CD Bioparticles

Particles Coating Procedures

Please read this instruction manual carefully before using the product

Page Finder

1. Introduction

The following information explains generalized protocols for the attachment of ligands to polystyrene particles. They are to be utilized only as initial conditions. CD Bioparticles encourages the optimization of the coating conditions by changing the buffer, pH or reagents concentration.

In general, polyclonal antibodies are coated to polystyrene particles by adsorption without using any coupling agents. The binding of polyclonal antibodies to polystyrene particles is strong. However, care should be taken not to overload the antibodies to the particles. If over loading occurs, leaching of the coated antibody will happen during storage. This is due to the weak interaction between antibody molecules compared to the interaction of antibody molecules to the surface of polystyrene particles.

If different size particles are used, the antibody to particle ratio will need to be adjusted according to the surface area of the particles. When the same solid weight of particles is used the total surface area is inversely proportional to the size of the particles.

The buffer pH during passive adsorption of antibody to the particles can range from pH 5 to pH 9, where pH 5.0 is sodium acetate, pH 6, 7 and 8 are phosphate and pH 9 and 10 are carbonate. An acidic buffer of pH 5.0 such as MES, phosphate or acetate buffer is preferred for covalent coupling of proteins to carboxyl particles.

If the particles have to be transferred into another medium or simply washed before the application, centrifugation is a standard method. The centrifugation parameters vary in dependence on the particle diameter and matrix material. After centrifugation the particles can be suspended in the new medium by shaking, vortexing or sonication. Factors that can impact centrifugation are particle density and size, as well as the force applied and viscosity of the solution. Sedimentation rates rapidly decrease with lower sizes. Gel filtration, dialysis or diafiltration is used for challenging smaller sizes.

2. Passive Adsorption for Particle Coating

This procedure is used for the passive adsorption of immunoglobulins, antigens or other ligands to polystyrene particles

1. Add the following to a 15 mL glass centrifuge tube:
   a. 1.8 mL of phosphate buffer, 0.1 M, pH 7.4
   b. 0.2 mL of 1 mg/mL protein solution
   c. 0.2 mL of 5% w/v 0.8 µm polystyrene particles
2. Vortex and incubate for at least one hour at ambient temperature.
3. Centrifuge at 10,000xg for 15 minutes.
4. Remove the supernatant carefully.
5. Add 4 mL of Isotonic Buffered Saline (IBS).
6. Mix well using a vortex mixer.
7. Centrifuge at 10,000xg for 15 minutes.
8. Remove the supernatant carefully.
9. Add 4 mL of IBS and mix well to obtain 0.25% w/v suspension.

3. Coating of Amine Particles with Ligands or Proteins Using EDC

Covalent Coupling (one step EDC coupling)

1. Add the following to a glass centrifuge tube:
   a. 2 mL of 0.05M MES buffer, pH 5.0
   b. 2 mg of ligands or proteins
   c. 2 mL of 5% w/v 0.8 µm Amino particles
   d. 20 mg of EDC (Sigma, E7750)
2. Vortex and incubate for two hours at ambient temperature on a rotary mixer or with occasional vortexing or shaking.
3. Centrifuge at 10,000xg for 15 minutes.
4. Remove the supernatant carefully.
5. Resuspend the pellet in 4 mL of Isotonic Buffered Saline.
6. Repeat Steps 3 and 4 and resuspend the pellet in 2 mL of IBS to obtain 2 mL of 5% w/v suspension.

4. Coating of Carboxyl Particles with Avidin Using EDC

Covalent Coupling (one step EDC coupling):

This procedure is also for covalent coupling of other proteins such as monoclonal or polyclonal antibodies, antigens or other ligands. Acidic buffers such as phosphate, 0.1M or MES, 0.05 M can be used instead of acetate buffer.

