Painless Particles®

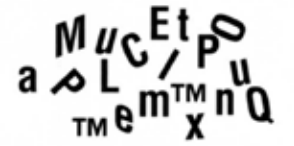
Global Newsletter
Volume 17, #3, Fall 2004

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

BEADS ● ABOVE THE REST™

In This Issue

We're beginning to develop a COMPLEX...



Admittedly, it was only a matter of time.... COMPEL™ uniform superparamagnetic microspheres meet the QuantumPlex™ platform for multianalyte analysis. Things will never be the same.....

The Latex Course™ 2004 Course Book

You missed the course? Don't fret! You can purchase the course book with all the information the attendees received.

The book is currently available for $395 USD + shipping. Please visit our website for more details, or contact us to reserve your copy.

BioMag® Solutions

BioMag ProMax Albumin Removal Kit

Changes that occur in serum and plasma proteins have long been recognized as a way to investigate and monitor physiological changes. This rich source of information does, however, present challenges for most of the analytical methods used. One of the reasons for this is that one-dimensional and two-dimensional electrophoresis, high performance liquid chromatography, and mass spectroscopy have a limited dynamic range for the amount of protein mass that can be loaded and resolved. In addition, greater than 50% of the protein in serum is represented by albumin. The presence of this and other highly abundant proteins lowers the detection threshold for the protein of interest.

The **BioMag ProMax Albumin Removal Kit** (Catalog Code **BP658**) is based on patented BioMag superparamagnetic particle technology, and this provides a rapid and simple protocol for serum albumin removal. The magnetically-responsive BioMag ProMax Albumin Removal Particles supplied in the kit, in combination with specific buffer conditions, allow the binding and release of less abundant proteins in serum, while minimizing the binding of albumin.

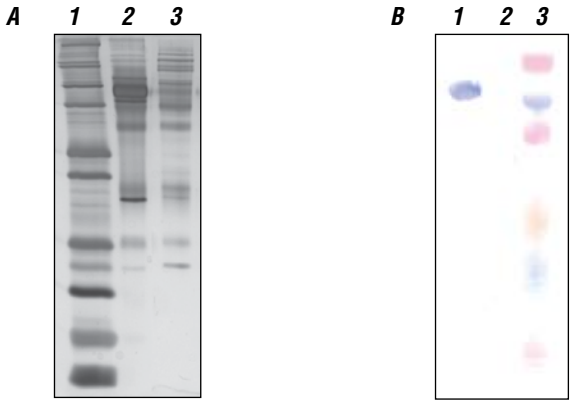


Figure 1. SDS-PAGE analysis and Western Blot showing enrichment of lower abundance proteins and depletion of albumin.

Panel A shows a silver stained SDS-PAGE gel. Lane 1, Molecular Weight Markers; Lane 2, untreated normal human serum; Lane 3, serum treated with BioMag® ProMax Albumin Removal Particles. Both Lanes 2 and 3 were loaded with the same amount of protein.

Panel B shows the depletion of albumin by Western Blot. Lane 1, normal serum; Lane 2, serum treated with the BioMag® ProMax Albumin Removal Kit; Lane 3, Molecular Weight Markers. Mouse anti-albumin was the primary antibody and the signal was visualized using an anti-mouse horseradish peroxidase conjugate and TMB as the chromagen. Both Lanes 1 and 2 were loaded with equal amounts of protein. Lane 2 shows that nearly all of the albumin has been depleted from the sample.

Bangs Labs' Custom Services

In addition to our catalog products, Bangs Laboratories offers a range of custom services to address the unique needs of our customers. Our capabilities include:

Coating
- Proteins
- Spacers / linkers
- Other molecules

Dyeing
- Surface-immobilized or internal fluorophore
- Visible colors
- Targeted or multiple intensities

OEM Production and Packaging
- Production to your specifications
- Bottling and labeling
- Package inserts

Other Services
- Dilutions
- Drying

Synthesis
- Polystyrene and methylmethacrylate polymers
- Functionalized and crosslinked polymers
- Silica

We welcome inquiries regarding these and other services. Please contact us to learn how we may be of assistance in formulating solutions to meet your specific requirements.

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

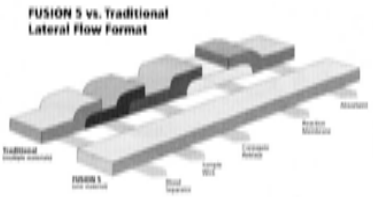
Boulders-in-the-Stream
Lateral Flow Test Format

Boulders-in-the-Stream is a technique that has been established as a useful tool to improve lateral flow tests. In this format, large particles are dried on a hydrophilic membrane and serve to capture the smaller dyed mobile phase.

There are 3 areas where this approach offers significant advantages:

1) There is a greater surface area for capture reagent immobilization on latex beads than seen on a fast wicking membrane.
2) The analyte and capture reagent are small when compared to the size of the pore. Filling the pores with beads increases the available surface area, and therefore the amount of capture reagent that can be bound.
3) With membranes, the ability for a protein to bind is size-dependent, and proteins <40k MW are very difficult to bind to nitrocellulose. Latex beads allow covalent linkage of these small proteins.

