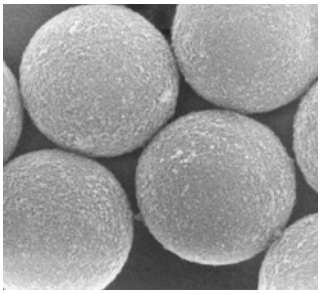
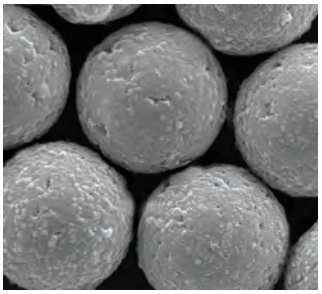
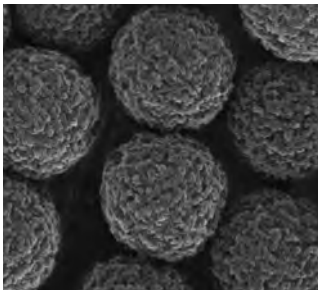
Bangs Laboratories, Inc.
BEADS ABOVE THE REST

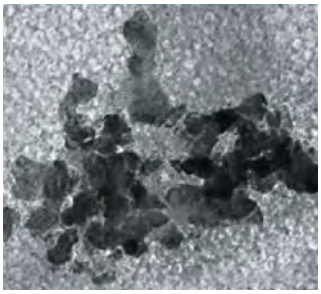
Magnetic Microspheres

TechNote 102


COMPEL™


ProMag® HP


ProMag®


BioMag™

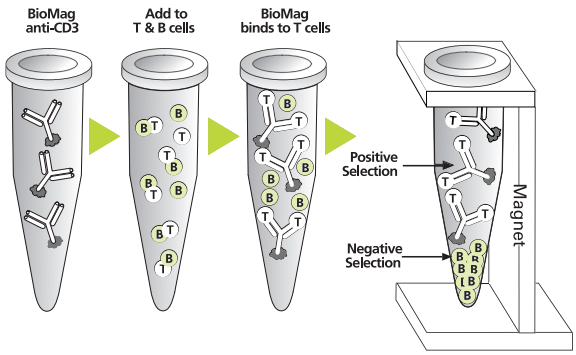
INTRODUCTION

Superparamagnetic particles have been utilized extensively in diagnostics and other research applications for the capture of biomolecules and cells. They confer a number of benefits, including ease of separation[33] and suitability for automation.[35] Highly efficient magnetic separations have also led to improvements in applications. PCR-related improvements include increased template amplification success, decreased inhibition, and improved recovery of product.[3, 12, 25] Gene detection and immunoassay have also seen increased sensitivity due to lowered nonspecific signal.[39, 40, 41]

As with other microspheres, magnetic particles may be coated with ligand for the capture of target in sample. Following incubation with sample, a magnet is applied for the separation of target-bound particles. Unwanted (unbound) sample constituents may then be efficiently washed away. Negative selections may also be performed for the isolation of 'untouched' cells.

As the particles are superparamagnetic, they are easily redispersed in buffer upon removal of the magnet. Successive washes may be simply and rapidly performed to ensure the removal of material that may be attached nonspecifically.

Figure 2: Positive and negative cell selection using BioMag® superparamagnetic particles.



PRODUCTS AND APPLICATIONS

Many assays and separations have been adapted to a magnetic particle format to take advantage of the benefits it confers. This is evidenced by the impressive array of magnetic particle applications that exist.

Table 1: Suggested Products for Various Applications

Application	Suggested Product
Cell separation (positive selection)[23, 27, 28]	BioMag® anti-CD (human and mouse) or secondary antibody particles
Cell separation (negative selection)[2, 5, 7, 10, 20, 37]	BioMag® enrichment systems (human) or secondary antibody particles
Subcellular organelle isolation[14, 44, 46]	BioMag®
Immunoprecipitation[6, 13, 32]	BioMag® or ProMag®
mRNA isolation[29, 30, 42]	BioMag® Oligo (dT)20 or mRNA isolation kit
Biotinylated nucleic acid capture or binding [8, 18, 24, 34, 38, 39, 40, 41]	BioMag® streptavidin or nuclease-free streptavidin, ProMag® streptavidin, or COMPEL™ streptavidin
Hybridization assays or separations [39, 40, 41]	ProMag® or COMPEL™
Immunoassays[1, 19, 21, 31]	ProMag®, ProMag® HP (chemiluminescence), COMPEL™, or BioMag®
Flow cytometric assays[9, 16, 26, 36]	COMPEL™ fluorescent or non-fluorescent, QuantumPlex™M
Biosensors[4, 17]	ProMag®, COMPEL™, or BioMag®
Biopanning[11, 22, 43]	ProMag®, COMPEL™, or BioMag®
SELEX[45]	ProMag® or ProMag® HP
Microfluidic chip, Lab-on-a-Chip[47,48]	ProMag® or ProMag® HP
Protein, glycoprotein isolation[49]	BioMag® or ProMag®

