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Chemistry beyond the ordinary

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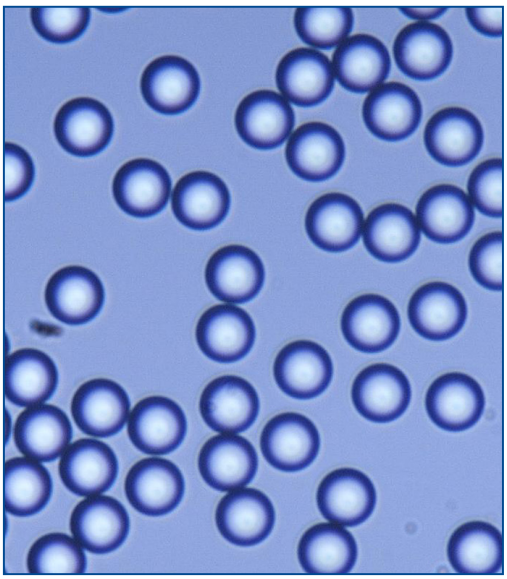
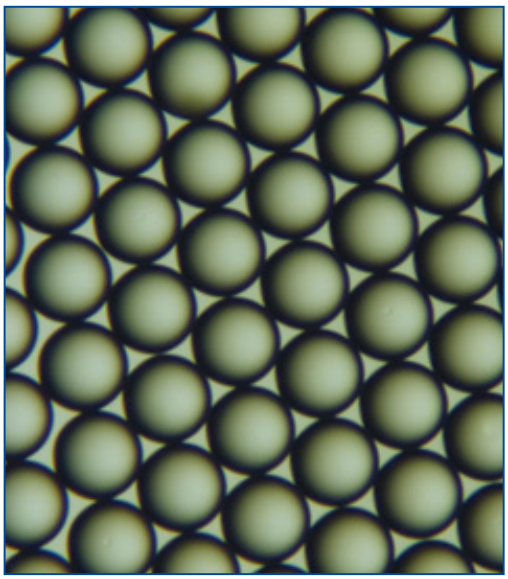
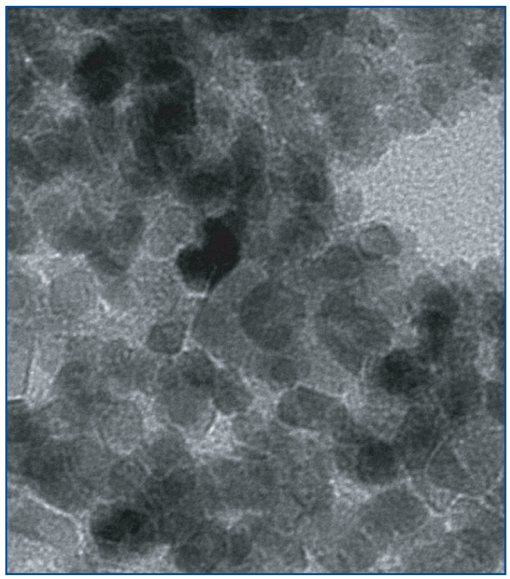
Microspheres & Particles Handling Guide

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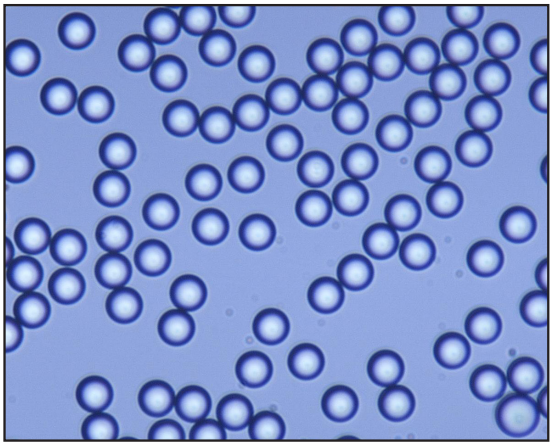
Technical Information

At Polysciences, we are committed to making the finest microspheres in the world, and providing the highest level of customer and technical service from initial discussions through the product lifecycle and beyond. We hope that you find this catalog to be helpful as you consider products for your work, and invite you to contact us if we may address any questions or be of assistance in formulating solutions to meet your specific needs.



