Magnetic Microspheres

9025 Technology Dr. • Fishers, IN 46038-2886
800.387.0672 • 317.570.7020 • Fax 317.570.7034
info@bangslabs.com • www.bangslabs.com

Bangs Laboratories, Inc.

B E A D S ● A B O V E T H E R E S T ™

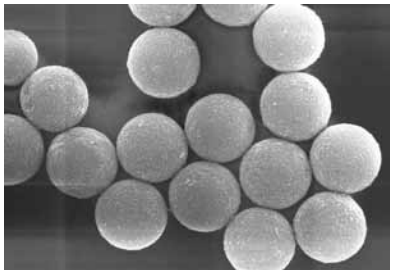

Figure 1: COMPEL™ 6µm magnetic spheres

CONTENTS

I. 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 recognition molecules 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.

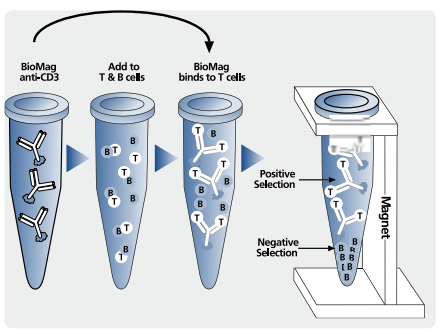

Figure 2: Positive and negative cell selection using BioMag® superparamagnetic particles.

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.

II. 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 presently 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]	BioMag®
Immunoprecipitation[6, 13, 32]	BioMag®
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™, 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®

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™, COMPEL™ and BioMag® Particle Characteristics

Parameter	ProMag™	COMPEL™	BioMag®
Diameter (µm)	1µm and 3µm	3, 6 and 8µm	~1.5µm
Density (g/cm^3)	1.33 (1µm); 1.22 (3µm)	1.1-1.2*	2.5
Composition	functionalized polymer impregnated with iron oxide	functionalized polymer impregnated with iron oxide	silanized iron oxide
Shape	spherical	spherical	cluster

** depends upon diameter*

We encourage investigators to contact us with any questions regarding product selection.

III. 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).

*Table 3: Magnetic Particle Surfaces for Coating**

Functional Groups	Affinity Binding Proteins
COOH	Streptavidin
NH_2	Biotin
	Protein A or G
COOH immobilization kit	Secondary antibodies
NH_2 immobilization kit	Goat anti-Mouse (IgG or IgM)
	Goat anti-Rat (IgG or IgM)
	Goat anti-Human (IgG or IgM)

* Contact us or see our website's Products & Ordering section for specific availability and pricing.

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

IV. 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*).

Magnetic separators often pull particles to the wall of the vessel or well to allow for aspiration of the liquid and particle retention. See the Products & Ordering section of our website for our range of magnetic particle separators. For technical information regarding magnetic particle separations, see Hatch and Stelter.[15]

Figure 3: BioMag® Multi-6 Microcentrifuge Tube Separator

V. STORAGE

Microsphere suspensions should NOT be frozen, as freezing is likely to cause irreversible aggregation. As with other types of microspheres, 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.

