News | Views | Insights *from...*

PolyFacts
Microspheres / Particles

Vol. 2 | No. 2

Polysciences, Inc.
Extraordinary chemistry. Superior beads.

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Working on Assay Development? See Page 3...

Clean It Up!

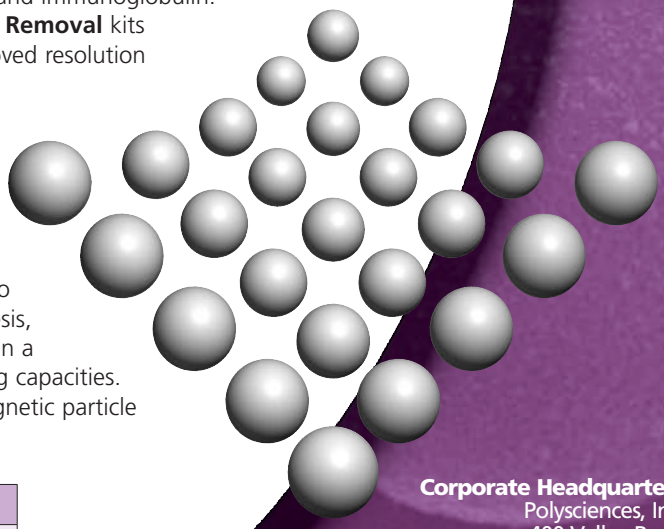
BioMag® particles to the rescue!

Optimized sample preparation is vital to the successful recovery and analysis of cellular and soluble proteins. Purification strategies involve a succession of techniques, from cell lysis through target recovery and analysis. Step-wise purifications of the lysate or serum sample may be performed to reduce the presence of components that can mask less abundant constituents, and to enrich specific binding classes of proteins or peptides.

Products based on patented BioMag® superparamagnetic particle technology have been developed for the reduction of highly abundant proteins, such as albumin and immunoglobulin. **BioMag® ProMax Albumin Removal** and **BioMag® ProMax Serum IgG Removal** kits provide rapid and simple protocols for removal of these proteins and improved resolution of less abundant targets.

Lectin-coated substrates may be used to enrich specific saccharide-binding factions. Selections of glycosylated targets may be accomplished using our surfactant-free **BioMag®Plus Wheat Germ Agglutinin (WGA)** or **BioMag®Plus Concanavalin A (Con A)** particles.

BioMag® magnetic particle technology can complement other techniques to prepare samples for downstream protein analyses, such as gel electrophoresis, western blotting and mass spectrometry. BioMag®'s irregular shape results in a greater surface area than its spherical counterparts resulting in high binding capacities. Just think - efficient target capture with minimal amounts of particles! Magnetic particle separations are rapid, easy and permit highly efficient recoveries.



Cat. No.	Description
24351	**BioMag® ProMax Albumin Removal Kit**
24352	**BioMag® ProMax Serum IgG Removal Kit**
86054	**BioMag®Plus Wheat Germ Agglutinin**
86057	**BioMag®Plus Concanavalin A**

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A New BioMag®Plus?

Introducing BioMag®Plus Wheat Germ Agglutinin (WGA)

Bead and column-based separation methods rely heavily on the speed and ease of affinity binding systems. Ligands such as streptavidin, antibodies and lectins are used both to capture specifically-tagged targets and for the isolation of cells and biomolecules that naturally express the ligand binding partner.

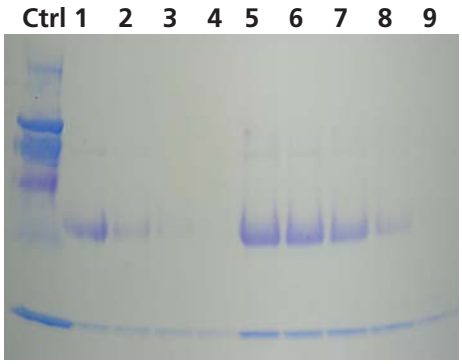
The unique saccharide-binding properties of plant lectins, such as wheat germ agglutinin (WGA), have made them useful for the study of glycosylated proteins. Lectins have been used in cell adhesion studies, to effect lymphocyte activation and to explore carbohydrate-based therapeutics.

WGA is a binder of gram-positive bacteria, via GlcNAc moieties in the peptidoglycan layer of the cell wall. WGA also interacts with saccharides with a terminal GlcNAc, chitobiose or sialic acid residue. WGA does not contain protein-bound carbohydrate, and is not blood group specific.

Our new WGA-coated BioMag®Plus microparticles provide a convenient means for isolating N-acetylglucosamine-containing glycoproteins from cell lysate or to explore other lectin/glycan-mediated processes. The BioMag®Plus magnetic particle format provides high surface area, and permits easy and efficient separations.