Well-dispersed 10µm Polybead® Microspheres

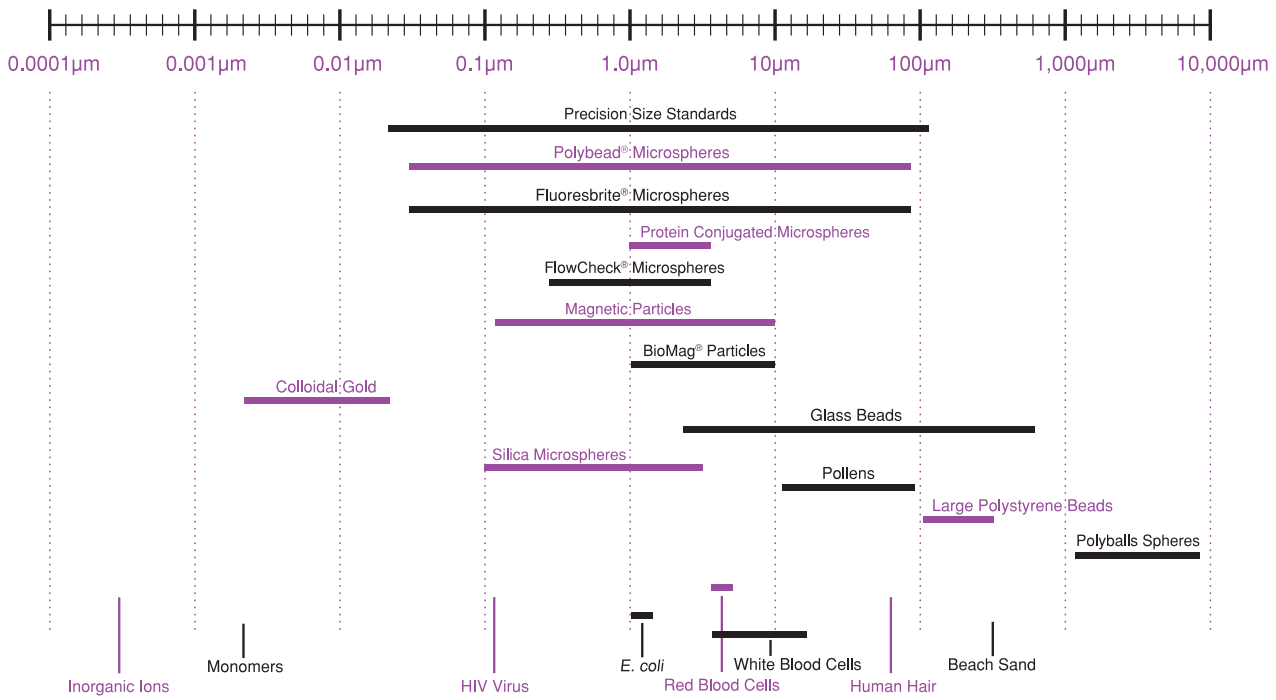
How Particles Measure Up

Precision Size Standards ..... 0.04 - 175µm
Polybead® Microspheres ..... 0.05 - 90µm
Fluoresbrite® Microspheres..... 0.05 - 90µm
Protein Conjugated Microspheres ..... 1 - 6µm
Flow Check™ Microspheres ..... 0.5 - 6.0µm
Magnetic Particles..... 0.2 - 10µm
BioMag® Particles..... 1.0 - 10µm
Colloidal Gold..... 0.005 - 0.06µm
Glass Beads ..... 3 - 850µm
Silica Microspheres..... 0.1 - 5.0µm
Pollens..... 12 - 90µm

Large Polystyrene Beads..... 106 - 600µm
Polyballs..... 1,587.5 - 9,525µm
Inorganic Ions..... 0.0006µm
Monomers..... 0.0045µm
HIV virus..... 0.12µm
E. coli..... 1 - 2µm
Red Blood Cells..... 6 - 8µm
White Blood Cells..... 7 - 25µm
Human Hair 80µm
Beach Sand 650µm

How Polysciences' Microspheres and Other Particles Measure Up

1 Micron (µm) = 1,000 Nanometers = 10,000 Angstroms



Microsphere Selection

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 of the application when selecting a microsphere. 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 / -philicity, Nonspecific binding, Autofluorescence
Surface chemistry	Reactive groups, Level of functionalization, Charge
Special properties	Visible dye / fluorophore, Superparamagnetic

Particle Size

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.

In magnetic separations, particularly those involving capture and elution of 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.

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

All sizes listed in this catalog are nominal. For most products, the mean diameter of your particles will be printed on the label with the standard deviation.

Particle 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 reactive surface groups. Functional groups may be used in covalent binding reactions and also aid in stabilizing the suspension.

Silica microspheres are inherently hydrophilic and negatively-charged. Consequently, aqueous silica suspensions rarely require the use of surfactants or other colloidal 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.

Composition	Refractive Index (589nm)	Density (g/cm^3)	Glass Transition Temperature (˚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.*

Common Test and Assay Formats

Test / Assay Format	Bead Size	Bead Type	Coating Strategy	Detection Strategy
Flow cytometric (suspension array)	2 - 15µm	QuantumPlex™, QuantumPlex™M (encoded populations for multiplexing), Non-fluorescent (simplex or multiplex with different bead sizes)	Covalent, Streptavidin / biotin	Flow cytometer
Lateral Flow	0.1 - 0.4µm	Dyed (visible or fluorescent)	Covalent, Adsorption	Visual or automated reader (absorbance, fluorescence), Visual
Lateral Flow - Boulders in the Stream	0.1 - 0.4µm mobile phase, ~2 - 3µm capture phase	Dyed (visible) mobile phase, Undyed capture beads	Covalent, Adsorption	Visual
Dipstick	0.1 - 0.4µm	Dyed (visible)	Covalent, Adsorption	Visual
Latex Agglutination Tests (LATs)	0.2 - 1.0µm	Undyed, Visibly dyed	Covalent, Adsorption	Visual (may be microscope-assisted)
Turbidimetric (Automated LAT)	50nm - 500nm	Undyed	Covalent	Turbidimetry

Magnetic Assays

Assays	Suggested Products
Immunoassays	ProMag™, ProMag™ HP, ProMag™ HC or BioMag®
Hybridization-based assays	ProMag™ and ProMag™ HC

Magnetic Separations

Magnetic Separations	Suggested Products
Cells	BioMag® anti-CD marker or secondary antibody
Subcellular organelles	BioMag®
Immunoprecipitates	BioMag® secondary antibody
mRNA	BioMag® Oligo dT(20) or mRNA Purification System
Biotinylated oligonucleotide capture or binding	ProMag™ or BioMag® Streptavidin
Biopanning	ProMag™ or BioMag®
Glycoproteins	BioMag® Wheat Germ Agglutinin or BioMag® Concanavalin A

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 some 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, with different matrices, magnetite content, surface groups, etc. For new assays or applications, magnetic beads should be evaluated with application demands in mind.

General Handling

Particle Suspension

The number of particles per ml will vary with the specified weight to volume (w / v) concentration, diameter of the particle and density of particle composition. The number of particles per milliliter can be calculated using the following equation:

$$\frac{6x \bullet 10^{12} \bullet \rho_L}{\rho_S \bullet \pi \bullet z^3}$$

x = solids content (g/ml)
ρ_S = density of solid sphere (g/cm^3)
z = diameter (µm)
ρ_L = density of bead suspension (g/ml)
$\rho_L = 100 \bullet \rho_S \; / \; [100 \times (1 - \rho_S) + (100 \bullet \rho_S)]$

The following grid gives the estimated particles per milliliter for common diameters of polystyrene beads (ρ = 1.05 g/ml) suspended at 2.5% solids (w / v) and silica beads (ρ = 2.0 g/ml) suspended at 10% solids, at common diameters:

Diameter (µm)	Polystyrene 2.5% Solids (particles/ml)	Silica 10% Solids (particles/ml)
0.05	3.64×10^{14}	N/A
0.10	4.55×10^{13}	1.00×10^{13}
0.20	5.68×10^{13}	1.26×10^{13}
0.35	1.06×10^{12}	2.34×10^{12}
0.50	3.64×10^{11}	8.04×10^{11}
0.75	1.08×10^{11}	2.38×10^{11}
1.00	4.55×10^{10}	1.00×10^{10}
1.50	1.35×10^{10}	2.98×10^{10}
2.00	5.68×10^{9}	1.26×10^{10}
3.00	1.68×10^{9}	3.72×10^{9}

