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Polystyrene Microspheres: Frequently Asked Questions

INTRODUCTION:

Polysciences, Inc. offers a wide selection of polystyrene microspheres. Diameters range from 0.05 microns to over 90 microns. We also offer particles with both visible and fluorescent dyes. Both our dyed and non-dyed particles are available with amino and carboxyl surface chemistries. Polystyrene microspheres are used for lateral flow tests, latex agglutination tests, flow cytometry, fluorescence microscopy, and as calibration particles to name just a few applications. The information contained in this technical data sheet covers many of the frequently asked questions about polystyrene microspheres. Please contact us with any questions you might have.

GENERAL CHARACTERISTICS:

Parameter	Benefits
Size	0.05 - 90µ for a wide range of applications
Monodispersity	Coefficient of variance < 3% for size range 0.5 - 1.0µ
Concentration	2.5% - 20%, according to customer requirements
Suspending Medium	DI water, to ensure stable dispersions
Color	No-dye, red, yellow, blue, violet; and several fluorescent colors.
Functionality	Plain, COOH, -NH_2, -OH, -CH_2Cl acrylated, and other functionalities to suit customer requirements
Stability	Inert, safe for handling and ideal for biological studies
Protein affinity	Covalent coupling or passive adsorption possible
Glass transition temperature	100°C, stable to moderate heating
Bead density	1.05g/ml, similar to cell densities
Refractive index	1.6000, ideally suited for instrumentation application
Additives or residues	No surfactant, or polymerization residues to interfere with biochemical reactions
Biocides	None (except where noted), particles are compatible with azide or thimerosal treatments

PARTICLE HANDLING AND PRINCIPLES:

MICROSPHERE MONODISPERSITY

The chart below 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.

Size in Microns	CV Maximum
0.05	≤ 15%
0.10	≤ 10%
0.20	≤ 5%
0.35	≤ 5%
0.50	≤ 3%
0.75	≤ 3%
1.00	≤ 3%
2.00	≤ 5%
3.00	≤ 5%
4.50	≤ 7%
6.00	≤ 10%
10.00	≤ 10%
>10.00	≤ 15%

MICROSPHERE SIZE

All sizes in this catalog are nominal sizes. The exact size for your particles will be printed on the label with the standard deviation.

Should any of our materials fail to perform to our specifications, we will be pleased to provide replacements or return the purchase price. We solicit your inquiries concerning all needs for life sciences work. The information given in this bulletin is to the best of our knowledge accurate, but no warranty is expressed or implied. It is the user's responsibility to determine the suitability for his own use of the products described herein, and since conditions of use are beyond our control, we disclaim all liability with respect to the use of any material supplied by us. Nothing contained herein shall be construed as a recommendation to use any product or to practice any process in violation of any law or any government regulation.

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PARTICLE SUSPENSION

For most of our products, 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. Number of particles per ml for frequently used sizes are:

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

The following equation can be used to calculate the number of particles per ml:

$$\text{Number of particles per ml} = \frac{6W \times 10^{12}}{p \times \pi \times \varnothing^3}$$

W = grams of particles per ml of suspension (0.025 g/ml for 2.5% solids)
∅ = diameter in microns of latex particles
p = density of polymer in grams per ml (1.05 for polystyrene)

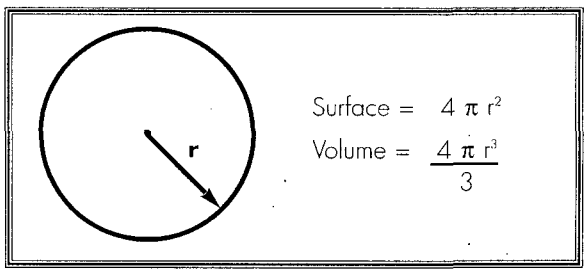
ARE THE MICROSPHERES CROSSLINKED WITH DVB (divinylbenzene)?

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, top right.

Particle Size	Catalog Number
15μ	18328
20μ	18329
25μ	07313
45μ	07314
75μ	24049
90μ	07315

SURFACE TO VOLUME RATIOS

Use the following formula as a rough guide to estimate the surface area or the volume of a sphere



The determination of the surface area of the 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 will be much greater than that predicted by the simple formula. This is particularly important for protein binding applications and charge calculations.

