News | Views | Insights *from...*

Polysciences, Inc.
Chemistry beyond the ordinary

PolyFacts
Microspheres / Particles

Vol. 3 | No. 1

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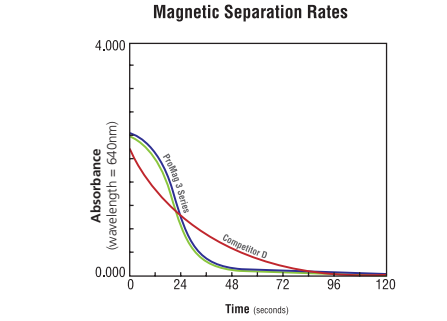
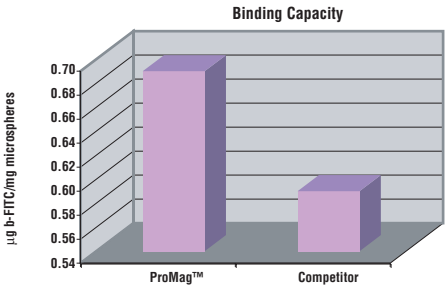
SuperparaMAGnificent!

Streptavidin-coated ProMag™

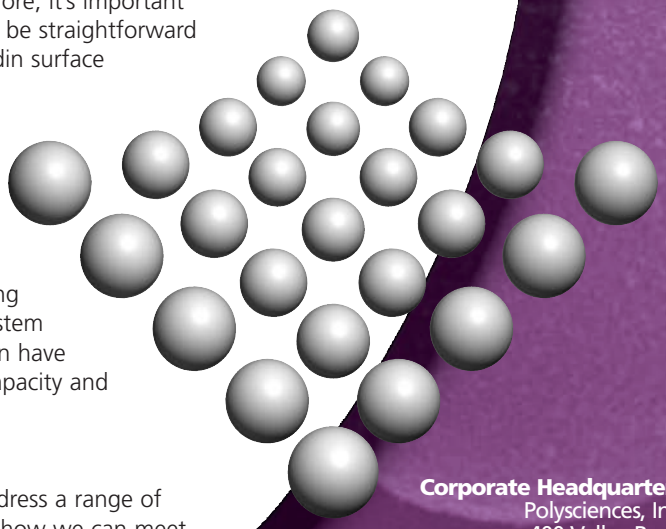
Superparamagnetic particles are used extensively for the capture and manipulation of biomolecular targets in diagnostic and research applications. They confer a number of benefits, including ease of separation and suitability for automation.





As with other solid phases, the successful use of magnetic microspheres is reliant on the quality of the ligand coating. The coating must be stable, and exhibit high specific binding and minimal nonspecific binding. Furthermore, it's important that the coating process itself be straightforward and reproducible. A streptavidin surface addresses this need.

ProMag™ Streptavidin offers the best of both worlds - uniform and rapid magnetic separations and the easy, reproducible and stable binding that the streptavidin/biotin system affords. ProMag™ Streptavidin have demonstrated high coating capacity and low nonspecific binding.



Our superparamagnetic microparticle product lines allow us to uniquely address a range of applications in the life sciences, and we welcome customer calls to discuss how we can meet your specific requirements.

Cat. No.	Description
86056	**ProMag™ Series 3 • Streptavidin**

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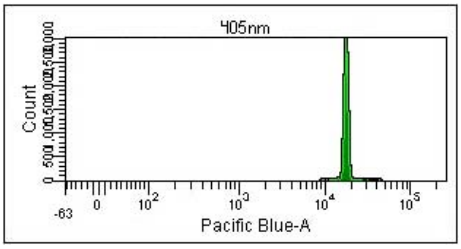
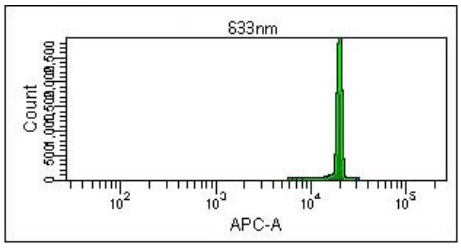
Full Spectrum™

A Multi-Color Fluorescent Reference Standard

The **Full Spectrum™ Multi-Color Fluorescence Reference Standard** is a highly uniform microbead product that fluoresces over the visible spectrum. Full Spectrum™ offers a convenient way to perform initial daily QC or instrument set-up on the flow cytometer.

As part of a QC program, these fluorophore-labeled microspheres offer a convenient means to check general instrument status and monitor stability over time. When beads are run, the channel values for pertinent fluorescent detectors are recorded, and data is monitored to identify outliers and trends. This type of check can alert the user to problems with the optical and fluidic systems (e.g. diminishing laser power or obstruction/ leakage) and the effect of environmental factors, such as temperature, humidity, and vibration on instrument performance.





