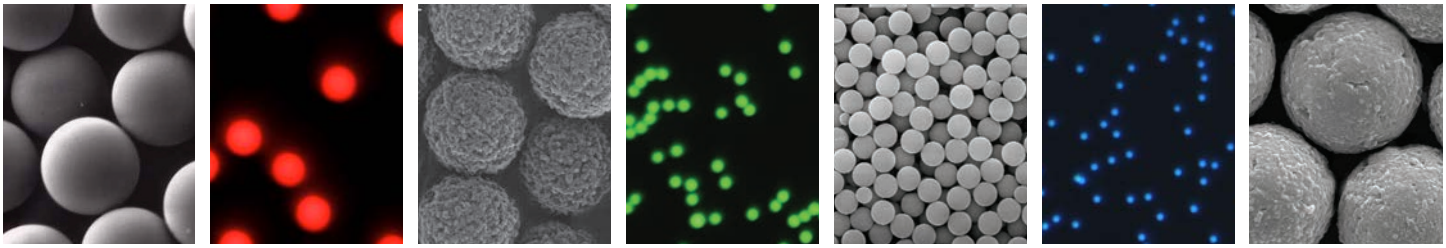
Bangs Laboratories, Inc.
BEADS ABOVE THE REST

Microsphere Selection

TechNote 201A



INTRODUCTION

Microspheres offer a highly convenient and flexible system for developing reagents for assays and bioseparations, and for use as instrument standards. As there are many varieties of microspheres available, it is important to think about the demands the application will place on them when making a base bead selection. Physical and optical properties should be considered in the context of handling and detection, and thought should also be given to requirements for diameter and size distribution, composition, surface chemistry, and any other needed properties.

PROPERTIES	Size	Composition	Surface chemistry	Special properties
CONSIDERATIONS	Diameter Uniformity / distribution	Density Refractive index Hydrophobicity / -philicity Nonspecific binding Autofluorescence	Reactive groups Level of functionalization Charge	Visible dye/fluorophore Superparamagnetic

DIAMETER

Microsphere size may be critical to the proper function of an assay, or it may be secondary to other characteristics. Considering traditional diagnostic methods, the test or assay format commonly dictates particle size, such as the use of very small spheres (~0.1-0.4µm) to ensure satisfactory wicking in lateral flow tests, or the use of larger, cell-sized spheres (~4-10µm) for bead-based flow cytometric assays. Also see application-specific TechNotes 301-304.

In magnetic separations, particularly those involving capture and elution of the target, the exact size of the magnetic particle may be unimportant provided that the particles are in some general size range, and offer desired separation characteristics. See TechNote 102 for additional details regarding our three magnetic particle lines.

Diameter also determines surface area. Small-diameter spheres present more surface area per unit weight, while larger spheres present more surface area per bead. Size also affects ease of handling, processing considerations (such as the method used for separations [centrifugation, dialysis, filtration]), and the amount of reagent needed for coating.

COMPOSITION

Common microsphere compositions include polystyrene (PS), poly(methyl methacrylate) (PMMA), and silica. These materials possess different physical and optical properties, which may present advantages or limitations for different applications.

Polymer beads are generally hydrophobic, and as such, have high protein binding abilities. However, they often require the use of some surfactant (e.g. 0.01-0.1% Tween® 20 or SDS) in the storage buffer to ensure ease of handling. During synthesis, functional monomers may be co-polymerized with styrene or methyl methacrylate to develop beads with surface reactive groups. Functional groups may be used in covalent binding reactions, and also aid in stabilizing the suspension.

COMPOSITION	**REFRACTIVE INDEX** (589nm)	**DENSITY** (g/cm^3)	**GLASS TRANSITION TEMP** (°C)
PS	1.59	1.05	95
PMMA	1.49	1.19	105
Silica	1.43-1.46*	2.0*	>>1000

** Determined using representative samples. Other values are as reported in the literature for bulk polymer or silica.*

Silica microspheres are inherently hydrophilic and negatively charged. Consequently, aqueous silica suspensions rarely require use of surfactants or other stabilizers. Carboxyl- and amine-functionalized silica spheres are available for use in common covalent coating protocols, and plain silica microspheres may be modified using a variety of silanes to generate functional groups or alter surface properties.

COATING

Microspheres may be coated with capture molecules, such as antibodies, oligonucleotides, peptides, etc. for use in diagnostic or separation applications. Microsphere coatings are typically optimized to achieve desired specific activity, while minimizing nonspecific interactions. Consideration should also be given to the required stability, development time frame and budget, and the specific biomolecule to be coated. These factors will aid in determining the most fitting coating strategy for both short- and long-term objectives.

Standard microsphere products support three basic coating strategies: adsorption, covalent coupling, and affinity binding.