VI. REFERENCES

1. **Alefantis, T., P. Grewal, J. Ashton, A.S. Khan, J.J. Valdes, V.G Del Vecchio.** 2004. A rapid and sensitive magnetic bead-based immunoassay for the detection of staphylococcal entertoxin B for high-through put screening. *Mol Cell Probes*, 18: 379-382.
2. **Anderson, B.E., J. McNiff, J. Yan, H. Doyle, M. Mamula, M.J. Shlomchik, W.D. Shlomchik.** 2003. Memory CD4+ T cells do not induce graft-versus-host disease. *J Clin Invest*, 12: 101-108.
3. **Andreadis, J.D., L.A. Chrisey.** 2000. Use of immobilized PCR primers to generate covalently immobilized DNAs for in vitro transcription/ translation reactions. *Nucleic Acids Res*, 28(2): e5.
4. **Baselt, D.R., G.U. Lee, M. Natesan, S.W. Metzger, P.E. Sheeham, R.J. Colton.** 1998. A biosensor based on magnetoresistance technology. *Biosens Bioelectron*, 13: 731-739.
5. **Blander, J.M., I. Visintin, C.A. Janeway Jr., R. Medzhitov.** 1999. α(1,3)-fucosyltransferase VII and α(2,3)-sialyltransferase IV are up-regulated in activated CD4 T cells and maintained after their differentiation into Th1 and migration into inflammatory sites. J Immunology, 163: 3746-3752.
6. **Bolster, D.R., S.J. Crozier, S.R. Kimball, L.S. Jefferson.** 2002. AMP-activated protein kinase suppresses protein synthesis in rat skeletal muscle through down-regulated mammalian target of rapamycin (mTOR) signaling. *J Biol Chem,* 277(27): 23977-23980.
7. **Boonstra, A., C. Asselin-Paturel, M. Gilliet, C. Crain, G. Trinchieri, Y-J Liu, A. O'Garra.** 2003. Flexibility of mouse classical and plasmacytoid-derived dendritic cells in directing T helper type 1 and 2 cell development: Dependency on antigen dose and differential toll-like receptor ligation. *J Exp Med,* 197(1): 101-109.
8. **Bukanov, N.O., V.V. Demidov, P.E. Nielsen, M.D. Frank-Kamenetskii.** 1998. PD-loop: A complex of duplex DNA with an oligonucleotide. *PNAS*, 95: 5516-5520.
9. **de Jager, W., H. te Velthuis, B.J. Prakken, W. Kuis, G.T. Rijkers.** 2003. Simultaneous detection of 15 human cytokines in a single sample of stimulated peripheral blood mononuclear cells. *Clin Diag Lab Immunol*, 10(1): 133-139.
10. **Eylar, E.H., C.E. Lefranc, Y. Yamamura, I. Baez, S.L. Colon-Martinez, N. Rodriguez, T.B. Breithaupt.** 2001. HIV infection and aging: Enhanced Interferon- and Tumor Necrosis Factor-alpha production by the CD8+ CD28 T subset. *BMC Immunol,* 2(1): 10.
11. **Feldhaus, M.J., R.W. Siegel, L.K. Opresko, J.R. Coleman, J.M. Weaver Feldhaus, Y.A. Yeung, J.R. Cochran, P. Heinzelman, D. Colby, J. Swers, C. Graff, H.S. Wiley, K.D. Wittrup.** 2003. Flow-cytometric isolation of human antibodies from a nonimmune Saccharomyces cerevisiae surface display library. *Nat Biotechnol,* 21(2): 163-170.
12. **Flagstad, O., K. Roed, J.E. Stacy, K.S. Jakobsen.** 1999. Reliable noninvasive genotyping based on excremental PCR of nuclear DNA purified with a magnetic bead protocol. *Mol Ecol,* 8(5): 879-883.
13. **Fox, H.L., P.T. Pham, S.R. Kimball, L.S. Jefferson, C.J. Lynch.** 1998. Amino acid effects on translational repressor 4E-BP1 are mediated primarily by L-leucine in isolated adipcyotes. *Am J Physiol,* 275(Cell Physiol 44): C1232-1238.
14. **Hammond, C., L.K. Denzin, M. Pan, J.M. Griffith, H.J. Geuze, P. Cresswell.** 1998. The tetraspan protein CD82 is a resident of MHC class II compartments where is associates with HLA-DR, -DM, and -DO molecules. *J Immunol,* 161: 3282-3291.
15. **Hatch, G.P., R.E. Stelter.** 2001. Magnetic design considerations for devices and particles used for biological high gradient magnetic separation (HGMS) systems. *J Magn Magn Mater,* 225: 262-276.
16. **Keller, K.L., M.A. Iannone.** 2002. Multiplexed microsphere-based flow cytometric assays. *Exp Hematol,* 30: 1227-1237.
17. **Kim, G-H., A.G. Rand, S.V. Letcher.** 2003. Impedance characterization of a piezoelectric immunosensor part II: Salmonella typhimurium detection using magnetic enhancement. *Biosens Bioelectron,* 18:91-99.
18. **Kim, S., J.R. Edwards, L. Deng, W. Chung, J. Ju.** 2002. Solid phase capturable dideoxynucleotides for multiplex genotyping using mass spectrometry. *Nucleic Acids Res,* 30(16): e85.
19. **Lee, G.U., S. Metzger, M. Natesan, C. Yanavich, Y.F. Dufrene.** 2000. Implementation of force differentiation in the immunoassay. *Anal Biochem,* 287(2): 261-271.
20. **Mazurek, G.H., V. Reddy, D. Murphy, T. Ansari.** 1996. Detection of Mycobacterium tuberculosis in cerebrospinal fluid following immunomagnetic enrichment. *J Clin Microbiol,* 34(2): 450-453.
21. **McConnell, D.S., Q. Wang, P.M. Sluss, N. Bolf, R.H. Khoury, A.L. Schneyer, A.R. Midgely Jr., N.E. Reame, W.F. Crowley Jr., V. Padmanabhan.** 1998. A two-site chemiluminescent assay for activin-free follistatin reveals that most follastatin circulating in men and normal cycling women is in an activin-bound state. *J Clin Endocrinol Metab,* 83(3): 851-858.
22. **McConnell, S.J., T. Dihn, M.H. Le, D.G. Spinella.** 1999. Biopanning phage display libraries using magnetic beads vs. polystyrene plates. *BioTechniques*, 26(2): 208-210, 214.
23. **Moss, D.M., M.J. Arrowood.** 2001. Quantification of Cryptosporidium parvum oocytes in mouse fecal specimens using immunomagnetic particles and two-color flow cytometry. *J Parasitol,* 87(2): 406-412.
24. **Nanda, S.K., J.L. Leibowitz.** 2001. Mitochondrial aconitase binds to the 3' untranslated region of the mouse hepatitis virus genome. *J Virol,* 75(7): 3352-3362.
25. **Osaki, T., H. Taguchi, H. Yamaguchi, S. Kamiya.** 1998.Detection of Helicobacter pylori in fecal samples of gnotobiotic mice infected with H. pylori by an immunomagnetic-bead separation technique. *J Clin Microbiol* 36(1): 321-323.
26. **Park, M.K., D.E. Briles, M.H. Nahm.** 2000. A latex bead-based flow cytometric immunoassay capable of simultaneous typing of multiple pneumococcal serotypes (multibead assay). *Clin Diagn Lab Immunol,* 7(3): 486-489.
27. **Przyborski, S.A.** 2001. Isolation of human embryonal carcinoma stem cells by immunomagnetic sorting. *Stem Cells*, 19: 500-504.
28. **Pyle, B.H., S.C. Broadaway, G.A. McFeters.** 1999. Sensitive detection of Escherichia coli O157:H7 in food and water by immunomagnetic separation and solid-phase laser cytometry. *Appl Environ Microbiol,* 65(5): 1966-1972.
29. **Saito, A., G. Fujii, Y. Sato, M. Gotoh, M. Sakamoto, G. Toda, S. Hirohashi.** 2002. Detection of genes expressed in primary colon cancers by in situ hybridisation: Overexpression of RACK 1. *J Clin Pathol: Mol Pathol,* 55: 34-39.
30. **Sangawa, H., T. Himeda, H. Shibata, T. Higuti.** 1997. Gene expression of subunit c(P1), subunit c(P2), and oligomycin sensitivity-conferring protein may play a key role in biogenesis of H+-ATP synthase in various rat tissues. *J Biol Chem*, 272(9): 6034-6037.
31. **Schweitzer, B., S. Wilshire, J. Lambert, S. O'Malley, K. Kukanskis, Z. Zhu, S.F. Kingsmore, P.M. Lizardi, D.C. Ward.** 2000. Immunoassays with rolling circle DNA amplification: A versatile platform for ultrasensitive antigen detection. *PNAS*, 97(18): 10113-10119.
32. **Sha, Q., K.L. Lansbery, D. Distefano, R.W. Mercer, C.G. Nichols.**

