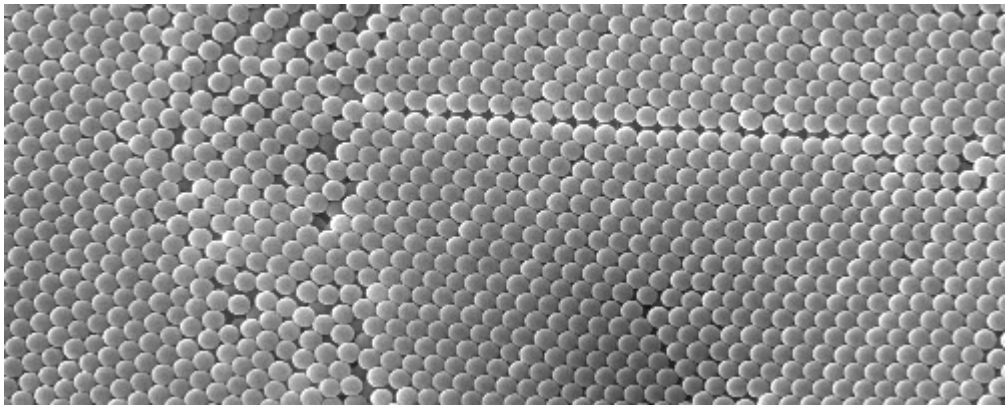
MonoPS Microspheres

Our standard polystyrene microspheres are extremely uniform with an excellent lot-to-lot reproducibility. Those microspheres are mainly devoted to hydrophobic or passive immobilization of molecules (polyclonal or monoclonal antibodies, proteins, haptens etc…) onto their surface.

The Microspheres are generally made by emulsion polymerization using different type of surfactants which usually are negatively charged. Once adsorbed onto the surface of the microspheres, those surfactants induce an increased colloidal stability. Surfactants are well known for interfering with the binding or coupling of proteins onto the microspheres. Then, it is recommended to remove all surfactant, by additional washing steps. The washing of polystyrene microspheres can be accomplished by centrifugation or cross flow filtration for particle with size of 0.4μm or larger. Most of the microspheres developers and users know how tedious washing steps are with small microspheres.

Recently, reagent microspheres are prepared without the use of surface-active agents or other contaminants, resulting in clean surfaces that are optimized for attachment of ligands. The microspheres are suspended in deionized water.



390nm Polystyrene microsphere

We offer a large range of size（0.1μm－5μm）, making this range suitable for most Microspheres-based technologies.

1. Immuno-Turbidimetric
2. Nephelemetric Assays
3. Enhanced Immuno-Turbidimetric Assays
4. Latex Agglutination Test "LAT"
5. Solid phase ImmunoAssays
6. Microspheres capture ELISA
7. Calibration

Reagent

MES Buffer
Prepare 10X stock buffer at 500 mM, pH 6.1. The pH will not change significantly on dilution. Store at 4°C and discard if yellowed or contaminated.

EDAC 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride
52 µmol/mL: Just before use, weigh approximately 10 mg of EDAC on an analytical balance: for each 10.0 mg weighed add 1.0 mL deionized water.

NHS N-hydroxysuccinimide
50 mg/mL in water (very soluble)

Protein Stocks
The protein should be completely dissolved and not too concentrated.The recommended concentration range is 1-10 mg/mL.

Adsorption of proteins and other ligands that have hydrophobic domains is the simplist and most straightforward method of attaching these substances to particle surfaces. In this method, microspheres are mixed with a solution of the desired protein and the excess unbound protein is removed by a washing procedure to obtain protein bound microspheres thar are ready for use.

Application and Advantages:

1. readily and permanently.
2. Coating the surface of polystyrene particles with proteins is successful most of the time using adsorption techniques.
3. Adsorption is adequate for the coating of most polyclonal IgG for assay systems. Other methods of coating particles can be considered if simple adsorption is inadequate.

Adsorption Procedure:

1. Set up binding reaction by pipetting into microcentrifuge tubes in the order given:
50 µL of 500 mM stock MES buffer
Water to make 1.0 mL final volume
Protein stock solution
200µL of 5％（or 100 µL of 10.0% ）solids stock microparticles

2. Mix tubes at room temperature for two hours.

3. First, pellet microparticles and decant the supernatant. Next, perform two washes with fresh 50 mM MES buffer, pH 6.1. Resuspend pellets between washes by ultrasonication.

4. Resuspend final pellet to 1.0% solids by adding 0.97 mL of the same buffer.

Technical Notes:

1. Since the total surface area of the microspheres is nversely proportional to the diameter of the particles, the amount of antibody to particles ratio needs to be adjusted accordingly.