The **FUSION 5™ membrane** was developed specifically for use as a matrix for the Boulders-in-the-Stream lateral test format. The material is hydrophilic and predominantly non-protein binding. FUSION 5 can function as a complete lateral flow strip, allowing a lateral flow test to be built on a single material for the first time.



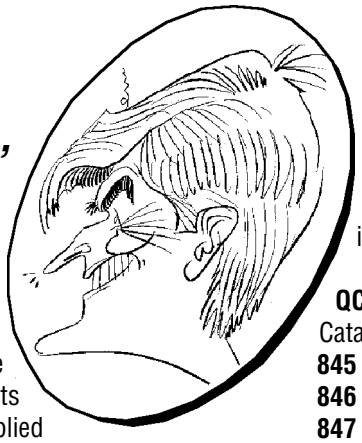
Undyed microspheres ~2.3-2.4µm (**PS05N** or **PC05N**) are suitable for use as the capture phase ("Boulders"), and smaller dyed particles, ~0.1-0.4µm (**DS02** or **DC02**) may be used for the mobile phase. For microsphere selection, see our online **Product Selection Guide**, or contact us at info@bangslabs.com. For further information regarding the **FUSION 5 membrane**, contact: diagnostics@whatman.com.

Boulders-in-the-Stream

--with apologies to Dolly Parton and Kenny Rogers (but we had to do it)

Boulders in the stream
That is what we are
No conjugate release pad in-between
How can we be wrong
Flow along with me
To the capture zone
And we rely on each other, ah-ah
From one bead to another, ah-ah

Ask *"The Particle Doctor®"*

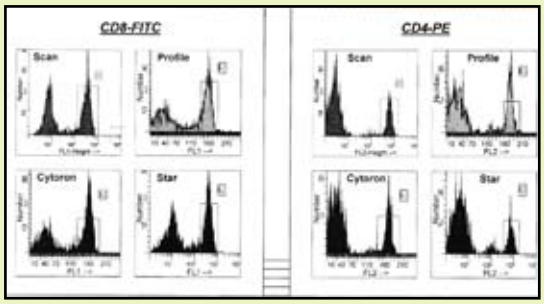


Q: What type of daily QC do you recommend for flow cytometers?

A: For typical QC needs, we recommend **QC Windows®**, which is the product that we use for daily set up and QC. The QC Windows kit consists of **QC3™** and **Certified Blank™** microbeads. It is supplied with "Initial Target Channels" that, when used in conjunction with labeled control cells, provide a unique approach to unified instrument setup and qualitative evaluation of instrument performance. QC Windows allows multiple users to establish a Common Window of Analysis with respect to the fluorescence intensity. Instruments that have been adjusted to a Common Window of Analysis produce histograms that are nearly identical. Such standardization may be critical when comparing the presence, absence or relative intensity of immunophenotyping cell-clusters in bone marrow or leukemia samples. It also allows data comparison independent of instrument make.

QC Windows is an essential part of a uniform set up protocol to establish a **Common Window of Analysis**. The QC3 standard has the same spectral properties and fluorescence intensity as the samples being analyzed. QC Windows establishes the position of the fluorescence intensity Window of Analysis before any samples are run. The Window positioning is accomplished by adjusting the PMT voltage (with the compensation turned off) such that the QC3 standards fall in predetermined Target Channels. After setting the Target Channels, the compensation may then be accurately adjusted using labeled control cells, as they represent the most accurate spectra of the sample cells. Re-running the QC3 standards at these settings (post-compensation) provides the Instrument-Specific Target Channels, which should be achieved daily if the instrument settings are not changed. The instrument noise may then be qualitatively assessed with the Certified Blank microbead standard, by comparing its position relative to the autofluorescence of non-labeled cells to ensure that noise does not interfere with the assay.

Figure 1: *The same sample of labeled blood was run after a Common Window of Analysis was established using QC Windows® on each instrument.*



QC Windows® may be ordered under the following Catalog Codes:

845 QC Windows® (FITC/PE)
846 QC Windows® (FITC/PE/PE-TR)
847 QC Windows® (FITC/PE/PE-Cy™5)
848 QC Windows® (FITC/PE, Cy™5/APC)

Q: I conduct many different types of cell separations. Do you offer any products that support this application?

A: Funny you should ask...! We were just talking about the variety of BioMag® kits and particles for human and mouse cell separations that we offer. (Well, as far as the boss knows, that's what we were talking about....) A list of our anti-leukocyte BioMag particles is provided below. We also offer BioMag T cell enrichment systems and a range of secondary antibody and other affinity coatings to capture cells that have been antibody-labeled.

Human		Mouse
anti-CD2	anti-CD16	anti-CD4
anti-CD3	anti-CD19	anti-CD8
anti-CD4	anti-CD34	anti-CD45R
anti-CD8	anti-CD45	
anti-CD11b	anti-CD56	
anti-CD14	anti-CD71	

Mail Bonding

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

- *I may say that Bangs Labs is, with a big difference, the most helpful company I have found. R.P., Australis*
- *I really appreciate your generosity in providing the excellent flow cytometry CD8 free of charge. J.W., Canada*
- *Thank you very much for your prompt reply! I really appreciate the help and advice from you and your fellow workers. R.Y., CA*

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"It is the tension between creativity and skepticism that has produced the stunning and unexpected findings of science." – Carl Sagan

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 steptavidin, 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.