Product information

Polystyrene Latex Beads

LB1, LB3, LB5, LB6, LB8, LB11, LB30, SD6A, SD26, SD91, CLB4, and CLB9

Product Description

Uniform latex particles were first discovered in 1947. Since then, they have been utilized in a wide variety of applications including electron microscopy and cell counter calibration, antibody mediated agglutination diagnostics, phagocytosis experiments, and many others.

Polystyrene microparticles are negative charge-stabilized colloidal particles. The microparticles are produced by polymerization of styrene under conditions that induce spontaneous coalescent bead formation. Polymerization is terminated when two chains react to make a sulfate-terminated polymer chain. These terminal sulfate groups locate on the particle surface where they interact with the aqueous phase.

Latex beads are supplied as aqueous suspensions. The percent solids concentrations are expressed as weight per weight (w/w) values.

The number of particles per milliliter (N) can be calculated using the following equation:

$$N = \frac{(6 \times 10^{10}) \times S \times P_L}{\Pi \times P_s \times d^3}$$

Where:

S = % solids (w/w)

d = diameter (µm)

P_s = density of bulk polymer (g/mL)

P_L = density of latex (g/mL)

This reduces to:

$$N = \frac{1.828 \times 1011}{d^3}$$

When:

S = 10% solids

P_s = 1.05 g/mL (polystyrene)

P_L = 1.005 g/mL

The refractive index at 589 nm for polystyrene is 1.5905, and 1.602 at 486 nm.

The latex bead suspensions are composed mainly of polymer particles and water, with small amounts of surfactant, sodium bicarbonate and potassium sulfate. A typical latex bead contains the following:

- Water >69.0%
- Polymer 30.0%
- Surfactant 0.1–0.5%
- Inorganic salts 0.2%

Carboxylate and amine modified latex bead suspensions also contain 0.3–1.5% water-soluble polymer. The surfactant is added to the suspension in order to stabilize the suspension. Removal of the surfactant will cause the beads to be more susceptible to flocculation.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Resuspension

Particles larger than 0.6 µm tend to settle on prolonged storage. Particles can be easily resuspended by gentle agitation until they are returned to a uniform suspension. Depending on the particle size uniform redispersion may take several hours. Mild ultrasonic agitation can also be used.

Diluting Particles

Particles can often be diluted with deionized water. However, some particles, especially at very dilute concentrations, may require additional surfactant. An anionic surfactant, especially alkyl sulfonates such as sodium dodecyl sulfate, can be used. Nonionic surfactants such as Triton™ X-100 and TWEEN® 20 can also be used. Suggested working concentration of surfactant should be <0.1% in the aqueous phase. Addition of electrolytes may cause destabilization of the particle suspension. In this case nonionic detergents may be more suitable in the diluent.

Concentrating Particles

Particles can be concentrated by gravity settling, centrifugation, diafiltration, or evaporation. One-micron particles will settle at an approximate rate of 1.57 mm/day. Larger particles will settle faster. Smaller particles (<0.5 µm) will never settle by gravity alone.

Centrifugation time can be calculated from the following equation:

$$V_m = 5.488 \times 10^{-5} \times (P_s - 1) \times d^2$$

Where:

V_m = maximum settling velocity

p_s = density of bulk polymer
(1.05 g/cm^3 for polystyrene)

d = particle diameter (µm)

For a 5% suspension:

$$V_h = 2/3 \times g \times V_m$$

Where:

V_h = True settling velocity of a 5% suspension (cm/hr)

g = g force

As an example, 1 mL of a 1% (w/w) 0.3 µm bead suspension can be spun down in about 10 minutes at 17,000 rpm. Longer centrifugation times will be required if a tightly compacted pellet is desired.

Centrifugation should be terminated without the use of the centrifuge brake. The supernatant should be siphoned off, not decanted. The addition of surfactant before settling or centrifugation may prevent over-compaction of the latex pellet. Resuspension can be accomplished by gentle agitation or by probe-type sonication.

Particles can also be concentrated by diafiltration. High molecular weight cut-off membranes (up to 1 million) are most efficient.

Storage/Stability

They may be stored at room temperature or refrigerated. However, they should be protected from freezing. Latex beads that have been frozen look like cottage cheese.

Latex beads are not considered sterile.

Procedures

Cleaning Latex Beads

Your protocol may require that the existing surfactant and/or inorganic salts be removed from the latex bead suspension. This may be accomplished by centrifugation, diafiltration or dialysis. High molecular weight cut off membranes (up to 1 million) should be used for these applications.

