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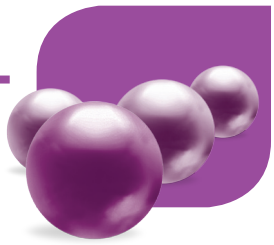
# Microsphere Selection

## Suggestions and Strategies for Choosing the Best Bead



Microspheres offer a highly convenient and flexible system for developing reagents for assays and bioseparations, and for use as instrument standards. With many varieties of microspheres available, it is important to think about the demands the application will place on them when choosing a base bead. 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.

| Property | Considerations |
|---|---|
| Size | Diameter |
| | Uniformity / distribution |
| Composition | Density |
| | Refractive Index |
| | Hydrophobicity / -philicty |
| | Nonspecific binding |
| | Autofluorescence |
| Surface chemistry | Reactive groups |
| | Level of functionalization |
| | Charge |
| Special properties | Visible dye / fluorophore |
| | Superparamagnetic |

### DIAMETER

Microsphere size may be critical to the proper function of an assay, or it may be a secondary consideration. Considering traditional diagnostic methods, the test or assay format commonly dictates particle size, such as the use of submicron spheres (≤0.5µm) in lateral flow tests and turbidimetric assays, or the use of cell-sized spheres (~2-10µm) for bead-based flow cytometric assays. In magnetic separations, 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.

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 impacts ease of handling, processing considerations, and the amount of reagent needed for coating.

### COMPOSITION

Common microsphere compositions include polystyrene (PS) 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 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 aid in stabilizing the suspension.

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 microspheres are available for use in common covalent coating protocols, and plain silica spheres may be modified using a variety of silanes to generate functional groups or alter surface properties.

| Composition | Refractive Index (589nm) | Density ($g/cm^3$) | Glass Transition Temperature (°C) |
|---|---|---|---|
| PS | 1.59 | 1.05 | 95 |
| Silica | 1.43-1.46* | 2.0* | >>1000 |

### COATING

Microspheres may be coated with ligands, such as antibodies or oligonucleotides, for use in diagnostic or separation applications. Microsphere coatings are typically optimized to achieve desired specific activity, while minimizing nonspecific interactions. Consideration should be given to the required stability, development time frame and budget, and the specific biomolecule to be coated. These factors will help determine the most fitting coating strategy (adsorption, covalent coupling or affinity binding) for short- and long-term objectives.

#### Adsorption

Adsorption relies primarily on hydrophobic interactions between the biomolecule and 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.

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### Covalent Coupling

Covalent coupling results in the permanent attachment of the molecule to the functionalized 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.

### 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.

| Biomolecule | Typical Coating Strategy | Notes |
|---|---|---|
| Peptides | Covalent<br>Streptavidin / biotin | End-point attachment to preserve the activity of the peptides. |
| Nucleic acids | Covalent<br>Streptavidin / biotin | End-point attachment to permit hybridization<br>with target sequence. |
| Proteins (e.g. antibodies) | Covalent<br>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. |

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.

## 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.

### Microsphere Selection for Common Test and Assay Formats:

| Test / Assay Format | Bead Size | Bead Type | Coating Strategy |
|---|---|---|---|
| Flow cytometric (suspension array) | 2 – 15µm | QuantumPlex™<br>QuantumPlex™M (for multiplexing)<br>or<br>Non-fluorescent (simplex or multiplex with different bead sizes) | Covalent or streptavidin / biotin |
| Lateral Flow | 0.1 – 0.4µm | Dyed (visible or fluorescent) | Covalent or adsorption |
| Lateral Flow – Boulders in the Stream | 0.1 – 0.4µm mobile phase | Dyed (visible) mobile phase | Covalent or adsorption |
| | ~2 – 3µm capture phase | Undyed capture beads | |
| Dipstick | 0.1 – 0.4µm | Dyed (visible) | Covalent or adsorption |
| Latex Agglutination Test (LAT) | 0.2 – 1.0µm | Undyed or visibly dyed | Covalent or adsorption |
| Turbidimetric | 50nm – 500nm | Undyed | Covalent |
| Magnetic Chemiluminescence | 1-5 µm | ProMag™ HP | Covalent |

| Separation | |
|---|---|
| Cells | BioMag® anti-CD marker or secondary antibody |
| Subcellular organelles | BioMag® |
| Immunoprecipitates | ProMag™ Protein G, BioMag® secondary antibody, or Protein A or G |
| mRNA | BioMag® Oligo dT (20) or mRNA Purification System |
| Biotinylated oligonucleotide capture or binding | ProMag™ or BioMag® Streptavidin |
| Biopanning | ProMag™ or BioMag® |
| **Assay** | |
| Immunoassays | ProMag™, ProMag™ HP, ProMag™ HC, or BioMag® |
| Hybridization-based assays | ProMag™ |
| Flow cytometric | QuantumPlex™M |

For additional information regarding microsphere selection, visit our website at www.polysciences.com.

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