For research applications, magnetic particle selection is often driven by practical matters, i.e. selection of an "off-the-shelf" product that will accomplish the task at hand (e.g. anti-CD34 for cell separation or oligo(dT) for mRNA isolation). If an appropriate product isn't readily available, or if a new application or assay is being developed, investigators typically select a base particle for customized coating. In these instances, further consideration may be given to characteristics of the base particle (such as size, surface area, density and composition) for tailored handling, binding capacity, etc. A comparison of magnetic particle characteristics is provided in Table 2. See also our magnetic particle data sheet for images of these particle types.

Table 2: Comparison of ProMag®, ProMag® HP, COMPEL™ & BioMag® Particle Characteristics

Parameter	ProMag®	ProMag® HP	COMPEL™	BioMag®
Diameter (µm)	1µm and 3µm	3µm	3, 6 and 8µm	~1.5µm
Density (g/cm^3)	1.8 (1µm); 1.6 (3µm)	1.4	1.1-1.2*	2.5
Composition	functionalized polymer impregnated iron oxide	functionalized polymer impregnated iron oxide	functionalized polymer impregnated with iron oxide	silanized iron oxide
Shape	spherical	spherical	spherical	cluster
* depends upon diameter				

We encourage investigators to contact us with any questions regarding product selection or try our Magnetic Particle Sampler Pack.

COATING STRATEGIES

For investigators who require customized particle reactivity, our magnetic product lines support a number of coating strategies. Particles are available with surface functional groups for covalent coupling, and immobilization starter kits are available for those who are new to the world of microspheres or bioconjugation.

Particles coated with affinity binding proteins are available for simplified coating (or for isolation of target, e.g. streptavidin-coated particles for capture of biotinylated DNA).

Technical information and general coating protocols may be downloaded from our website (www.bangslabs.com). See *TechNote 205, Covalent Coupling*, and *TechNote 101, Affinity Ligand Microspheres*, in addition to our collection of Product Data Sheets. We also welcome inquiries about our custom coating services.

Table 3: Magnetic Particle Surfaces for Coating*

Functional Groups	Affinity Binding Proteins
COOH	Streptavidin
NH_2	Biotin
COOH immobilization kit	Protein A or G
NH_2 immobilization kit	Lectins
	Secondary antibodies: Goat anti-Mouse (IgG or IgM) , Goat anti-Rat (IgG or IgM), Goat anti-Human) (IgG or IgM)
* Contact us or go to bangslabs.com for specific availability and pricing.	

MAGNETIC SEPARATIONS

Magnetic particles are handled in much the same manner as other microspheres, with magnetic separation replacing traditional forms of separation (centrifugation, filtration). Separations are often performed using specially designed laboratory magnets, i.e. rare earth magnets embedded in a tube or microplate holder. Complete separation of the magnetic particles from the liquid generally occurs within seconds or minutes of placement on the magnet (depending upon bead concentration/volume of suspension). Particles should not be left on the magnet longer than required, as they will pack more tightly over time, potentially leading to aggregation. If aggregation occurs, standard methods for resolution may be followed (e.g. surfactant, sonication, pipetting, mixing - see also *TechNote 202, Microsphere Aggregation*).

Figure 3: BioMag® Multi-6 Microcentrifuge Tube Separator

Magnetic separators often pull particles to the wall of the vessel or well to allow for aspiration of the liquid and particle retention. Go to bangslabs.com for our range of magnetic particle separators. For technical information regarding magnetic particle separations, see Hatch and Stelter.[15]

STORAGE

Microsphere suspensions should NOT be frozen, as freezing is likely to cause irreversible aggregation. As with other types of microsphere suspensions, cold storage (2-8°C) is recommended to deter microbial growth. Most as-supplied 'standard' (non-protein coated) microsphere suspensions do not contain an antimicrobial agent. It is recommended that all suspensions be handled using aseptic technique.

If possible, continuous rolling (e.g. 3-5 rpm on a cell culture roller) is recommended to keep microspheres in suspension, without generating foam (foam may cause particle loss through bead entrapment). If continuous rolling is not possible, particles should be thoroughly resuspended before use. Our experience indicates that higher speed rolling (30-60 rpm for ~2-4 hours) is effective for the resuspension of settled material. Again, rolling speed is intended to effectively resuspend the beads without generation of foam.

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