Cat. No.	Description
86054	**BioMag®Plus Wheat Germ Agglutinin (WGA)**

BioMag®Plus Wheat Germ Agglutinin

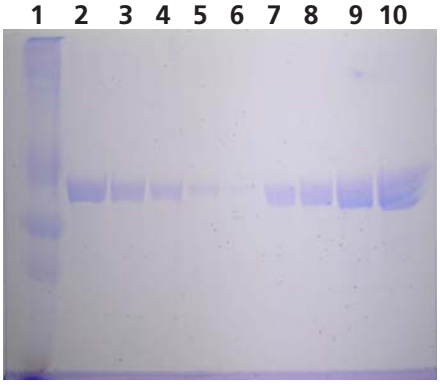


4-20% Tris Glycerine SDS-PAGE electrophoresis gels displaying staining of eluted tryspin inhibitor (Lanes 5, 6, 7, 8 and 9) using different volumes of BioMag®Plus WGA particles (1ml, 0.75ml, 0.5ml, 0.25ml and 0.1ml). Lanes numbered 1, 2, 3 and 4 are titrated tryspin inhibitor control samples.

And Another?

Introducing BioMag®Plus Concanavalin A (Con A)

BioMag®Plus Concanavalin A



Binding and elution of apo-transferrin using BioMag®Plus Con A magnetic particles. Sample eluates (lanes 2-6) from 1ml, 0.5ml, 0.25ml, 0.125ml and 0.05ml of BioMag®Plus Con A particles, respectively, are compared with aliquots of 0.3 mg/ml apo-transferrin stock (lanes 7-10) from 5µl, 10µl, 20µl and 30µL.

Concanavalin A (Con A) is a protein derived from the jackbean. Like other lectins, it has characteristic saccharide-binding properties.

Con A is one of the most widely used and well characterized lectins because it recognizes α-linked mannose, a commonly occurring sugar structure. Since many serum and membrane glycoproteins have a structure that includes α-linked mannose residues, Con A can be utilized to examine or purify a wide range of glycoproteins.

Our new BioMag®Plus Con A microparticles provide a convenient means for isolation of mannosyl and glucosyl-containing glycoproteins and polysaccharides from serum or cell lysate, or for investigating other lectin/glycan-mediated processes. For example, Con A agglutinates red blood cells (RBCs), interacts with immunoglobulin glycopeptides and is a lymphocyte mitogen. It also binds some bacteria.

Rapid and easy separations of our BioMag®Plus Con A particles may be conducted using one of our rare-earth (Nd-Fe-B) magnetic separation units.

Cat. No.	Description
86057	**BioMag®Plus Concanavalin A (Con A)**

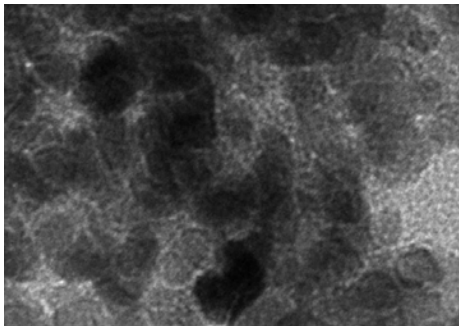
For a complete list of our rare-earth magnets, please visit our website at www.polysciences.com.

Particle Perplexities

Questions & Answers pertaining to Polysciences' Microspheres / Particles

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: **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 ~1.6µm 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.

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

Q: How do I coat your beads with lectins, such as wheat germ agglutinin (WGA)?

A: For the immobilization of WGA, a number of strategies may be utilized. For one common method see: **Ertl B., F. Heigl, M. Wirth, F. Gabor.** 2000. Lectin-mediated bioadhesion: preparation, stability and caco-2 binding of wheat germ agglutinin-functionalized Poly(D,L-lactic-co-glycolic acid)-microspheres. *J Drug Target,* 8(3): 173-184. They used the "carbodiimide/N-hydroxysuccinimide method," which is a fairly standard method for coupling ligands to COOH-functionalized microspheres. In any case, lectins will have COOH and NH_2 termini that could be utilized for immobilization.

WGA also possesses numerous cysteine residues, which could be utilized for the formation of disulfide bonds, i.e., a bead could be modified using a hetero-bifunctional crosslinker that will react with amines (bead) and sulfhydryls (WGA). EDAC is a zero-length crosslinker for joining COOH and NH_2 groups; glutaraldehyde is a homobifunctional crosslinker utilized for binding NH_2 groups. Please visit our website, www.polysciences.com, for glutaraldehyde and other reagents such as formamide, nylon wool or ethanol.