Sterilization

Pasteurization

Heating beads for 24 hours at 70 °C has been shown to be an effective method for killing most microbes in latex preparations.

Tantalization

Several cycles alternating between 37 °C and 60 °C have been reported to kill bacteria by induction of spore vegetation at 37 °C and killing vegetative cells at 60 °C.

γ-Irradiation

A dose of 0.03 megarad/hr for 24 hours (0.72 megarad) has been shown to control most microbes. However, a few yeast cells may survive up to 3.0 megarads.

UV Irradiation

UV irradiation can be used on very dilute suspensions (<0.1% solids). Suspensions should appear clear for this method to work.

Antimicrobials

The addition of common antimicrobials such as Sodium azide, thimerosal, and formaldehyde can be used to prevent microbial growth.

Coupling Procedures

Passive Adsorption

Many proteins can be passively adsorbed on the particle surface. The most popular use of latex beads is in the diagnostic use of adsorbed antibodies or antigens. The multivalent nature of antibodies allows them to bind to antigens on more than one latex bead simultaneously, causing the latex beads to agglutinate. Other proteins and ligands can be covalently bound to the particle surface.

Proteins and other biochemicals can be passively adsorbed on the bead surface by simply dissolving in a buffer, adding to a bead suspension, and stirring for times ranging from a few minutes to more than a day.[1–4]

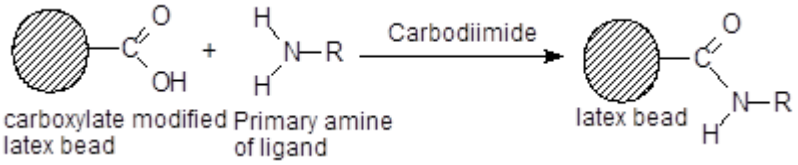
A typical protein adsorption procedure would use 1 mL of 1% bead suspension in a buffer such as 25–50 mM MES, pH 6.1, containing a final protein concentration up to 2 mg/mL. The adsorption reaction is stopped by centrifuging the suspension and resuspending the pellet in 1 mL of buffer. Centrifugation and resuspension should be repeated until the unbound protein is no longer present in the supernatant.

Protein coupling tends to decrease with increasing pH due to anionic repulsion. MES buffer typically gives good results. Phosphate buffer under acidic conditions typically gives poor results. Low protein binding can possibly be increased by adding more protein, decreasing surfactant concentration, use of beads with a different surface charge density, changing buffer, or precipitating the protein onto the bead surface ("forced" absorption).[1,5]

Covalent Coupling – Carboxylate-Modified beads

If passive adsorption is not suitable, covalent coupling of proteins and other ligands may be a viable alternative.

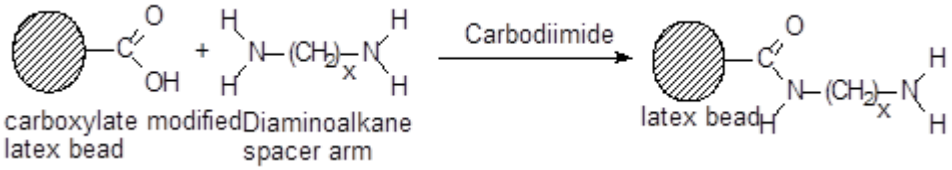
Water-soluble carbodiimide coupling agents, such as EDAC [1-ethyl-3-(3-dimethylaminopropyl) carbodiimide] can be used with carboxylate modified beads to covalently link ligands directly through primary amine groups on the ligand.[2,6,7]



In a typical carbodiimide coupling reaction 1 mL of a 1% bead suspension containing 25–50 mM MES buffer, pH 6.1, 0.15–2.6 mM EDAC (Catalog Number E7750) and up to 2 mg/mL of protein. Reaction termination and washing steps would be the same as mentioned above in the passive adsorption method.

An alternate method involves the formation of an intermediate active ester.[8] One advantage of the active ester method is that it allows separation of the ligand from the carbodiimide, thus preventing possible crosslinking of the ligand.