As a tool for instrument set-up, a reference peak may be positioned for each detector to achieve initial settings.

A single test requires one drop (50µl) of particle suspension, which is equivalent to ~100,000 particles. Full Spectrum™ particles are available in three sizes: 1ml, 5ml, and 14ml. Call for more details!

Excitation (nm): 355, 405, 408, 633
Emission: Full Spectrum
Diameter: ~7-9µm

Cat. No.	Description
BLI885	**Full Spectrum™ Multi-Color Fluorescent Reference Standard**

Quantum™ MESF

Ensuring Consistent Results Each and Every Time

Fluorescence cytometry is an important tool for investigations in cell and molecular biology. This technology is routinely used for immunophenotyping and for an expansive array of research applications, such as the study of protein phosphorylation and the determination of telomere length.

Although fluorescence cytometry has proven to be a very powerful and versatile technology, it is not without limitations. Notably, without a standardized measure of fluorescence intensity, results of analyses can only be described in relative terms. The interpretation of fluorescence intensity measurements can be further complicated by factors such as daily instrument variation and differences in hardware (laser power, filter sets), PMT settings, software, environmental factors and fluorochrome labeling density of antibodies.

Our **Quantum™ MESF** microspheres provide the means to standardize fluorescence intensity measurements, thereby permitting truly quantitative analysis. Moreover, they are labeled with the actual fluorochromes used to label cells, for synchronous response to the environment (consider the pH-responsive fluorescence intensity of fluorescein, see Figure 1). The beads are run on the same day and at the same settings as samples to establish a calibration curve relating instrument channel values and standardized fluorescence intensity units. Unknowns may then be read against the curve for determination of expression (i.e. quantitation of the signal from each cell population).

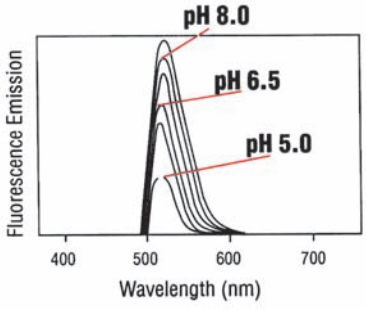


Figure 1: *pH-responsive fluorescence intensity of fluorescein (FITC).*

For a complete listing of our Quantum™ MESF products, please visit our website at www.polysciences.com.

Particle Perplexities

Questions & Answers pertaining to Polysciences' Microspheres / Particles

Q: I plan to bind IgG to protein A beads. How stable is the attachment? Do I need to covalently crosslink the antibody to the coating on the beads?

A: The affinity of protein A for IgG varies by antibody species and subclass (see Technical Data Sheet 554). As an affinity interaction, it may be susceptible to competitive binding (displacement of the intended antibody), and the beads should be used in an environment that is otherwise antibody-free. Consideration should be given to the intended shelf-life and other stability requirements of the application. For example, for quantitative assays or long-term stability, crosslinking is advisable. If the beads simply need to isolate a target (non-quantitative) and a lengthy shelf-life isn't required, then crosslinking may not be so important.

Q: We have purchased **BioMag®** and **ProMag™** carboxylic acid modified beads. In the protocols, it always says to use BSA in the buffers. However, we only want to attach small molecules (such as toxins) to the beads, not proteins. Why is BSA needed, and do we require it despite the fact that we are not using protein?

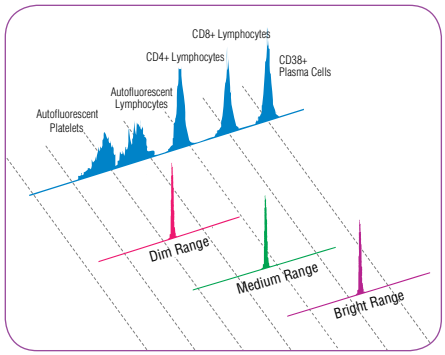
A: Coating protocols generally involve a series of steps, which may include:

- Coupling of the specific biomolecule
- Quenching (discharging unreacted surface groups)
- Blocking (adsorption of a blocking molecule, such as BSA, in an attempt to cover any bare patches on the surface of the microsphere)
- Suspension in a storage buffer that contains blocker and possibly surfactant (some surfactant molecules are also expected to adsorb to the beads)

Use of blockers and surfactants are intended to produce a well-coated bead that will have good handling and low nonspecific binding. You can certainly omit steps or substitute reagents (e.g. use a different irrelevant protein or surfactant) to tailor the coating for your specific application. In your case, another strategy might be to couple the small molecule toxin to a carrier protein (e.g. BSA), which will then serve to coat and block the microsphere surface.

For a complete listing of our magnetic particle products, please visit our website at www.polysciences.com.