Surface area of particles per gram: A/M = 6/PD (m^2/g)

Where D= diameter in microns of latex particles

P = density of polymer in grams per ml (1.05 for polystyrene)

For example : 0.8μm polystyrene microsphere，A/M = 6/1.05×0.8 = 7.14 m^2/g

1.6μm polystyrene microsphere，A/M = 6/1.05×1.6 = 3.57 m^2/g

Use 125－250μL polyclonal antibody for 0.8μm，10mg polystyrene microsphere

Use 62.5－125μL polyclonal antibody for 1.6μm，10mg polystyrene microsphere

2. Althrough hydrophobic adsorption is not pH dependent, the pH of the reaction buffer can have a strong effect on the conformation of the protein being adsorpted, and thus affect the efficiency of protein adsorption. Proteins tend to adsorb more efficiently at their isoelectic point, because the molecule usually has more hydrophobic sites exposed under this condition. In general, proteins have been shown to bind most efficiently at or near their isoelectric point.

3. The microsphere suspension should be added to the protein solution rapidly and with very efficient mixing.

4.Although the vast majority of ligand adsorption occurs very rapidly, the extended incubation seems to aid in achieving correct orientation by allowing an equilibrium to be reached.

Although adsorption of hydrophobic ligands to polymeric microspheres is advantageous in many situations, there are times when the hydrophobic attractive forces may not be strong enough to resist the incubation and wash steps included in many assay procedures. In other cases, the antibody in question might not be able to be adsorbed and still retain its immunoreactivity. One answer to such situations is to modify the surface of the microspheres so that covalent coupling becomes an option. Following are approaches that can be taken for such modification.

Application and Advantages:

1. More protein can be covalent bound on the microsphere surface .
2. After covalent attachment, the protein will not come off the surface. It is possible to add as much surfactant as necessary to eliminate nonspecific binding. Lot-to-lot reproducibility should be improved.
3. More thermally stable. This could be very important if the particles are to be used in PCR work, or other applications requiring thermocycling.
4. For small molecules, precious reagents and antibody pieces, the covalent coupling is the best method.

One-Step Covalent Procedure:

1. Calculate the amount of EDAC required.(0.5 to 2.5 fold molar excess over microparticle carboxyl concentration be used)

(Acid content, μmol/mg) (10 mg microparticles) (desired ratio)= μmol EDAC required
(μmol EDAC required) / (52 μmol/mL) = mL EDAC stock per mL of reaction.

2. Set up binding reaction by pipetting into microcentrifuge tubes in the order given:
50 μL of 500 mM stock buffer:
Water to make 1.0 mL final volume
Protein stock solution
200μL of 5％（or 100 μL of 10.0% ）solids stock microparticles
Mix the tubes for about 15 minutes at room temperature.

3. Prepare the EDAC solution immediately before use and mix the calculated volume rapidly into the reaction by syringing repeatedly with the pipettor.

4. Mix tubes at room temperature for one hour.

5. Remove unbound protein: first, pellet microparticles and decant the supernatant. Next, perform three washes with fresh 50 mM buffer. Resuspend pellets between washes by ultrasonication.

6. Resuspend final pellet to 1.0% solids by adding 0.97 mL of a buffer that does not contain blocking proteins. This may be the MES buffer or a higher pH buffer of your choice.

7. BCA Protein Assay: microparticle preparation can be assayed for total and covalently bound protein

8. For long term colloidal stability, a stabilizing storage buffer will be needed .

Preactivation Covalent Procedure:

1. Preactivation step, pipet into microcentrifuge tubes in the order given:
100 µL of 500 mM MES buffer
Water to make 1.0 mL final volume
200µL of 5％（or 100 µL of 10.0% ）solids stock microparticles
230 µL NHS solution: 100 mM final
EDAC solution, calculated amount

2. Mix tubes at room temperature for 30 minutes.
Centrifuge and discard supernatant. Resuspend microspheres with 1 mL 50 mM MES buffer, pH 6.1. Centrifuge again, discard supernatant.

4. Resuspend pellet by adding the following and sonicating:
100 µL 500 mM MES buffer
Water to make 1.0 mL final volume
Protein stock solution

5. Mix tubes at room temperature for 1 hour.

6. Remove unbound protein:
First, pellet microparticles and decant the supernatant. Next, perform three washes with fresh 50 mM buffer. Resuspend pellets between washes by ultrasonication.

7. Resuspend final pellet to 1.0% solids by adding 0.97 mL of a buffer that does not contain blocking proteins. This may be the MES buffer or a higher pH buffer of your choice.

8. BCA Protein Assay:
microparticle preparation can be assayed for total and covalently bound protein

9. For long term colloidal stability, a stabilizing storage buffer will be needed .

Important Notes:
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. This protocol is offered as a guide. Specific situations may require one or more alterations of this protocol.