2001. Heterologous expression of the Na+, K+-ATPase γ subunit in Xenopus oocyts induces an endogenous, voltage-gated large diameter pore. *J Phys,* 535(2): 407-417.
33. Shah, V.D., et al. 1993. Myocardial infarction immunoassay. U.S Patent 5,202,234.
34. **Shepard, A.R., J.L. Rae.** 1997. Magnetic bead capture of cDNAs from double-stranded plasmid cDNA libraries. *Nucleic Acids Res,* 25(15): 3183-3185.
35. **Sinclair, B.** 1998. To bead or not to bead: Applications of magnetic bead technology. *The Scientist,* 12(13): 17.
36. **Spiro, A., M. Lowe, D. Brown.** 2000. A bead-based method for multiplexed identification and quantitation of DNA sequences using flow cytometry. *Appl Environ Microbiol,* 66(10): 4258-4265.
37. **Summers, K.L., B.D. Hock, J.L. McKenzie, D.N.J. Hart.** 2001. Phenotypic characterization of five dendritic cell subsets in human tonsils. *Am J Pathol,* 159(1): 285-295.
38. **Tong, A.K., J. Ju.** 2002. Single nucleotide polymorphism detection by combinatorial fluorescence energy transfer tags and biotinylated dideoxynucleotides. *Nucleic Acids Res,* 30(5): e19.
39. **Wang, J., A.N. Kawde.** 2002. Amplified label-free electrical detection of DNA hybridization. *Analyst*, 127(3): 383-386.
40. **Wang, J., A.N. Kawde, A. Erdem, M. Salazar.** 2001. Magnetic bead-based label-free electrochemical detection of DNA hybridization. *Analyst*, 126(11): 2020-2024.
41. **Wang, J., R. Polsky, A. Merkoci, K.L. Turner.** 2003. 'Electroactive beads' for ultrasensitive DNA detection. *Langmuir*, 19(4): 989-991.
42. **Wang, P., P. Wu, K.M. Ohleth, R.W. Egan, M.M. Billah.** 1999. Phosphodiesterase 4B2 is the predominant phosphodiesterase species and undergoes differential regulation of gene expression in human monocytes and neutrophils. *Molec Pharmacol,* 56: 170-174.
43. **Yeung, Y.A., K.D. Wittrup.** 2002. Quantitative screening of yeast surface-displayed polypeptide libraries by magnetic bead capture. *Biotechnol Prog,* 18(2): 212-220.
44. **Zuk, P.A., L.A. Elferink.** 1999. Rab 15 mediates an early endocytic event in Chinese hamster ovary cells. *J Biol Chem,* 274(32):22303-22312.