Painless Particles®

Global Newsletter
Volume 18, #3, Fall 2005

Bangs Laboratories, Inc.
A DIVISION OF POLYSCIENCES, INC.

BEADS ● ABOVE THE REST™

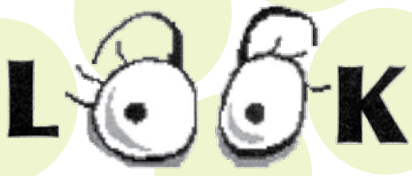
In This Issue

ATTENTION

QuickCal® v. 2.1 Users

QuickCal v. 2.1 diskettes have been discontinued. Please note that our current version, QuickCal v. 2.3, is available online for FREE! Please visit our website for further information about its latest capabilities.

Remember our NIST Traceable Particle Size Standards for your calibration needs!



... at what we're planning!!

The Latex Course™ 2006

More details soon to follow.

PolyLink
Protein Coupling Kit

Making it Even Easier to Use Our Beads!

The PolyLink Protein Coupling Kit is used for covalent attachment of proteins to carboxyl (COOH) modified microspheres. The kit contents are sufficient for 50 coupling reactions using 1µm+ polymer (or magnetic) microspheres and 200-500µg of protein per reaction.

Water Soluble Chemistry

When the microsphere carboxyl groups are activated with water-soluble carbodiimide, they become highly reactive toward primary amines on the protein of interest. A step-by-step procedure is provided in our Product Data Sheet 644, *PolyLink Protein Coupling Kit for COOH Microscopes*, (which may be downloaded from our website). Everything you need has been combined into one easy-to-use kit; catalog code **PL01N**.

Kit Components:

PolyLink Coupling Buffer
PolyLink Wash/Storage Buffer
PolyLink EDAC (Carbodiimide)

So there you have it... Painless Particles – just like we said.

The Value of a Great Copycat
The Benefit of Quantum™ MESF

On the school playground, copycats can be really annoying. They repeat everything that you say or do. This sort of copycat can be a bad thing. However, in the world of cytometry, a really good copycat cell can mean the difference between OK data and great data. True quantitative cytometry requires the comparison of fluorescently labeled cells to fluorescently labeled standards, with the standards being assigned numeric values with some meaningful unit of measure.

Molecules of Equivalent Soluble Fluorochrome (MESF) is the unit of measurement accepted by the National Institute of Standards and Technology (NIST).

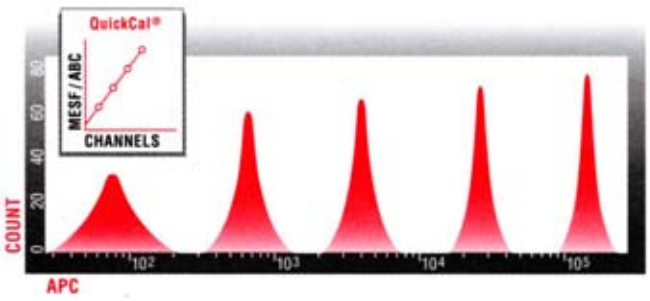
[continued on page 2]