Diameter (µm)	Polystyrene 2.5% Solids (particles/ml)	Silica 10% Solids (particles/ml)
4.50	4.99×10^{8}	1.10×10^{9}
6.00	2.10×10^{8}	4.65×10^{8}
10.0	4.55×10^{7}	N/A
15.0	1.35×10^{7}	N/A
20.0	5.68×10^{6}	N/A
25.0	2.91×10^{6}	N/A
45.0	4.99×10^{5}	N/A
75.0	1.08×10^{5}	N/A
90.0	6.24×10^{4}	N/A

Surface to Volume Ratios

Use these formulas as a rough guide to estimate the surface area or the volume of a sphere. Determination of the surface area of polystyrene spheres is complicated by the unique form of the polymer. These beads are made by the formation of many single chain polymers which may be likened to a ball of wool. Thus, the surface area may be much greater than that predicted by the simple formula. This is particularly important for protein binding applications and charge calculations.

Surface Area = $4 \pi r^2$

Volume = $4 / 3 \pi r^3$

Handling and Storage

Our microspheres are synthesized in water and should be stored in aqueous environments. Deionized water is the best suspending medium for uncoated spheres as high concentrations of ions may result in aggregation. Coated microspheres should be stored in buffers that are appropriate for the ligand that is bound to the surface. Storage of particles over long periods of time should be at 4°C to deter the growth of microbes, and the particle suspensions must not be allowed to freeze. Dyed and fluorescent particles should be protected from light. Biocides may be added for extended storage.

Washing

Microspheres sold as instrument standards can often be used as-is, or simply diluted in an appropriate buffer or aqueous solution. Conversely, microspheres that will be coated or otherwise modified should be washed to remove additives and residuals that could interfere with the binding reactions or other processes.

Common washing and separation methods for non-magnetic beads include centrifugation, filtration and dialysis. Selection of the "best" method will depend on scale, required throughput and microsphere characteristics. Centrifugation is often used for small-scale separations of ≥0.5µm, dialysis for spheres <0.5µm and filtration for small spheres <0.5µm, or to achieve higher throughput. Superparamagnetic microparticles are separated using rare earth or electro-magnets.

Centrifugation

Particle washing may be conducted via centrifugation. This procedure must be performed carefully as excessive centrifugation may result in resuspension difficulties. Though centrifugation of BioMag® magnetic particles is not recommended, ProMag™ may be processed in this way. For the purposes of pelleting, it is important to understand the settling velocities of particles. For spherical particles, settling velocity can be calculated using Stokes' Law:

$$V = \frac{2Ga^2 (\rho_1 - \rho_2)}{9n}$$

V = Velocity (cm/sec)
G = G force (1G = 980.7 cm/sec^2)
ρ_1 = density of particle (g/cm^3)
ρ_2 = density of suspending media (g/cm^3)
n = coefficient of viscosity (poise; g/cm-sec)
a = radius of spherical particle in cm

For calculating the settling velocity of polystyrene spheres at 1 G in 20°C water, Stokes' Law can be expressed in the following formula where d = diameter (µm) (ρ_1 = 1.05 g/cm^3, ρ_2 = 1.00 g/cm^3 and n = 1.002 cp); V= 2.77 x 10^{-6} d^2. To estimate appropriate times for centrifugation, settling velocity is multiplied by the G forces generated by the centrifuge. The resultant velocity is then compared to the height of the centrifuge tube.

For example:
A 1.0µm polystyrene particle placed in a microcentrifuge generating 10,000 G will settle at a velocity of 2.72 x 10^{-2} cm/sec.

Pelleting the particle in a 4cm high tube would require a 144 second (minimum) centrifuge run. The actual time required to form an acceptable pellet could possibly be 50% longer. These calculations are intended to be used as guidelines to assist in determining centrifugation time. Different size particles yield dramatically different settling velocities. A 10.0µm particle could settle in 2 seconds under the aforementioned conditions, whereas a 0.01µm particle could take at least 4 hours to settle. Brownian motion and particle concentration also affect the settling rate.

Aggregation

Our microspheres are available in a variety of compositions, including polystyrene, poly(methyl methacrylate), and silica. Though polymer microspheres are more susceptible to hydrophobic-mediated aggregation, there are several factors that may influence the dispersity of the suspension. For example, low surface charge, small diameter (high surface area : volume ratio), high microsphere concentration and suboptimal buffer composition or pH may promote aggregation. Strategies that are effective in addressing aggregation thus counter these conditions, i.e. use of surfactant to reduce hydrophobicity (e.g. 0.01 - 0.1% Tween® 20 or SDS), sonication to disrupt aggregates and adjusting microsphere concentration or buffer pH to deter contact between individual spheres.

Coating Microspheres

General Information

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: passive adsorption, covalent coupling and affinity binding. 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 upon the finished reagent.

Passive Adsorption

Passive 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 ligand. 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 the 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. Most techniques using passive adsorption technology report four to six months of bead stability. The reagent may be lyophilized for extended stability.

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. If surfactant is required as an additive in the assay, covalent coupling procedures are recommended as surfactants can displace adsorbed proteins from the surface. Covalent binding is also important for the immobilization of oligonucleotides or peptides, where end-point attachment is required. Although covalent binding protocols often involve a higher level of optimization than other approaches, coupling kits are available to simplify the process.

When Coupling to Particles Less Than 0.5µm

The chemical aspects of the protocols are universally applied, but the mechanical separations of these particles must be adapted for specific sizes. Most protocols suggest centrifugation to separate the particles from the reagents. This is not practical for particle sizes less than 0.5µm, as most microcentrifuges cannot spin these particles down within 30 minutes. Even extremely high G forces are not recommended, as resuspension becomes arduous. Other separation techniques may be utilized, such as dialysis, forced membrane filtration or centrifugal filter devices. Polysciences also offers coupling kits that use hollow fiber filtration techniques in addition to Vivaspin® Ultrafiltration devices to effect separation of 0.1-0.5µm particles.

