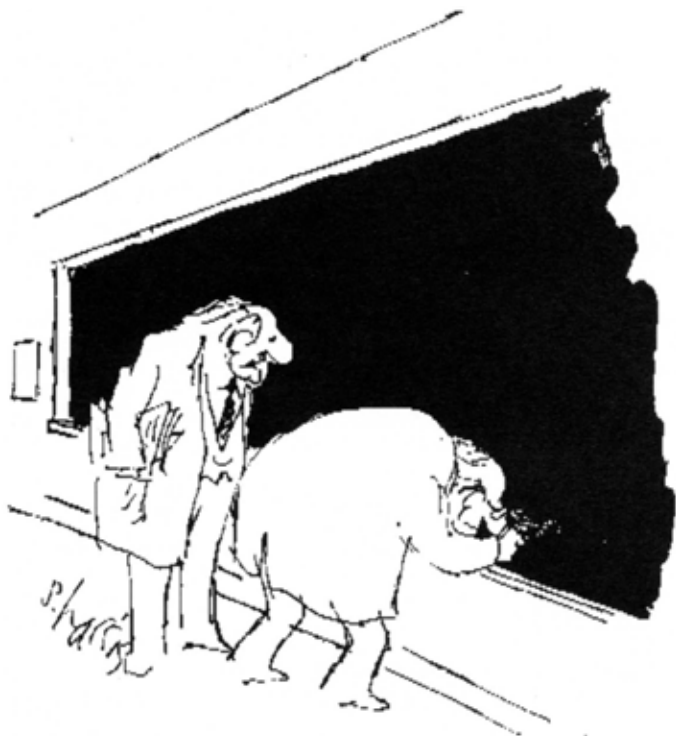
Quantum™ MESF Kits each consist of 5 bead populations – 1 blank population and 4 populations labeled with different amounts of the fluorochrome, and calibrated to MESF (Molecules of Equivalent Soluble Fluorochrome) units.



P(articles)₂ = Particle Articles

❖ Electroactive Microspheres or "E-beads"

Wang J, Polsky R, Merkoci A, Turner KL. "Electroactive beads for ultrasensitive DNA detection," *Langmuir*, **19**, #4, 989-91 (2003). Electrical transduction of DNA hybridization events is a major challenge in genelectronics. A new strategy for amplifying electrical DNA sensing, based on the use of microsphere tags loaded internally with a redox marker, yielded "electroactive beads" capable of carrying a huge number of the ferrocenecarboxyaldehyde marker molecules, and hence offering a remarkable amplification of single hybridization events. This allows chronopotentiometric detection of target DNA down to 5.1×10^{-21} mol (~31,000 molecules) after 20 min. of hybridization and "release" of the marker in an organic medium. The dramatic signal amplification advantage is combined with a remarkable discrimination against a huge excess (10^7 X) of noncomplementary nucleic acids. Such electroactive beads (microspheres loaded with different redox markers) hold great promise for multitarget detection and for enhancing the sensitivity of other bioassays. (Patent pending).



Sure, we're dealing with tiny particles, but your formula is just a *symbolic* representation.

❖ Flow Cytometry, Blood Platelets, and Beads

Hagberg IA, Lyberg T, "Blood platelet activation evaluated by flow cytometry: optimised methods for clinical studies," *Platelets*, **7**, 137-150 (2000). Quantum™ MESF kits were used to quantitate P-selectin expression of activated platelets in whole blood. QC Windows™ was utilized to ensure uniform daily instrument set-up.



On the Road Again

BLI's Trade Show Schedule: Stop by and see us!

- ❖ **AACR 94th Annual Meeting**, April 6-9, 2003. Toronto, Ontario. Booth 907. <http://www.aacr.org/2003am/2003am.asp>
- ❖ **ASM 103rd General Meeting**, May 18-21, 2003, Washington, DC. Booth 2037. <http://www.asmtg.org/mtgs/mtgs.htm>
- ❖ **AACC Clinical Lab Expo**, July 22-24, 2003. Philadelphia, PA. Booth # forthcoming. <http://www.aacc.org/2003am/>

"I'm confused. What Time Is It in Indiana?"

Well, that depends on where you are in the state. Most of Indiana observes Eastern Standard Time (EST) year-round. Since we do not observe daylight savings time, we never change our clocks. Therefore, in the Winter, we are on NY time and in the Summer, we are on Chicago time.

Blue Light Specials Now Include Silica Beads!

(We call them 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."



If You Need Help "by the book", just *Buy the Book!* **The Latex Course™ 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.)

Please ask if you need any help with the fine print in any "symbolic representation" – like the Microsphere Selection Guide to our "tiny particles." 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"®

Correlation between Flow Cytometers?

Q : We have flow cytometers in labs at several different facilities. Is there a way to correlate the sample values between the different instruments?

A : Yes! You can use **Quantum™ MESF** standards to establish standard curves relating channel values to fluorescence intensity on each instrument. By determining the intensity of your unknowns in **MESF (Molecules of Equivalent Soluble Fluorochrome)** units, you will be able to compare the results of samples run on different machines.

