Polysciences, Inc.
Chemistry beyond the ordinary

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Polybead® Polystyrene Microspheres: Frequently Asked Questions

Introduction

Polystyrene microspheres are used in many applications, including lateral flow tests, latex agglutination tests, flow cytometry, fluorescence microscopy, and as calibration particles. Polysciences, Inc. offers a wide selection of polystyrene-based microspheres, with diameters ranging from 0.05µm to 90µm within our Polybead® line. We also offer particles with visible and fluorescent dyes. Both our dyed and undyed particles are available with amino and carboxyl surface chemistries.

The information contained in this section covers many of the frequently asked questions about polystyrene microspheres. Please contact us with any further questions you might have.

General Characteristics

Parameter	Description
Size	0.05-90µm for Polybead®; 40nm-175µm NIST Traceable, some very large diameters (200µm +) are also available
Monodispersity	Coefficient of variance ≤ 10% for size range 0.5-90.0µm
Concentration	2.5% (w/v)
Suspending Medium	DI water with residual surfactant
Color	Available undyed, or as visibly dyed or fluorescent preparations
Functionality	Plain, COOH, $-NH_2$, -OH, and $-CH_2Cl$ acrylated
Chemical Stability	Inert, safe for handling and ideal for biological studies
Protein affinity	Covalent coupling or passive adsorption possible
Glass transition (*T*g)	~94˚C, stable to moderate heating temperature; some diameters feature a low-level of divinylbenzene (DVB) crosslinking, which may raise the *T*g
Bead density	~1.05g/cm^3, similar to cell densities
Refractive index at 589nm	~1.59-1.60, ideally suited for instrumentation applications
Biocides	None (except where noted), particles are compatible with azide, thimerosal, and other treatments

Custom preparations are available.

Particle Handling and Principles

Microsphere Monodispersity

The chart below lists our specifications for the uniformity of our Polybead® microspheres, expressed as the coefficient of variance (CV). The actual diameter (D) and the standard deviation (SD) for each lot are printed on the label. The % CV is expressed as the SD/D x100.

Diameter (µm)	*CV Maximum*	*Diameter (µm)*	*CV Maximum*
0.05	≤ 15%	0.20	≤ 5%
0.10	≤ 15%	0.35	≤ 5%

Diameter (µm)	CV Maximum	Diameter (µm)	CV Maximum
0.50	≤ 3%	3.00	≤ 5%
0.75	≤ 3%	4.50	≤ 7%
1.00	≤ 3%	6.00	≤ 10%
1.50	≤ 5%	10.00	≤ 10%
2.00	≤ 5%	15.00 - 90.00	≤ 10%

Microsphere Size

Diameters specified in product descriptions reflect nominal sizes. For most products, the specific diameter and standard deviation will be printed on the label.

Microsphere Stability

Polysciences offers a one year shelf life. Unless noted, neither biocides nor stabilizers are added and the particles are shipped in DI water with residual surfactant. All polystyrene products should be stored at 4˚C to deter 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.

Particle Suspension

Most of our products are supplied at 2.5% solids, i.e. every 100ml of the latex suspension contains 2.5 grams of polymer spheres. Higher concentrations are available for bulk shipments. Weight to volume packaging means that the number of particles per ml will vary with the diameter of the particle. The approximate number of particles per ml for common diameters follow. Bead counts were calculated using the nominal diameter and equation provided below.

Diameter (µm)	Particles/ml (2.5% solids-suspension)	Diameter (µm)	Particles/ml (2.5% solids-suspension)
0.05	3.64×10^{14}	4.50	4.99×10^{8}
0.10	4.55×10^{13}	6.00	2.10×10^{8}
0.20	5.68×10^{12}	10.0	4.55×10^{7}
0.35	1.06×10^{12}	15.0	1.35×10^{7}
0.50	3.64×10^{11}	20.0	5.68×10^{6}
0.75	1.08×10^{11}	25.0	2.91×10^{6}
1.00	4.55×10^{10}	45.0	4.99×10^{5}
1.50	1.35×10^{10}	75.0	1.08×10^{5}
2.00	5.68×10^{9}	90.0	6.24×10^{4}
3.00	1.68×10^{9}		

The following equation can be used to calculate the number of particles per ml: $\frac{6W \times 10^{12}}{\rho \times \pi \times \phi^3}$

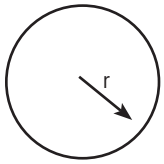
where: W = grams of polymer per ml in latex (0.025g for a 2.5% latex)
ϕ = diameter in microns of latex particles (consult label)
ρ = density of polymer in grams per ml (1.05 for polystyrene)

Are the Microspheres Crosslinked with DVB (divinylbenzene)?