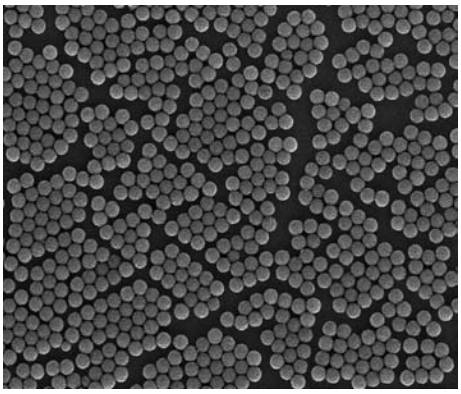
Adsorption of WGA to non-functionalized microspheres might also be considered. Yet another strategy would be to immobilize the lectin through affinity binding. For example, biotinylated WGA (which is commercially available) may be bound to streptavidin-coated spheres.

While you may insist on coating the beads yourself, you could also try our newest **BioMag®Plus** product, **Wheat Germ Agglutinin** beads. Ready to go, we've done the work for you - at least this step.

Cat. No.	Description
86054	**BioMag®Plus Wheat Germ Agglutinin (WGA)**

Q: I'm planning the development of a magnetic immunoassay, but I've never worked with particles before. Where do I begin?

A: You might consider our new **ProMag™** microspheres for development of the bead conjugate. ProMag™'s high surface functionality permits thorough antibody coating, and their uniform size and rapid separation times allow the development of miniaturized assays that are amenable to automation.



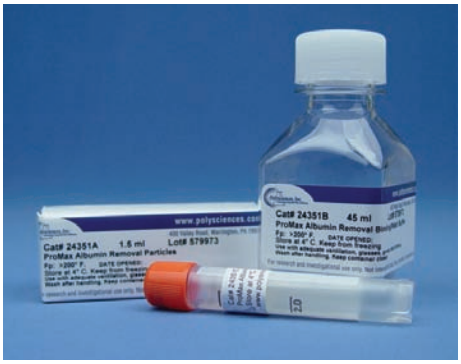
To simplify coating, ProMag™ may be used in conjunction with our **PolyLink Protein Coupling Kit**. The PolyLink kit is supplied with a detailed protocol providing step-by-step instructions for coupling the protein of choice to the spheres, and for determining coupling efficiency.

Cat. No.	Description
86055	**ProMag™ 3 Series • COOH Surfactant-Free**
86056	**ProMag™ 3 Series • Streptavidin**
24350	**PolyLink Protein Coupling Kit**
24818	**PolyLink Kit with Hollow Fiber Filtering System**

Protein Enrichment

Quick...simple...and proven. It's BioMag® ProMax.

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 spectrometry have limited dynamic range for the amount of protein mass that can be loaded and resolved. This limited dynamic range affects the resolution of less abundant proteins. Albumin can represent 50-70% total protein in serum and IgG can represent 10-20% total protein in serum, masking the ability to detect less abundant proteins of interest. If the majority of these two proteins can be removed from serum samples, a significant improvement in resolution of less abundant proteins can be obtained.



Our **BioMag® ProMax Albumin Removal Kit** enables the quick removal of human serum albumin from samples. Based on BioMag® superparamagnetic particle technology, the ProMax Albumin Removal Particles supplied in the kit, along with optimized buffers, allow for the selective binding and release of the less abundant proteins in serum. The protocol can be completed in 30 minutes or less, so researchers can move on to analyzing important biomarkers in the serum.

The **BioMag® ProMax Serum IgG Removal Kit** enables protein enrichment through serum IgG removal. The ProMax Serum IgG Removal Particles, in combination with specific buffer conditions, bind the IgG from the serum, enabling it to be removed. Using magnetically responsive particles for depletion of IgG has advantages over other systems. The ProMax Serum IgG Removal Kit is a rapid and simple procedure that requires no pretreatment of the sample. In addition, the ProMax system does not require the use of time consuming columns or centrifugation.

Each of these kits are scalable and can be used separately or in conjunction with each other.

Cat. No.	Description
24351	**BioMag® ProMax Albumin Removal Kit**
24352	**BioMag® ProMax Serum IgG Removal Kit**

Polybeads®

Now available in Orange and Green

As you know, Polysciences is the leading supplier of visibly dyed polystyrene microspheres. We have always offered a wide variety of colors including: blue, red, violet and yellow. Now, in order to serve you better, we have added two new colors to our standard line of Polybeads® - orange and green! Our orange beads are ~0.20µm in diameter, while the green beads are ~1.0µm. If you still don't see the color you are looking for, just ask. Other colors and intensities can be created just for you!

Cat. No.	Description
13369	**Polybead® Carboxylate Orange Dyed Microspheres**
13370	**Polybead® Carboxylate Green Dyed Microspheres**