Affinity Binding

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

Biomolecule	Coating Strategy	Notes
Peptides	Covalent Streptavidin / biotin	End-point attachment to preserve the activity of the peptides.
Nucleic acids	Covalent Streptavidin / biotin	End-point attachment to permit hybridization of probe sequence with target sequence.
Proteins (e.g. antibodies)	Covalent Adsorption Streptavidin / biotin Proteins A / G	Common proteins are generally large enough that multi-point attachment and nonspecific orientation do not compromise their activity. However, linkers, spacers (covalent or SA / B) or affinity ligands may be employed to address steric effects or sub-optimal orientation.

Alternative for BSA as a Blocking Agent

Any innocuous protein may be used to block the effects of non-specific adsorption. In selecting an alternative to BSA, it is suggested that the size of the active protein and the size of the blocking protein be compared. BSA is highly recommended for IgG coupling. However, the large size of BSA could obscure the activity of smaller active proteins. Glycine or small polypeptides may be used as alternatives.

Protein Coupling Efficiency Determination

Coupling efficiency can be determined by measuring the change in absorbance of the supernatant before and after coupling.

1. Set spectrophotometer wavelength to 280nm. Blank with the Coupling Buffer.
2. Measure the absorbance of the Pre-Coupling Solution. A further dilution may be necessary to read an absorbance depending upon the amount of protein added (D = dilution factor).
3. Measure the absorbance of the Post-Coupling Solution. A dilution may be necessary to read the absorbance (D = dilution factor).
4. Calculate the coupling efficiency, expressed as the % Protein Uptake, as follows. Typical values of Protein Uptake are >60%.

$$\left[\frac{(A_{280}\ \text{Pre-Coupling Solution x D}) - (A_{280}\ \text{Post-Coupling Solution x D})}{(A_{280}\ \text{Pre-Coupling Solution x D})} \times 100 \right]$$

Protein Binding Protocols

The following sequences serve as guidelines for protein binding. Technical Data Sheets (TDS) with detailed step-by-step protocols can be downloaded from our website, and ligand-specific immobilization protocols may be found in the literature.

Adsorbing Protein on Particles TDS #238E	Coupling by Carbodiimide TDS #238C & #644	Coupling by Glutaraldehyde TDS #238D & #238G
Plain Polystyrene	**Carboxylate Functional Particles**	**Amino or Blue Dyed Particles**
• Initial Buffer: 0.1M Borate Buffer, pH 8.5	• Initial Buffer: 0.1M Carbonate Buffer	• Initial Buffer: 0.02M PBS, pH 7.4
• Suspend in buffer, spin down and resuspend 2 or 3 times.	• Suspend in buffer, spin down and resuspend 2 or 3 times.	• Suspend in buffer, spin down and resuspend 2 or 3 times.
• Suspend in borate buffer.	• Suspend in MES buffer.	• Suspend in PBS.
• Add protein and mix end-to-end overnight.	• Add fresh carbodiimide solution dropwise and incubate for 15 - 30 minutes.	• Suspend in 8% glutaraldehyde in PBS, pH 7.4 and mix end-to-end for 4 - 6 hours.
• Spin and save supernatant for protein determination.	• Wash to remove excess carbodiimide, then resuspend in borate buffer.	• Wash to remove excess glutaraldehyde and resuspend in PBS buffer.
• Re-suspend in BSA in appropriate buffer and spin down twice.	• Add protein and mix end-to-end for 2 - 4 hours.	• Add protein and mix end-to-end overnight.
• Re-suspend in PBS, pH 7.4, containing BSA (storage buffer).	• Add ethanolamine, mix for 30 minutes.	• Add ethanolamine, mix for 30 minutes.
• Protein bound directly on surface.	• Spin and save supernatant for protein determination.	• Spin and save supernatant for protein determination.
	• Re-suspend in BSA-containing buffer and spin down twice.	• Re-suspend in BSA-containing buffer and spin down twice
	• Re-suspend in PBS, pH 7.4, containing BSA (storage buffer).	• Re-suspend in PBS, pH 7.4, containing BSA (storage buffer).
	• Protein bound 2 - 3 carbon atoms from surface.	• Protein bound 5 carbon atoms from surface of blue dyed beads and 11-12 carbon atoms from surface of amino beads.

Protein Coupling Troubleshooting

Below are some solutions for protein coupling troubleshooting. If you do not see a solution to the problem you have encountered, please email us at **info@polysciences.com**.

Problem	Solution
Clumping prior to use	Careful sonication
Clumping after procedure • Carbodiimide addition causes clumping • Glutaraldehyde addition causes clumping • Protein addition causes clumping • Washing causes clumping	Isolate the step that causes clumping • Add slowly, agitate beads, decrease bead concentration • Add slowly, agitate beads, decrease bead concentration; add an excess of glutaraldehyde to avoid chemical crosslinking of particles; clumping will typically resolve by the conclusion of protein coupling • Increase protein concentration • Add surfactant or reduce number of washing steps
Low binding	Move pH closer to protein isoelectric point
Variable coating	Use pure water – no contaminants; use fresh reagents
Coating, but no reaction	Optimize pH away from isoelectric point
Centrifuge not practical	Use membrane filtration, dialysis, or spin filters for small particles
Nonspecific adsorption	Use an alternative for BSA (glycine, casein)
Small proteins bound, but not reactive	Use a crosslinking reagent to extend coupling away from the surface of the bead
Long-term storage leaches protein	Try covalent attachment or lyophilize final product

Polystyrene Microspheres

Polystyrene Microsphere General Characteristics

Parameter	Description
Size	0.05 - 150µm for a wide range of applications
Monodispersity	Coefficient of variance ≤15% for most size ranges, 0.05 - 90µm
Concentration	2.5%
Suspending medium	DI water with residual surfactant to ensure stable dispersions
Color	Undyed, red, yellow, black, blue, violet, orange, green and several fluorescent colors
Functionality	Plain, COOH, -NH_2 and -OH.
Stability	Inert, safe for handling and ideal for biological studies
Protein Affinity	Covalent coupling for functionalized spheres or passive adsorption possible
Glass Transition	~94˚C, stable to moderate heating temperature; some diameters feature a low level of DVB crosslinking
Bead Density	~1.05 g/cm^3, similar to cell densities
Refractive Index (589nm)	~1.59 - 1.60, ideally suited for applications
Biocides	None (except where noted); particles are compatible with azide, ProClin® and other treatments.