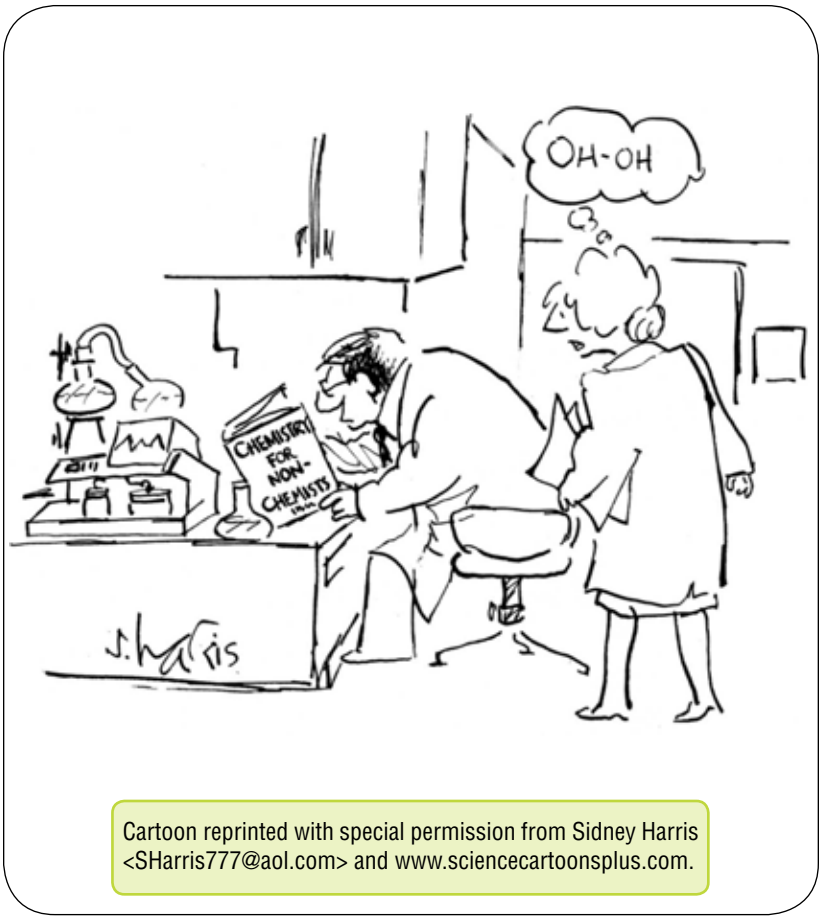
Quantum™ MESF APC

*A*pplause, *P*raise, and *C*ompliments

Allophycocyanine, or APC, is the newest member of our Quantum™ MESF line of products. We've even gone so far as to add the **FACSDiva** scale to our free quantitative QuickCal® v. 2.3 options, available on our website.

The Quantum APC MESF kit is available in 20- 100-, and 280-test quantities under Catalog Code **823.**





Cartoon reprinted with special permission from Sidney Harris <SHarris777@aol.com> and www.sciencecartoonsplus.com.

[The Values of a Great Copycat, continued]

At first, labeled cells seem like easy things to copy. And they would be, if they would just look the same all the time. The trouble is, that fluorophores often respond differently in response to changes in the environment. The simplest example is the response of fluorescein to pH (Figure 1).

In order to be a truly great copycat, the labeled beads standard must copy the cell in all environments. The only way to do this is to attach the fluorophore to the surface of the bead. In fact, MESF units of fluorescence measurement are only applicable to environmentally responsive standards.

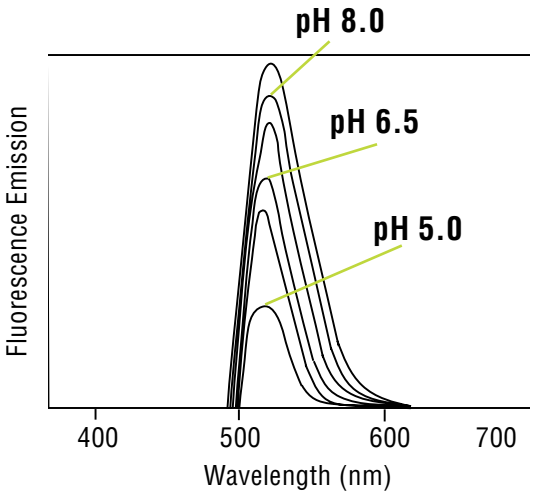
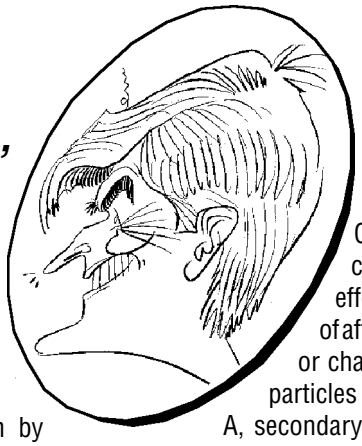


Figure 1: pH-dependent intensity of Fluorescein

Bangs Labs' Quantum™ MESF products are the best cell copycats around, and are available with FITC, PE, PE-Cy5, and APC surfaces. For more information on the use of fluorescence standards for quantitative fluorescence measurement, see the Product Selection Guide (insert), and the Flow Cytometry section of our website.