The carboxylate modified beads can also be converted to amine modified beads containing a spacer arm via the carbodiimide method.[7,9,10]



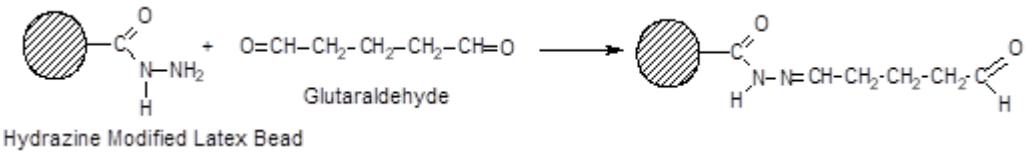
This is accomplished by reacting the beads with spacers such as diamino pentane. These spacer arms are particularly useful in antibody and other protein applications where stearic hinderance is a common problem. Subsequent protein coupling can be accomplished by glutaraldehyde and other crosslinking methods (see coupling procedures for amine-modified beads below).

Similarly, 6-aminocaproic acid can be used to create particles with carboxylate modified surfaces attached to the bead surface through spacer arms.[9]

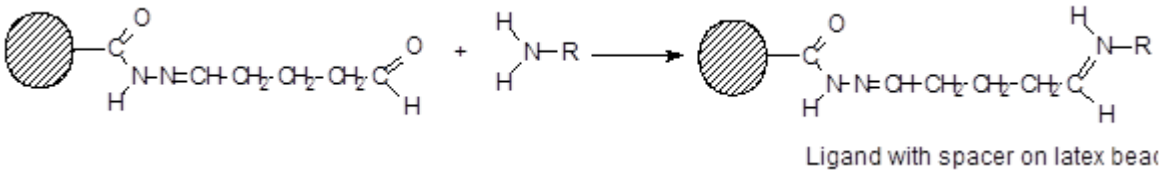
Covalent Coupling – Amine-Modified beads

Because of the need to maintain the stability of the latex suspension in aqueous systems, amine modified beads are typically first treated in aqueous systems with hydrazine, yielding an active hydrazide form.[11]

The hydrazine-modified particles are very versatile since so many different reactions can be performed on their surfaces.[12] The easiest methods for subsequent coupling employ bifunctional compounds such as glutaraldehyde.[12] In this two-step reaction, hydrazine-modified beads are first treated with glutaraldehyde at 25 °C for four hours.



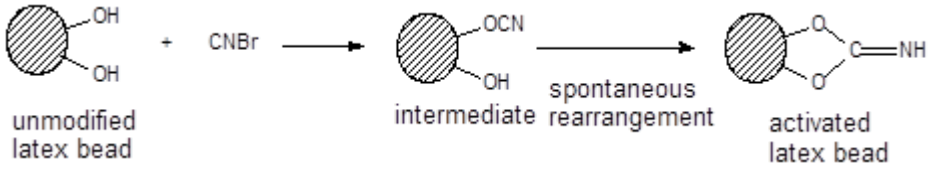
The second reaction couples the ligand to the beads and can be performed a 2–5 °C for 4 to 7 days.



Glutaraldehyde can also be used to couple ligands directly to the amine groups on amine modified beads.[9]

Covalent Coupling to Polyhydroxyl Modified Polystyrene Beads

Ligands with primary amine groups can be coupled to the surface hydroxyls of Polyhydroxyl modified beads via cyanogen bromide activation.[13–15]



Other methods include tosyl chloride or trifluoroethane sulfonyl chloride activation,[16–18] carbonyl diimidazole,[18–20] and direct nitration/amination.[21]

Dyeing Particles

Fluorescent dyes can be easily covalently coupled to the surface of modified particles.[9] Dyeing the inside of the particle may be desired in order to minimize interference with exterior-coupled ligands. Oil soluble dyes are typically used. Large, crosslinked particles (>5 µm) are dried and added directly to the dye dissolved in an organic solvent.[22] Smaller particles require careful adjustment of dye quantities in a biphasic coupling reaction.[23]

Applications

The most popular use of latex beads today is in the field of immunodiagnostics, particularly in latex agglutination tests. This method allows the detection of minute amounts of antigens or antibodies in serum, urine, and cerebrospinal fluids.[1]

Antigen-coated latex beads will agglutinate in the presence of minute amounts of antibody forming visible aggregates. The same visible agglutination occurs when the latex beads are coated with antibodies and exposed to antigens. Another popular method is the latex agglutination inhibition test. This method uses the indication that agglutination inhibition is observed in mixtures of antigen-bound beads and antibodies when free antigens are introduced.

Latex beads are also being used in phagocytosis research.[24–27] Typically latex beads being used in these applications range from <0.05 µm to 3 µm in size.

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