MESF Correlation to Antibodies on a Cell

Q : How do MESF units relate to the number of antibodies binding to a cell?

A : There is a direct relationship between the MESF value of a cell and the number of antibodies bound to it. The MESF value, however, does not equal the number of bound antibodies. An antibody may have any number of fluorescent molecules (fluorochromes) conjugated to it. The number of fluorochromes per antibody is known as the MESF value of the antibody, or more commonly, the "effective fluorochrome to protein ratio" or simply the "F/P ratio." The F/P ratio of an antibody can usually be obtained from the antibody manufacturer. Alternatively, the F/P ratio may be determined by using the antibody to label a cell or laboratory standard known to bind a specific number of antibodies, and then comparing the MESF value of the labeled sample to its binding capacity. Once the F/P ratio is obtained, it is easy to determine the number of antibodies bound to the cell: simply divide the MESF number by the F/P ratio.

Stability of [Strept]avidin/Biotin Binding for PCR Thermocycling

Q : Will streptavidin/biotin binding be stable enough to survive PCR thermocycling?

A : *Short answer:* Several customers have reported that our SA beads work well for PCR work.

Very long answer: Here are some references from my exhausting (but not exhaustive) literature search regarding stability of [strept]avidin and the [strept]avidin/biotin complex:

1. Bayer, E.A., *et al.* 1996. Sodium dodecyl sulfate-polyacrylamide gel electrophoretic method for assessing the quaternary state and comparative thermostability of avidin and streptavidin. *Electrophoresis*, **17**(8):1319-1324. PubMed: 8874057. On heating "...in the absence of biotin, the quaternary structure of streptavidin is more stable than that of avidin."
2. Wang, C., *et al.* 1996. Influence of the carbohydrate moiety on the stability of glycoproteins. *Biochemistry*, **35**(23):7299-7307. PubMed: 8652506. Apparently, deglycosylated avidin (SuperAvidin, NeutrAvidin) is less stable than avidin, the carbohydrate moieties conferring greater stability to the protein. If unbound streptavidin is more heat stable



- than avidin, then bound streptavidin should be more stable than bound deglycosylated avidins, although the next reference describes somewhat different findings.
3. Gonzalez, M., *et al.* 1997. Interaction of biotin with streptavidin. Thermostability and conformational changes upon binding. *J Biol Chem*, **272**(17):11288-11294. PubMed: 9111033. Also, Gonzalez, M., *et al.* 1999. Extremely high thermal stability of streptavidin and avidin upon biotin binding. *Biomol Eng*, **16**(1-4):67-72. PubMed:10796986. Biotin binding increases the midpoint temperature of thermal denaturation of streptavidin from 75°C (unbound) to 112°C at full saturation (4 biotin: 1 streptavidin); and for avidin, from 83°C (unbound) to 117°C at full saturation, i.e., that in both scenarios (unbound and saturated), avidin possesses greater thermal stability. Again, however, deglycosylated avidin is expected to be less thermally stable than native avidin.
 4. We expect that 1-2 binding sites are available for binding on each molecule of (strept)avidin (assuming that at least two sites are inaccessible due to immobilization of the molecule on the bead). So, biotin-bound immobilized (strept)avidin (1-2X biotin) should have stability intermediate between that of unbound (0X) and saturated (4X) (strept)avidin. One might speculate as to whether immobilization will confer further stability to the (strept)avidin molecule (we know that many enzymes are more stable once immobilized).
 5. As mentioned before, we have had clients successfully use our streptavidin-coated microspheres during thermocycling. We are not aware of any studies that cite use of deglycosylated avidin-coated beads during PCR thermocycling. Nor do we know of any studies that evaluated the activity of streptavidin, avidin or modified avidin molecules after denaturation and subsequent "renaturation," i.e., studies that investigated "folding errors" during renaturation, or the specific effects of different denaturation procedures.
 6. Reznik, *et al.* 1996. Streptavidins with intersubunit crosslinks have enhanced stability. *Nat Biotechnol*, **14**(8):1007-1011. PubMed: 9631041.
 7. "Avidin-Biotin" in Pierce Catalog and Handbook, Pierce Chemical Company.

Please note: We do recommend that beads be washed prior to use, as some preservatives and stabilizers can inhibit PCR.



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

- ❖ "Thanks so much for the concise, informative, and authoritative response to my question! It simply amazes me to get this level of technical support." (AC, NYC) We appreciate your comments.
- ❖ "Thanks. I just received my copy of "Painless Particles" and the Selection Guide. I'm beginning a new project with microspheres and your technical references will give me a huge leap up the learning curve that I'm facing. How can I get some?" (DZ, Seattle). We're happy to offer a boost. You can download references fast from our website.

"No pessimist ever discovered the secrets of the stars, or sailed to an uncharted land, or opened a new heaven to the human spirit." – Helen Keller

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.

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. Priority will be given to requests accompanied by chocolate chip cookies. *Since the last issue, we have received many tech support requests, but have yet to receive a single chocolate chip. Given the present economy, we are willing to temporarily relax our standards: we will now accept peanut-butter cookies and pecan sandies, which tech support requests. (Oh, the sacrifices we make for our customers!)**

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.