A. Adsorption

Adsorption relies primarily on hydrophobic interactions between the biomolecule and the polymer particle. Such coatings are fairly simple to conduct, involving incubation of the microspheres with the purified biomolecule. They typically require little optimization, and reagents may be developed relatively quickly. However, as adsorption relies on the formation of multiple attachment points between the molecule and particle, this strategy is typically reserved for use with proteins and non-functionalized polymer spheres. Adsorption is generally not suitable for hormones, peptides, or nucleic acids in hybridization-based applications, and protein adsorption to silica is expected to be less efficient than to polymer. See TechNotes 201 and 204.

B. Covalent Coupling

Covalent coupling results in the permanent attachment of the molecule to the functionalized (e.g. carboxyl or amine) microsphere. It can provide needed stability when developing a commercial reagent, and for multiplexed assays, where analyte-specific bead populations are mixed. Additionally, specialized chemical linkers may be employed to address steric effects or to optimally orient the molecule. Although covalent binding protocols often involve a higher level of optimization than other approaches, coupling kits are available to simplify the process. See TechNotes 201 and 205.

C. Affinity Binding

Affinity binding is a straightforward method for immobilizing primary antibodies or biotinylated molecules. Proteins A and G and Fc-specific antibody coatings permit the directed immobilization of primary antibodies, and streptavidin is used extensively for the binding of biotinylated molecules, such as antibodies, peptides, and oligonucleotides. See TechNotes 101 and 302.

It is important to note that each binding strategy has benefits and limitations, which should be weighed in the context of study objectives and the demands that will be placed on the finished reagent.

BIOMOLECULE	TYPICAL COATING STRATEGY	NOTES
Peptides	Covalent Streptavidin/Biotin	End-point attachment to preserve the activity of the peptide
Nucleic acids	Covalent Streptavidin/Biotin	End-point attachment to permit hybridization with target sequence
Proteins (e.g. antibodies)	Covalent Adsorption	Common proteins are generally large enough that multi-point attachment and nonspecific orientation do not compromise activity. However, linkers or spacers (covalent or SA/B) may be employed to address steric effects or sub-optimal orientation.

SPECIAL PROPERTIES

Many applications in the life sciences demand added properties, such as fluorescence or a visible color, or iron oxide inclusions for magnetic separations. Polymer spheres (and polymer-based magnetic spheres) are often internally dyed via organic solvent swelling, and many standard products are available. Dye concentrations can be adjusted to produce beads with different intensities to meet special needs, such as QuantumPlex™ for multiplexed flow cytometric assays, or our Dragon Green or Flash Red Intensity Standards, which support imaging applications and associated instrument QC. Many surface- or internally-labeled fluorescent beads are also available as specialized flow cytometry standards.

Various types of superparamagnetic microparticles are available as well – with different matrices, magnetite content, surface groups, etc. For new assays or applications, magnetic beads should be evaluated with application demands in mind.

The following tables provide product suggestions for common microsphere applications. These are offered as general guidelines only. Further literature research and screening experiments may be appropriate.

TEST / ASSAY FORMAT	BEAD SIZE	BEAD TYPE	COATING STRATEGY	DETECTION STRATEGY	DOC.
Turbidimetric (Automated LAT)	50nm – 500nm	Undyed	Covalent	Turbidimetry	TN304
Magnetic Chemiluminescence	1-5 µm	ProMag® HP	Covalent	Luminescence	-
Flow cytometric (suspension array)	2µm – 15µm	QuantumPlex™ QuantumPlex™M (encoded populations for multiplexing) or Non-fluorescent (simplex or multiplex with different bead sizes)	Covalent or streptavidin / biotin	Flow cytometer	TN305
Bead "ELISA"	1-5 µm	ProMag®, ProMag® HP,	Covalent	Spectrophotometer	TN301
Lateral Flow	0.1µm – 0.4µm	Dyed (visible or fluorescent)	Covalent or adsorption	Visual or Automated Reader (absorbance, fluorescence)	TN303
Lateral Flow – Boulders in the Stream	0.1µm – 0.4µm mobile phase	Dyed (visible) mobile phase	Covalent or adsorption	Visual	TN303
	~2µm – 3µm capture phase	Undyed capture beads			
Dipstick	0.1µm – 0.4µm	Dyed (visible)	Covalent or adsorption	Visual	TN303
Latex Agglutination Test (LAT)	0.2µm – 1.0µm	Undyed or visibly dyed	Covalent or adsorption	Visual (may be microscope-assisted)	TN201 TN301

Separation	
Antibodies	BioMag® Protein A or Protein G and ProMag® Protein G
Proteins	BioMag®, ProMag®, ProMag® HP or COMPEL
Glycans, glycoproteins	BioMag® WGA or ConA
Cells	BioMag® anti-CD marker or secondary antibody
Subcellular organelles	BioMag® (antibody-coated)
Immunoprecipitates	BioMag® secondary antibody
mRNA	BioMag® Oligo dT(20) or mRNA Purification System
DNA (total-SPRI)	Magnefy™ or BioMag® COOH
DNA (specific sequence)	ProMag® ProMag® HP, Magnefy™, COMPEL™, or BioMag® Streptavidin or COOH (oligo attachment)
Biopanning	ProMag®, COMPEL™, or BioMag®