1. Add the following to a 15 mL glass centrifuge tube:
   a. 2 mL of sodium acetate buffer, 0.01 M, pH 5.0

b. 2 mg of Avidin or Streptavidin
c. 2 mL of 5% w/v 0.8 µm Carboxyl particles
d. 20 mg of EDC (Sigma, E7750)

2. Vortex and incubate for two hours at ambient temperature on a rotary mixer or with occasional vortexing or shaking.
3. Centrifuge at 10,000xg for 15 minutes.
4. Remove the supernatant carefully.
5. Resuspend the pellet in 4 mL of Isotonic Buffered Saline.
6. Repeat Steps 3 and 4 and resuspend the pellet in 2 mL of IBS to obtain 2 mL of 5% w/v suspension.

Covalent Coupling (two step EDC coupling):

1. For two step EDC coupling, wash the particles with coupling buffer, centrifuge and remove ~80% of the supernatant.
2. Add EDC to the pellet, mix, and incubate for 1 hour.
3. Wash the particles with coupling buffer and resuspend with protein solution.
4. Continue with Steps 2 to 6 of the Covalent Coupling (one step) procedure.

5. Coating of Avidin Particles with Biotinylated Proteins Affinity Coupling

1. Add the following to a 15 mL glass centrifuge tube:
a. 2.0 mL of biotinylated protein (100 µg/mL protein) in sodium phosphate buffer (PB), 0.1 M, pH 5.5
b. 0.2 mL of Avidin coated polystyrene particles, 5% w/v
2. Vortex and incubate for at least one hour at ambient temperature.
3. Centrifuge at 10,000xg for 10 minutes.
4. Remove the supernatant carefully.
5. Resuspend the pellet in 4 mL of 0.1M PB.
6. Repeat Steps 3 and 4 and resuspend the pellet in 4 mL of PB to obtain 4 mL of 0.25% w/v suspension.

Note: *This procedure is also used for coating biotin-polystyrene or magnetic particles with various avidin-protein conjugates or other avidin-ligand conjugates.*

6. Coating of Protein to Hydroxyl Particles Covalent Coupling using Cyanogen Bromide (CNBr)

1. Add 2 mL of 1.25% w/v 0.8 µm hydroxyl polystyrene particles to a centrifuge tube:
2. Adjust the pH to 10.5 with 1N NaOH.
3. Add 10 mg of CNBr in a fume hood.
4. Readjust the pH to 10.5 with 1N NaOH.
5. Incubate for 15 minutes.
6. Add 2 mL of cold borate buffer (0.1M, pH 8.5).
7. Cool to 4°C and add 1 mL of protein at a concentration of 1 mg/mL.
8. Incubate at 4°C for at least four hours.
9. Add 5 mL of glycine buffer (0.1M, pH 8.5).
10. Centrifuge for 30 minutes at 5,000 xg.
11. Remove the supernatant and resuspend the pellet in 10 mL of 0.1M phosphate buffer, pH 7.2.
12. Repeat Steps 10 and 11 twice to give 10 mL of particles at 0.25%.

7. Coating of Dimethylamino Particles with DNA Ionic Interaction Coupling

1. Add the following to a 15 mL glass centrifuge tube:
a. 100 µL of 0.25% w/v 0.8 µm dimethylamino particles in carbonate buffer, 0.1 M, pH 9.0
b. 5.0 µL of DNA (200 ng/mL) in 0.1M carbonate buffer, pH 9.0
2. Vortex and incubate for three hours at ambient temperature.
3. Centrifuge at 10,000xg for 15 minutes.
4. Remove the supernatant and resuspend the pellet in 150.0 µL of 0.1M carbonate buffer, pH 9.0.
5. Centrifuge at 10,000xg for 15 minutes.
6. Remove the supernatant and resuspend the pellet in 100.0 µL of 0.1M tris buffer, pH 7.5. Final particle concentration is 0.25%w/v. Store refrigerated.