Polystyrene Microsphere Stability

Polysciences offers a one year shelf life for most products. Unless noted, biocides are not added and the particles are shipped in DI water with residual surfactant. All polystyrene products should be stored at 4˚C to prevent microbial growth. Microsphere suspensions must be protected from freezing to safeguard against irreversible aggregation. If long-term storage is required, the addition of biocide is recommended.

Polystyrene Microsphere Monodispersity

The following chart lists our specifications for the uniformity of our particles, expressed as the coefficient of variance (CV). The actual diameter (D) and the standard deviation (SD) for each lot is printed on the label. The % CV is expressed as the SD / D x 100.

Diameter (µm)	CV Maximum (%)
0.05	≤15
0.10	≤15
0.20	≤8
0.35	≤5
0.50	≤3

Diameter (µm)	CV Maximum (%)
0.75	≤3
1.00	≤3
1.5	≤5
2.00	≤5
3.00	≤5

Diameter (µm)	CV Maximum (%)
4.50	≤7
6.00	≤10
10.00	≤10
15.00 - 90.00	≤15

Polystyrene Microsphere Sterility and Shelf Life

Our polystyrene microspheres are packaged as non-sterile suspensions. We have made the decision to give the customer the option of adding biocides or preservatives into the product upon receipt. The particles will be stable for up to one year after the date of sale. Degradation of the particles, their functional groups or the incorporated dyes is not expected under normal conditions and our primary concern is the quality of the DI water. We make every effort to ensure that our water source and packaging procedures will allow us to meet our one year shelf life. If a sterile product is necessary, then the particles may be gamma irradiated. Additions of biocides, such as thimerosol or sodium azide, are common. For research applications involving *in vivo* studies or live cells, the particles can be suspended in alcohols prior to use. See Technical Data Sheet #670, *Decontaminating Microspheres,* for more information.

Embedding Tissues Containing Polystyrene Microspheres

Polystyrene microspheres have been visualized by light microscopy in unembedded coverslip monolayers, in fixed or unfixed frozen sections, in paraffin sections and in glycol methacrylate kits. For paraffin sections, n-butyl alcohol must be used for clearing and deparaffination since typical organic solvents (e.g. toluene, THF, or ethyl acetate) will destroy the beads. The beads cannot be embedded in methyl or butyl methacrylate media. TEM embedments in Epon and Spurrs have been successful.

Polybead® and Fluoresbrite® Dyed Particles

Types of Dyes Used

Our Fluoresbrite®, Polybead® and some Flow Check™ product feature water insoluble dyes*. This minimizes the incidence of dye leaching from the particles into aqueous buffers. Our visibly dyed microspheres are available as black, blue, red, violet, orange, green and yellow. Other colors and intensities are available on a custom basis. Many of the Bangs Flow Cytometry Standards are "surface dyed" with the same fluorochromes used for making antibody conjugates. Polysciences can custom manufacture Fluoresbrite® particles with a customer's dye of choice. Polysciences' most popular fluorescent dyes match the following filter settings:

Fluorescent Particles	Filter Setting	Dyed Particle	Excitation Max. (nm)	Emission Max. (nm)
BB (Bright Blue)	Coumarin	BB	360	407
YG (Yellow Green)	Fluorescein	YG	441	486
YO (Yellow Orange)*	Rhodamine	YO	529	546
PC (Polychromatic Red)	Phycoerythrin	PC	491; 512	554
		Ruby Red	475; 590	663
		FITC	492	512
		R-PE	480; 565	578
		Sulforhodamine	540	645
		APC	650	660

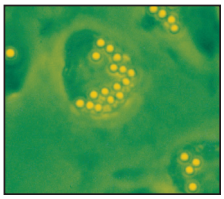
** YO has limited water solubility; some leaching may occur with rigorous washing.*

Dyed Microspheres and Microscopy

A 6µm visibly dyed (non-fluorescent) particle is the smallest particle whose color can reasonably be observed under light microscopy conditions (400x). Infinite magnification of a dyed particle will result in an undyed appearance. Fluorescently-labeled Fluoresbrite® microspheres are recommended for microscopic viewing of particles smaller than 6µm. Fluoresbrite® 0.05µm particles have been identified using a fluorescent microscope set at 100x objective and 10x ocular magnification.

Fluoresbrite® for Phagocytosis or Retrograde Transport

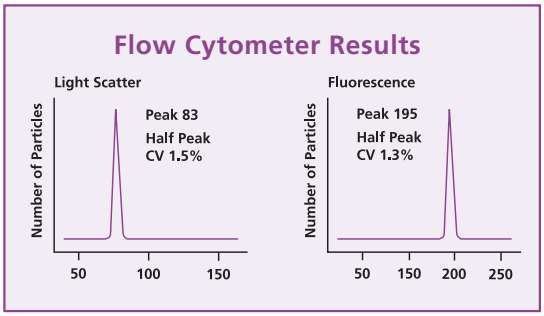
Uniform polystyrene particles are ideal for cellular studies. Microspheres are easily identified by their intense fluorescence, and polystyrene has long been recognized as a biologically active surface for cell attachment. See *Technical Data Sheet #430* for more information on phagocytosis studies.



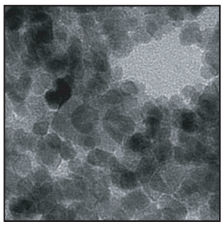
Fluoresbrite® to Calibrate Flow Cytometers

Our Fluoresbrite® Calibration Grade particles have had a long history of use as flow cytometry standards. We also carry the extensive line of Bangs Flow Cytometry Standards. See *Technical Data Sheet #431* for more information.

Data for the chart to the right was obtained on a EPICS V instrument with fluorescent polystyrene beads, 2µm in diameter. Data courtesy of Kristi Harkins, University of Nebraska / Lincoln.