CENTRIFUGATION

Washing particles may be done via 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/sec²
ρ_1 = density of particle in g/cm³
ρ_2 = density of suspending media in g/cm³
n = coefficient of viscosity in poises (g/cm-sec)
a = radius of spherical particle in cm

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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/cm3, iρ_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 1.0µ 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 10.0µ particle could settle in 2 seconds under the aforementioned conditions, whereas a 0.01µ particle could take at least 4 hours to settle. Brownian motion and particle concentration also affect settling rate.

ARE THE PARTICLES STABLE?

The particles have a one year shelf life. Unless noted, neither biocides nor stabilizers are added and the particles are shipped in DI water only. All latex products should be stored at 4°C and protected from freezing. If long storage is required, the addition of biocides is recommended.

WHAT ABOUT STERILITY AND SHELF LIFE?

Our latex particles 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. 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 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 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.

CAN TISSUES WHICH 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 glycol methacrylate kits. For paraffin sections, n-butyl alcohol must be used for clearing and deparaffination since the typical organic 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 in the syntheses of Polybead dyed and Fluoresbrite™ particles. This minimizes incidents of dye leaching from the particles into aqueous buffers or dyed microspheres are available as blue, red, violet, and yellow. Other colors and intensities are available at the customers 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:

Polyscience's Fluorescent Particles		Filter Setting
BB	≈	Coumarin
YG	≈	Fluorescein
YO	≈	Rhodamine
Polychromatic	≈	Phycoerythrin

Dye	Excitation Max. (nm)	Emission Max. (nm)
BB	365	435
YG	445	500
YO	535	570
Polychromatic	535	588

Excitation and emission data listed are for dyes only. The exact excitation and emission maxima of the particles have not been determined.

WHERE IS THE DYE?

By embedding the beads and observing thin sections of the bead, it has been determined that, more often than not, the dye is on the outside 10% radii of the particles. However, for some of the yellow-dyed Polybeads, we find that the particle is intensely dyed throughout the entire particle.

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WHAT IS THE SMALLEST DYED PARTICLE THAT CAN BE SEEN UNDER LIGHT MICROSCOPY CONDITIONS?

A 6μ visibly dyed (non-flourescent) particle is the smallest colored particle that can reasonably be observed under light microscopy conditions. 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 6μ. Fluoresbrite 0.05μ particles have been identified using a fluorescent microscope set at 100x objective and 10x ocular magnification.

COUPLING TO MICROSPHERES:

IS PASSIVE ADSORPTION STABLE?

Most techniques in today's market using passive adsorption technology report four to six months of bead stability. This technology is the basis for most commercialized latex assays. The passively-bound proteins may eventually are lost from the surface of the particle.

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. Many attempts to couple DNA have been successful only with covalent coupling or with a covalently coupled linking group. 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.

WHAT ADVANTAGES DOES AMINO FUNCTIONALITY OFFER?

Amino functional beads couple proteins with glutaraldehyde. Glutaraldehyde is more stable than the carbodiimide reagents used with carboxylate beads. Coupling with glutaraldehyde results in the 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, and drugs of abuse. Coupling is possible, but modifications of binding protocols may be necessary.

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 0.5μ?

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 0.5μ, 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 or forced membrane filtration. Polysciences offers coupling kits that use hollow fiber filtration techniques to effect separation of 0.1 - 0.5μ particles.

CAN PROTEINS BE COUPLED TO FLUORESBRITE™ PARTICLES?

The surface of dyed beads are 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.

BINDING PROTOCOLS:

The following sequences serve as guidelines for protein binding. Data sheets with detailed step-by-step protocols will gladly be provided upon request.

Adsorbing Protein on Beads (TDS 238E)

Plain Beads

Initial Buffer: 0.1M Borate Buffer, pH 8.5

Suspend in buffer, spin down and resuspend 2 or 3 times.

Suspend in borate buffer.

Add protein and mix end-to-end overnight.

Spin and save supernatant for protein determination.

Resuspend in BSA in appropriate buffer and spin down twice.

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