Q: We require **Quantum™ FITC MESF** calibration beads for the quantitation of FITC fluorescence intensity in MESF units. It seems three types of Quantum™ FITC beads (low, medium, and high) are available. I am confused in choosing the right one for my calibration work. Can you help?



A: The different levels of kits are intended to span the intensity range of common cellular analyses. Low level kits are commonly used for low expression levels, or for small cells that will be dimmer due to their size. Examples include telomere length determination and some cell surface markers (e.g. CD34). Medium level kits are used for many types of analyses and nicely span the range of typical cell samples. Common analyses include those for many surface markers, including CD4 / CD8. High level kits are often used for cells with very high expression or high autofluorescence, e.g. analysis of tumor cells.

If you're still wondering which kit is best suited for your assay, you might start with the mid-level kit, which overlaps areas of the low and high kits.

Cat. No.	Description
BLI824	**Quantum™ FITC MESF (low level)**
BLI824p	**Quantum™ FITC MESF (low level) premixed**
BLI826	**Quantum™ FITC MESF (medium level)**
BLI826p	**Quantum™ FITC MESF (medium level) premixed**
BLI825	**Quantum™ FITC MESF (high level)**
BLI825p	**Quantum™ FITC MESF (high level) premixed**

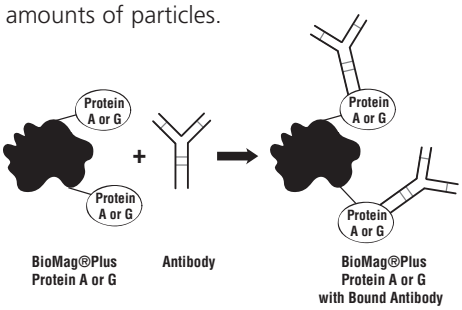
Q: I would like to bind peptide to microspheres. What type of microspheres do you recommend?

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 the reactive groups 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.

Binding with BioMag®

BioMag®Plus Protein A and Protein G

BioMag®Plus superparamagnetic particles are utilized in the magnetic separation of cells, organelles, proteins, immunoglobulins, nucleic acids and many other types of molecules in biological and non-biological systems. The irregular shape of the BioMag®Plus particle allows for a much greater surface area, 20 to 30 times that of the same size spherical particle. This large surface area results in high binding capacities, allowing for efficient target capture with minimal amounts of particles.



Protein A and protein G are commonly used to purify, immobilize, or detect immunoglobulins. Protein A is a polypeptide that is a normal constituent of the cell wall of *S. Aureus* and was discovered when researchers noticed that one fraction, isolated during the purification of the individual components of the cell wall, contained a protein that would bind to rabbit and human antibodies. Although protein A has four binding sites, only two of these can be used at any one time. It is known that there are at least two protein A binding sites on any antibody and that these are located in the Fc region of the antibody. Because Fc-directed binding is desired in order to maximize the antibody's biological activity, protein A pre-conjugated to a solid support, like our microspheres, has become an important reagent in many immunochemical applications.

Protein G is an immunoglobulin-binding protein expressed in group C and G *Streptococcal* bacteria and is much like protein A, but with differing specificities. It has a higher affinity for IgG than protein A and is useful as an IgG binding reagent to bind all human, mouse, rat and horse IgG subclasses, as well as bovine and sheep IgG. Protein G has been genetically engineered so as to not bind human IgA and IgM and does not bind serum albumin.

Polysciences, Inc. offers **BioMag®Plus Protein A** and **Protein G Antibody Isolation Kits** for the isolation of antibodies from serum and cell culture supernatants. The contents of the kit are sufficient for five coupling reactions. To use the kits for smaller or larger samples, simply adjust all volumes in a proportional manner.

For more information on these kits, as well as the relative degree of binding various antibodies using both protein A and protein G, please consult our Technical Data Sheet 620 or visit our website at **www.polysciences.com**.

Cat. No.	Description
86041	**BioMag®Plus Protein A Particles**
86040	**BioMag®Plus Protein A Particle Antibody Isolation Kit**
86051	**BioMag®Plus Protein G Particles**
86050	**BioMag®Plus Protein G Particle Antibody Isolation Kit**

Catalog Release

The new 2008 - 2009 Catalog is now available!

Polysciences, Inc. is proud to announce the release of our new **2008-2009 Catalog**. With more than 3000 products and over 200 new additions, this catalog is something to behold! In its pages, you will find detailed information on Biosciences, Monomers and Polymers, Microspheres and Particles, and Electronics Applications. All of our products are covered in the new catalog, including a wide range of products for:

- Cell Separation
- Lab Reagents
- Flow Cytometry
- Molecular Diagnostics
- Agglutination Tests / Assays
- Fluorescence Microscopy

To request your free copy of our new catalog, please contact our Customer Service Department at (800) 523-2575 or visit our website at www.polysciences.com today.