BioMag®, BioMag®Plus and BioMag® Maxi



BioMag® Physical Characteristics

Our conventional BioMag® are irregularly-shaped iron oxide particles that are approximately 1-2µm in size. BioMag®Plus are similar, but have undergone additional processing for the reduction of outliers. BioMag® Maxi are ~6µm. Functionalized versions are covered with a silane coating that provides functional groups for the attachment of proteins or antibodies. The irregular shape of the BioMag®, BioMag®Plus and BioMag® Maxi particles provide increased surface area and therefore increased binding capacity per unit mass.

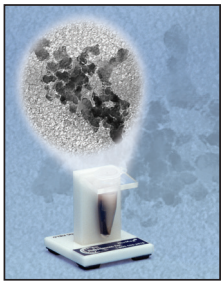
BioMag® particle surface area:	>100 m^2/g
BioMag® particle density:	>>2.5 g/cc
BioMag® settling rate:	4% in 30 minutes

BioMag® Stability

As the BioMag® base particle is composed of coated iron oxide, the particle itself is very stable. However, any proteins or antibodies attached to BioMag® particles are susceptible to degradation over time. BioMag® should not be frozen or exposed to elevated temperatures.

BioMag® Stability in Solvents

BioMag® particles have been used in various coupling buffers at pH ranging from 5.5 to 8.0. Low pH buffers can be problematic for BioMag®. It is best to test BioMag® in advance of exposure to organic solvents or extreme pH conditions.



BioMag® Magnetic Responsiveness

BioMag® particles are superparamagnetic. In other words, they have no magnetic memory and will readily re-suspend if the magnetic force is removed. The particles are greater than 90% magnetite in composition and have a magnetization of 25 - 35 emu/g (Electromagnetic Units).

Positive and Negative Selection with BioMag®

BioMag® particles can be used for both positive and negative selections. In negative selection, the unwanted components are bound and pulled out of solution by the BioMag® particles. After magnetic separation, the resulting supernatant is enriched for the target cells or molecules. In positive selection schemes, the BioMag® particles are used to pull out of solution only the target cells or molecules of interest. Unwanted cell populations and other sample constituents will be discarded with the supernatant, resulting in a purified suspension of the target components.

Magnetic Separator for BioMag®

Small superparamagnetic particles such as BioMag® require a strong magnetic field for efficient separation. Polysciences' magnets offer optimal performance, featuring rare earth (Neodymium-Iron-Boron) magnets embedded in plastic housings, with magnetic strengths ranging from 27 - 35 megagauss Oersteds. See *Technical Data Sheet #796* for additional information on the magnetic separators offered.

For complete technical information for each BioMag® product, refer to the appropriate Technical Data Sheet.

Flow Cytometry Quality Control

Validation / Quality Control

An instrument validation / Quality Control (QC) program will depend on the type and complexity of the work being performed on the instrument. A multi-fluorescent bead such as Full Spectrum™ allows operators to run a single product to check basic function and track general stability of all of the lasers / detectors. It will also be important to understand the sensitivity, resolution and linearity of different detectors. Linearity determinations are particularly important for quantitative fluorescence analyses.

Instrument Set-Up

Flow cytometers are highly configurable, and results can vary dramatically with different instrument settings. Establishing a common "Window of Analysis" for each detector with the upper and lower fluorescence limits defined, allows reference populations to be positioned in approximately the same place on the same scale. This may be accomplished with the aid of Quantum™ QC or Full Spectrum™. If multi-color analyses are being performed, compensation standards will likely be required to tailor settings.

Applications

There are many different types of studies that can be conducted on a flow cytometer. This might include quantitative surface marker expression analysis (Quantum™ MESF, Quantum™ Simply Cellular®), absolute counting (Flow Cytometry Absolute Count Standard™), size estimation (Small Bead Calibration Kits, Size Calibration Standard Kits), or various fluorescence analysis (Fluorescence Reference Standards).

The chart that follows provides additional product recommendations for specific tasks / objectives, and we additionally invite you to contact us to discuss the specific requirements of your program.

Quantum™ APC MESF Kit

Category	Purpose	Frequency	Products
Daily QC	General check of instrument stability / status	Daily	Full Spectrum™ (multi) Ultra Rainbow Fluorescent Particles (multi) Fluorescence Reference Standards (single) Flow Check™ Ruby Red Fluorescent Microspheres (single) Quantum™ QC
Daily QC	General check of instrument optical system	Daily	Full Spectrum™ (multi) Ultra Rainbow Fluorescent Particles (multi) Fluorescence Reference Standards (single) Flow Check™ Ruby Red Fluorescent Microspheres (single) Quantum™ QC
Daily QC	Optical alignment	Daily	Right Reference Standard™ Flow Check™ Alignment and Compensation Particle Sets
Daily QC	Fluidics check	Daily	Surface-labeled fluorescent microspheres, e.g. Fluorescence Reference Standards, Quantum™ MESF
Weekly QC	Optical system sensitivity, resolution for linearity (for specific lasers / PMTs)	Weekly	Quantum™ QC
Daily Set-Up	Standardized instrument set-up (PMTs)	Daily or between runs if settings are changed	Quantum™ QC
Daily Set-Up	Standardized compensation settings for multi-color analyses	Daily or between runs if settings are changed	FITC/PE Compensation Standard Simply Cellular® Compensation Standard, Quantum™ Simply Cellular® Viability Dye Compensation Standard Simply Cellular for Violet Laser
Application	Fluorescence quantitation in cellular expression studies or bead-based assays	Daily when quantitative analyses are performed or between different applications, if fluorescence PMT settings are changed	Quantum™ MESF Quantum™ Simply Cellular®
Application	F:P ratio determination for quantitative fluorescence analyses	As needed, i.e. with each new lot of fluorochrome-conjugated antibody	Simply Cellular® (used in conjunction with Quantum™ MESF)
Application	Compensation for multi-color flow cytometry	Daily or between different applications if fluorescence PMT settings are changed	FITC / PE Compensation Standard Simply Cellular® Compensation Standard Quantum™ Simply Cellular® Viability Dye Compensation Standard Simply Cellular ® for Violet Laser, Fluorescence Reference Standards (single) Flow Cytometry Antibody Binding Beads (Protein A and G)
Application	Cell counting	As needed	Flow Cytometry Absolute Count Standard™
Application	Cell size estimation	As needed	Size Calibration Standards Kit Submicron Bead Calibration Kit Micron Bead Calibration Kit
Application	Suspension array	Platform for development of bead-based flow cytometric assays	QuantumPlex™ QuantumPlex™M