Some of the larger size polystyrene particles are in fact a copolymer of polystyrene and DVB. For a listing of which catalog numbers contain DVB, please consult the chart.

Particle Size	Catalog Number	Particle Size	Catalog Number
4.5µm	17135	20.0µm	18329
6.0µm	07312	25.0µm	07313
10.0µm	17136	45.0µm	07312
15.0µm	18328	75.0µm	24049
20µm	18329	90.0µm	07315
25µm	07313		

Surface to Volume Ratios
Use the formula to the right as a rough guide to estimate the surface area or the volume of a sphere.



Surface = $4\pi r^2$

Volume = $\frac{4\pi r^3}{3}$

Centrifugation
Washing particles may be accomplished using centrifugation. This procedure must be performed carefully. Excess centrifugation will result in resuspension difficulties. For the purposes of pelletizing, 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 in cm/sec
g = g force in cm/sec^2
ρ^1 = density of particle in g/cm^3
ρ^2 = density of suspending media in g/cm^3
n = coefficient of viscosity in poises (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 in micron, ρ_1 = 1.05 g/cm^3, ρ_2 = 1.00g/cm^3 and n = 1.002 cp.

$$V = 2.77 \times 10^{-6} d^2$$

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 particle placed in a microcentrifuge generating 10,000 G will settle at a velocity of 2.77 x 10^{-2} cm/sec. Pelletizing 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 settling rate.

Are the Particles Stable?
Particles are stable as shipped if kept free of microbial contamination. Unless noted, neither biocides nor stabilizers are added and the particles are shipped in DI water with residual surfactant. All polystyrene microspheres should be stored at 4˚C and protected from freezing. If long term storage is required, the addition of biocides is recommended. Microspheres may settle over time and rolling / rotation is recommended to ensure a monodisperse suspension. Surfactant and careful sonication may be used to address stickiness or aggregation.

What about 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 putting biocides or preservatives into the product upon receipt. Degradation of the particles, their functional groups, or the incorporated dyes are rare and our primary concern is the DI water. We make every effort to insure 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 can be gamma irradiated, which results in some darkening of the products. Additions of biocides, such as thimerosal 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.

Can Tissues That Contain Particles Be Embedded?
Latex microspheres have been visualized by light microscopy in unembedded coverslip monolayers, in fixed or unfixed frozen sections, in paraffin sections, and glycerol methacrylate kits. For paraffin sections, n-butyl alcohol must be used for clearing and deparaffination

since the typical solvents, such as 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.

Dyed Particles

What Types of Dyes are Used?
Non-water soluble dyes are used to internally dye Polybead® visibly dyed and Fluoresbrite® particles. This minimizes incidents of dye leaching from the particles into aqueous buffers. Visibly dyed microspheres are available as green, orange, black, blue, red, violet and yellow. Other colors and intensities are available at the customer's request.

Several dyes are used to fabricate Fluoresbrite® particles. Polysciences can custom manufacture a Fluoresbrite® particle with a customer's dye of choice. Polyscience's most popular dyed particles match the following filter settings:

Dyed and Fluorescent Particle	*Filter Setting*	*Excitation Max. (nm)*	*Emission Max. (nm)*
BB (Bright Blue)	Coumarin	360	407
YG (Yellow Green)	Fluorescein	441	486
YO (Yellow Orange)	Rhodamine	529	546
Polychromatic Red	Phycoerythrin	491 & 512	554
Ruby Red		475	663

What is the Smallest Dyed Particle That Can Be Seen Under Light Microscopy Conditions?
A 6µm visibly dyed (non-fluorescent) particle is the smallest colored particle that 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.

See also TDS 431, *Fluoresbrite® Microparticles: Frequently Asked Questions,* TDS 745, *Microsphere Excitation and Emission Spectra*, and TDS 788, *Polybead® Microspheres* for further information regarding dyed and fluorescent microspheres.

Coating to Microspheres

Is Passive Adsorption Stable?
Most techniques in today's market using passive adsorption technology report 4-6 months of bead stability. Covalent binding may be used for longest stability.

Which Method Should Be Used - Passive Adsorption or Covalent Attachment?
Some applications demand covalent techniques. Two major areas include materials with low affinity for polystyrene and cases where a component of the assay will displace passively adsorbed material. Surfactant is a notorious example of a material that can displace proteins from the bead's surface. If surfactant is required as an additive in the assay, covalent coupling procedures are recommended. Additionally, ligands such as ssDNA and peptides require single-point attachment to ensure that they will bind to their complement or target. Synthetic sequences may be designed to include a reactive group for end-point covalent attachment.