8. Coating of Epoxy Particles with Proteins

1. Add 1 mL of 5 µm, 5% w/v epoxy polystyrene particles to a microfuge tube.
2. Microfuge at 15000 rpm for 20 seconds.
3. Aspirate the supernatant and resuspend the pellet in 1 mL of 0.1M carbonate buffer, pH 9.0.
4. Add 0.25 mL of proteins in 0.25 mL of carbonate buffer.
5. Rotate at 60°C for at least 20 hours.
6. Microfuge at 15000 rpm for 20 seconds and wash two times with PBS (0.1M, pH 7.4). Resuspend to 5 mL with PBS to get 5 mL of 1% particles.

Note: *For 3.0 µm epoxy polystyrene particles use 0.5 mL of proteins in 0.5 mL of carbonate buffer.*

9. Periodate Oxidation of Polysaccharide and Coupling to Amino Particles

1. Add a solution containing 1 mg of sodium m-periodate (Sigma S-1878) in 1 mL of deionized water dropwise with stiring to a solution containing 10 mg of polysaccharide in 2 ml of deionized water.
2. Stir the mixture at room temperature for 30 minutes and add 10 µL of 1 M ethylene glycol to the mixture. After five minutes, add the mixture to the packed 0.8 µm amino polystyrene particles obtained from 5 mL of 5% w/v suspension by centrifugation at 10,000xg for for 10 minutes.
3. Adjust the pH of the mixture between 9.0 to 9.5 with 10% K2CO3 and stir the mixture at room temperature for at least 45 minutes.
4. Add 6 mg of sodium cyanoborohydride (Sigma S-8628) to the mixture and stir the mixture at room temperature overnight.
5. Wash the particles twice with 5 mL of deionized water and resuspend the particles in 5 mL of 0.1 M PBS containing 100 mg of BSA.
6. Stir the mixture at room temperature for two hours and wash the particles twice as before.
7. Resuspend the particles in 5 mL of 0.1M PBS to give 5 mL of 5% w/v suspension.

10. Coating of Carboxyl Polystyrene Particles with Amino Modified Oligonucleotides

1. Add 2.5x106 carboxyl polystyrene particles to 62 µL of 0.1M MES (2-[N-morpholino]ethanesulfonic acid)
2. Add 5 nmoles of amino modified oligonucleotide in 25 µL of 0.1M MES
3. Add 0.3 mg of EDC(1-ethyl-3(-3-dimethylaminopropyl) carbomiimide hydrochloride)
4. Vortex and incubate for 20 minutes at ambient temperature.
5. Add 0.3 mg of EDC.
6. Repeat Steps 4 and 5.
7. Incubate for another 80 minutes on a rotary mixer.
8. Centrifuge and remove the supernatant carefully.
9. Resuspend the pellet in 1 mL of 0.1M PBS containing 0.02%Tween-20.
10. Repeat Step 8 and resuspend the pellet in 150 µL of 10mM Tris [hydroxymethyl]aminomethane hydrochloride / 1 mL EDTA (ethylenediamine-tetraacetic acid) pH 8.0 (TE)
11. Centrifuge and remove the supernatant carefully.
12. Resuspend the pellet in 200 µL of TE or IBS. Store at 4°C.

11. Important Notes

1. In this technical note, CD Bioparticles has recommended different initial procedures to produce tests and assays that provide good sensitivity and stability using polystyrene particles. Since the quality of the coated particles depends on the quality of reagents and on the coating procedures, high quality reagents should be used while optimizing the coating conditions. As a result of CD Bioparticles's lack of control over the reagents and coating condition, CD Bioparticles can not guarantee the quality or performance of the coated particles even if the provided procedures are followed.
2. Isotonic Buffered Saline (IBS) is prepared using the following formula:

NaCl	8.0g
KCl	0.28g
NaHPO4	0.275g
Na2HPO4	2.021g
Sodium Azide	0.2g
Deionized Water	1000mL