Technical Data Sheets

Accessory Reagents

- TDS #606 *MicroKros Hollow Fiber Filter*
- TDS #793 *Polysciences Bead Solution*
- TDS #794 *Polysciences Bead Coupling and Storage Buffers*
- TDS #911 *Accessory Reagents*
- TDS #912 *Surfactants*

Additional Microparticles

- TDS #281 *Sporopollenin Microparticles*
- TDS #604 *Iron Powder*
- TDS #605 *Black Iron Oxide Particles*
- TDS #607 *Red Iron Oxide Particles*
- TDS #744 *Hollow Glass Beads*
- TDS #758 *Glass Beads*
- TDS #783 *Phenolic Beads, Hollow*
- TDS #784 *Polybead® Hollow Microspheres*
- TDS #857 *Glass Beads*
- TDS #858 *PLGA Uniform Dry Microspheres*

Dyed Microspheres

- TDS #808 *Polybead® Dyed Microspheres*

Flow Cytometry Products

- TDS #508 *FlowCheck™ Microspheres*
- TDS #613 *FlowCheck™: Flow Cytometry Particles and Sets*
- TDS #624 *FlowCheck™ Ruby Red Fluorescent Microspheres*
- TDS #914 *Flow Cytometry Instrument Quality Assurance / Quality Control Program*
- TDS #916 *Small Bead Calibration Kits*
- TDS #PDS 214 *QuantumPlex™ SP Streptavidin*
- TDS #PDS 215 *QuantumPlex™ Streptavidin*
- TDS #PDS 234 *QuantumPlex™ SP Carboxyl*
- TDS #PDS 235 *QuantumPlex™ Carboxyl*
- TDS #PDS 250 *QuantumPlex™M Carboxyl*
- TDS #PDS 251 *QuantumPlex™M SP Carboxyl*
- TDS #PDS 252 *QuantumPlex™M Streptavidin*
- TDS #PDS 253 *QuantumPlex™M SP Streptavidin*
- TDS #PDS 510 *Right Reference Standard™*
- TDS #PDS 612 *Ultra Rainbow Fluorescent Particles*
- TDS #PDS 704 *Fluorescence Intensity Standards*
- TDS #PDS 725 *Quantum™ QC*
- TDS #PDS 810 *Simply Cellular® anti-Mouse IgG*
- TDS #PDS 812 *Simply Cellular® anti-Human IgG*
- TDS #PDS 813 *Simply Cellular® anti-Rat IgG*
- TDS #PDS 814 *Quantum™ Simply Cellular®*
- TDS #PDS 820 *FITC / PE Compensation Standard*
- TDS #PDS 821 *Quantum™ MESF Kits*
- TDS #PDS 829 *Size Calibration Standards Kit*
- TDS #PDS 831 *Time Delay Calibration Standard*
- TDS #PDS 835 *Simply Cellular® anti-Mouse for Violet Laser*
- TDS #PDS 850 *Simply Cellular® anti-Mouse Compensation Standard*
- TDS #PDS 851 *Simply Cellular® anti-Rat Compensation Standard*
- TDS #PDS 852 *Simply Cellular® anti-Human Compensation Standard*

Flow Cytometry Products con't.

- TDS #PDS 853 *Viability Dye Compensation Standard*
- TDS #PDS 854 *Flow Cytometry Protein A and Protein G Antibody Binding Beads*
- TDS #PDS 880 *Flow Cytometry Absolute Count Standard™*
- TDS #PDS 885 *Full Spectrum™*
- TDS #PDS 890 *Fluorescence Reference Standards*
- TDS #PDS 892 *Alexa Fluor® Reference Standards*
- TDS #917 *Quantitative Cytometry*
- TDS #PDS 818 *Quantum™ Simply Cellular® and Quantum™ MESF Tips and Techniques*
- TDS #PDS 819 *QuickCal®, v 2.3 Data Analysis Program*

Fluorescent Microspheres

- TDS #431 *Fluoresbrite® Microparticles – Frequently Asked Questions*
- TDS #745 *Microsphere Excitation and Emission Spectra*
- TDS #913 *StarLight™ Calibration Slides*
- TDS #915 *Fluoresbrite® Europium Chelate Microspheres*

Informational Data Sheets

- TDS #410 *Microsphere Coating Reagents*
- TDS #430 *Phagocytosis and Microparticles*
- TDS #670 *Decontaminating Microspheres*
- TDS #753 *Streptavidin-Coated Microspheres Binding Biotinylated DNA*
- TDS #788 *Microsphere Selection*

Magnetic Microparticles

- TDS #438 *Magnetic Microparticles*
- TDS #528 *BioMag® and Cell Sorting*
- TDS #528A *BioMag® and Cell Sorting References*
- TDS #529 *BioMag® Oligo (dT) 20*
- TDS #530 *BioMag® Nuclease-free Streptavidin*
- TDS #531 *BioMag® Coupling Procedures for Attaching Oligonucleotides*
- TDS #546 *BioMag® Magnetic Immobilization Kit & BioMag® Amine*
- TDS #547 *BioMag® Superparamagnetic Iron Oxide*
- TDS #548 *BioMag® Goat anti-Rat IgG (Fc Specific)*
- TDS #549 *BioMag® Goat anti-Mouse IgG*
- TDS #550 *BioMag® Goat anti-Mouse IgG (Fc Specific)*
- TDS #551 *BioMag® Streptavidin*
- TDS #552 *BioMag® Biotin*
- TDS #553 *BioMag® Protein A*
- TDS #554 *BioMag® Protein G*
- TDS #555 *BioMag® Dextran-coated Charcoal*
- TDS #557 *BioMag® Goat anti-Rat IgM*
- TDS #558 *BioMag® Goat anti-Mouse IgM*
- TDS #559 *BioMag® Goat anti-Rabbit IgG*
- TDS #560 *BioMag® Goat anti-Rat IgG*
- TDS #561 *BioMag® Goat anti-Human IgM*
- TDS #562 *BioMag® Goat anti-Human IgG (Fc Specific)*
- TDS #563 *BioMag® Goat anti-Human IgG*
- TDS #569 *BioMag® SelectaPure mRNA Purification System*
- TDS #570 *BioMag® Carboxyl*

Magnetic Microparticles con't.