What Advantages Does Amino Functionality Offer?
Amino functional beads couple proteins with glutaraldehyde. Glutaraldehyde is more stable than carbodiimide reagents used with carboxylate beads. Coupling with glutaraldehyde results in proteins being bound 11-12 carbon atoms away from the surface of the bead versus 2-3 carbon atoms, as in the case of carboxylate beads coupled using carbodiimide.

In Addition to Proteins, Can Any Other Biological Materials Be Coupled?
Particles have been used to bind DNA, lectins, enzymes, peptides, etc.

Is There a Recommended 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 will obscure the activity of smaller, active proteins. Glycine or small polypeptides may be used as alternatives.

Can Coupling Protocols Be Used for 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 residual reagents. This is not practical for particle sizes less than 0.5µm, since 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 can be utilized, such as dialysis, spin filters, or forced membrane filtration. Polysciences offers coupling kits that use hollow fiber filtration techniques to effect separations of 0.1-0.5µm particles.

Can Proteins Be Coupled to Fluoresbrite® Particles?
As internally dyed beads, the surfaces remain available for protein adsorption or covalent linking via functional groups. Polysciences' capability to manufacture custom lots with exact sizing and relative control of dye content per bead has enhanced its prominence as a world leader in supplying the diagnostic assay market.

See also TDS 778, *Microsphere Selection*, for further discussion of coating strategy selection.

Adsorbing Protein on Beads
(TDS 238E)

Plain Beads
Initial Buffer: 0.1 M Borate Buffer, pH 8.5

1. Suspend in buffer, spin down and resuspend 2-3 times.
2. Suspend in borate buffer.
3. Add protein and mix end-to-end overnight.
4. Spin and save supernatant for protein determination.
5. Resuspend in BSA in appropriate buffer and spin down 2 times.
6. Resuspend in PBS, pH 7.4, containing BSA and glycerol (storage buffer).

Result: Protein bound directly on surface.

Coupling by Carbodiimide
(TDS 238C & 644)

Carboxylate Functional Beads
0.1 M Carbonate Buffer

1. Suspend in buffer, spin down and resuspend 2-3 times.
2. Suspend in phosphate buffer.
3. Add fresh carbodiimide solution dropwise and incubate 20-30 minutes, end to end.
4. Wash to remove excess carbodiimide, then resuspend in borate buffer.
5. Add protein and mix end-to-end overnight.
6. Add ethanolamine, mix for 30 minutes.
7. Spin and save supernatant for protein determination.
8. Resuspend in BSA in appropriate buffer and spin down 2 times.
9. Resuspend in PBS, pH 7.4, containing BSA and glycerol (storage buffer).

Result: Protein bound 2-3 carbon atoms from surface.

Coupling by Glutaraldehyde
(TDS 238D & 238G)

Amino or Blue Dyed Beads
0.02 M PBS, pH 7.4

1. Suspend in buffer, spin down and resuspend 2-3 times.
2. Suspend in PBS.
3. Suspend in 8% glutaraldehyde in PBS, pH 7.4, and mix 4-6 hours maximum, end to end.
4. Wash to remove excess glutaraldehyde, then resuspend in PBS buffer.
5. Add protein and mix end-to-end overnight.
6. Add ethanolamine, mix for 30 minutes.
7. Spin and save supernatant for protein determination.
8. Resuspend in BSA in appropriate buffer and spin down 2 times.
9. Resuspend in PBS, pH 7.4, containing BSA and glycerol (storage buffer).

Result: Protein bound 5 carbon atoms from surface of blue dyed beads and 11-12 carbon atoms from surface of amino beads.

Protein Coupling Troubleshooting

Problem	Solution
Clumping prior to use	Careful sonication
Clumping after procedure	Isolate which step causes clumping
• Carbodiimide addition causes clumping	Add slowly, agitate beads, decrease bead concentration
• Glutaraldehyde addition causes clumping	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 the protein coupling
• Protein addition causes clumping	Increase protein concentration
• Washing causes clumping	Add surfactant or reduce washing steps
Low binding	Move pH of binding 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 fibers for small particles
Nonspecific adsorption	Use an alternative for BSA (glycine, casein)
Small proteins bound, but not reactive	Use a crosslinking agent with a spacer to extend coupling away from the surface of the bead
Long-term storage leaches protein	Try covalent attachment or lyophilize final product

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