Ask *"The Particle Doctor®"*



Q: I want to purify my target cells from a fairly nasty sample matrix. Ultra-high purity isn't necessary, but I want to capture as much target as possible. Which type of magnetic bead should I use?

A: Magnetic particle selection is often driven by practical matters, such as availability of an off-the-shelf product for the intended separation. In these instances, further consideration may be given to characteristics of the base particle (such as size, surface area, density, composition) for tailored handling, binding capacity, etc.

Our uniform **COMPEL™** microspheres are well suited for the development of flow cytometric and other bead-based assays. The low density of the polymer matrix permits binding kinetics that approach those of solution- based systems. The polymer matrix is also amenable to dyeing, and the high surface charge allows binding of large amounts of ligand.

BioMag® microparticles are ideal for isolation of cell fractions or purification of target from complex samples. Their tremendous surface area and greater density allow rapid and highly efficient capture of the target species. For this specific application, BioMag is our recommendation.

BioMag® and **BioMag®Plus** are ~1.5µm high-performance superparamagnetic microparticles widely used for the efficient separation of cells and purification of biomolecules. Their irregular shape provides a much greater surface area than similarly-sized spherical particles, resulting in high binding capacities and efficient capture of target with conservative use of particles. The high iron oxide content allows for rapid and efficient magnetic separations, even from difficult, e.g. highly viscous, samples. BioMagPlus particles undergo additional processing for removal of fines.

Composition:	Silanized iron oxide
Morphology:	Irregular/Cluster
Surface groups:	COOH and NH_2 available
Density (g/cm^3):	>2.5
Iron oxide content (%):	>90
Magnetization (emu/g):	25-35
Surface area (m^2/g):	>100
Particles / g:	~$1x10^8$

Once you've determined which type of bead to use, consideration moves to the surface that will be most effective. Often, separation is performed using some sort of affinity system, for example antibody/antigen interaction or charge mediated purification. Protein coated magnetic particles are available off-the-shelf, with streptavidin, protein A, secondary antibody, primary antibody, oligo (dT)20, or anti-CD marker surfaces. Functionalized particles are used in situations requiring the attachment of less common ligands.

Mail Bonding

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

I appreciate your diligence of customer service. I would like to pass along that for my applications, Bangs Labs supplies a product that outperforms any others I've tried. I would attribute this to your attention to detail and cleanliness of the spheres. J.P.J., MD

P(articles)$_2$ = Particle Articles

Cool Article Citing the Use of Microspheres

Essential to consistent flow cytometry results on a single instrument, or consistency across instruments and locations, is an established instrument standardization and daily quality control program. Used daily, Bangs' **QC3™** and **QC Windows®** products establish consistent instrument detector settings and a common window of analysis.

First, the QC Windows product is run (containing two beads; the first a Certified Blank, the second a QC3 bead with your fluorophores of choice attached to the surface). With compensation turned off, PMT voltages and amplifier settings for the fluorescence detectors are set to achieve predetermined target channels.

Then, with compensation turned on, the instrument-specific target channels for the QC3 beads are set and form the basis for the daily quality control program. Additional details on both instrument set-up and quantitative fluorescence calibration can be found in **Purvis N, Stelzer G. (1998) Multi-platform, multi-site instrumentation and reagent standardization. Cytometry; 33:156-165.**

Bangs Laboratories, Inc.
9025 Technology Dr. • Fishers, IN 46038-2886
Address Service Requested

"Every strike brings me closer to the next home run." – Babe Ruth

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.

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).
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 Jenski, *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 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.