- TDS #580 *BioMag® SelectaPure anti-Human CD3*
- TDS #581 *BioMag® SelectaPure anti-Human CD4*
- TDS #583 *BioMag® SelectaPure anti-Human CD8*
- TDS #584 *BioMag® SelectaPure anti-Human CD14*
- TDS #585 *BioMag® SelectaPure anti-Human CD16*
- TDS #586 *BioMag® SelectaPure anti-Human CD19*
- TDS #587 *BioMag® SelectaPure anti-Human CD34*
- TDS #588 *BioMag® SelectaPure anti-Human CD45*
- TDS #589 *BioMag® SelectaPure anti-Human CD56*
- TDS #590 *BioMag® SelectaPure anti-Human CD71*
- TDS #591 *Cell Sorting Using BioMag® SelectaPure anti-Human Leukocyte Particles*
- TDS #592 *BioMag® SelectaPure anti-Mouse CD4*
- TDS #593 *BioMag® SelectaPure anti-Mouse CD8a*
- TDS #594 *BioMag® SelectaPure anti-Mouse CD45R*
- TDS #595 *BioMag® SelectaPure anti-Human CD2*
- TDS #596 *BioMag® SelectaPure anti-Human CD11b*
- TDS #597 *BioMag® SelectaPure Human T cell Enrichment System*
- TDS #617 *BioMag®Plus Amine & BioMag®Plus Amine Protein Coupling Kit*
- TDS #618 *BioMag®Plus Carboxyl & BioMag®Plus Carboxyl Protein Coupling Kit*
- TDS #619 *BioMag®Plus Goat anti-Mouse IgG Particle Antibody Coupling Starter Kit*
- TDS #620 *BioMag®Plus Protein A and G Particle Antibody Isolation Starter Kit*
- TDS #621 *BioMag®Plus Streptavidin & BioMag®Plus Streptavidin / Biotin Binding Starter Kit*
- TDS #658 *ProMax Albumin Removal Kit*
- TDS #659 *ProMax Serum IgG Removal Kit*
- TDS #692 *BioMag®Plus Mouse anti-Fluorescein IgG*
- TDS #721 *BioMag® Maxi Carboxyl*
- TDS #722 *BioMag® Maxi Amine*
- TDS #745 *ProMag™ HC High Capacity Magnetic Microspheres*
- TDS #755 *ProMag™ Magnetic Microspheres*
- TDS #759 *BioMag®Plus Wheat Germ Agglutinin*
- TDS #766 *BioMag®Plus Concanavalin A*
- TDS #855 *Magnetic Particles – ProMag™ and BioMag®*
- TDS #1003 *ProMag™ High Performance Magnetic Microspheres*

Magnetic Separators

- TDS #571 *BioMag® Flask Separator*
- TDS #572 *BioMag® 15ml / 50ml Tube Separator*
- TDS #573 *BioMag® 12mm x 75mm Test Tube Separator*
- TDS #574 *BioMag® Multi-32 Microcentrifuge Tube Separator*
- TDS #575 *BioMag® 96-Well Plate Separator*
- TDS #575A *BioMag® 96-Well Plate Side Pull Magnetic Separator*
- TDS #576 *BioMag® Multi-6 Microcentrifuge Tube Separator*
- TDS #577 *BioMag® Solo-Sep Microcentrifuge Tube Separator*
- TDS #791 *BioMag® MultiSep Magnetic Separator*
- TDS #796 *Biomagnetic Separators*

NIST Traceable Particle Size Standards

- TDS #623 *Precision Particles: NIST Traceable Size Standards*

Polymer Microspheres

- TDS #238 *Polybead® Polystyrene Microspheres: FAQ*
- TDS #238C *Covalent Coupling of Proteins to Carboxylated Polystyrene Microparticles by the "Carbodiimide" Method*
- TDS #238D *Covalent Coupling of Proteins to Amino & Blue Dyed Microspheres*
- TDS #238E *Protocol for Adsorbing Proteins on Polystyrene Microspheres*
- TDS #238G *Glutaraldehyde Kit for Amino & Blue Dyed Beads*
- TDS #404 *Polyballs*
- TDS #644 *PolyLink Protein Coupling Kit for COOH Microparticles*
- TDS #788 *Polybead® Microspheres*
- TDS #853 *PolyLink Protein Coupling Kit with Hollow Fiber Filtering System*
- TDS #854 *Glutaraldehyde Coupling Kit with Hollow Fiber Filtering System*
- TDS #856 *Polystyrene Beads, Large*

Protein Coated Microspheres

- TDS #615 *Protein Conjugated Microspheres*
- TDS #616 *Streptavidin & Biotin Conjugated Microspheres*

SNARe™ DNA Purification Systems

- TDS #710 *SNARe™ Whole Blood Genomic DNA Purification System*
- TDS #710A *SNARe™ Whole Blood Genomic DNA Purification 96-Well Microtiter Plate Protocol*
- TDS #711 *SNARe™ Plasmid DNA Purification System*
- TDS #712 *SNARe™ Plant Genomic DNA Purification System*

SureCount™ Particle Count Standards

- TDS #852 *SureCount™ Particle Count Standards*

Uniform Silica Microspheres

- TDS #635 *Uniform Silica Microspheres*
- TDS #792 *Silica Microspheres, Colloidal*

ViaCheck™ Viability Instrument Standards

- TDS #729 *ViaCheck™ 0% Viability Control*
- TDS #729 *ViaCheck™ 25% Viability Control*
- TDS #729 *ViaCheck™ 50% Viability Control*
- TDS #729 *ViaCheck™ 75% Viability Control*
- TDS #729 *ViaCheck™ 90% Viability Control*
- TDS #729 *ViaCheck™ 100% Viability Control*
- TDS #734 *ViaCheck™ Concentration Control (1 x 10^6)*
- TDS #734 *ViaCheck™ Concentration Control (4 x 10^6)*
- TDS #734 *ViaCheck™ Concentration Control (8 x 10^6)*