Protocol for Coupling of Proteins to Sulfate Latex Beads by Passive Adsorption

Sulfate

Type ID: 1 (pKa ~2) 8% solids Hydrophobic

These latex microspheres are stabilized by sulfate charges. In some cases, hydroxyl and carboxyl groups are also present in small amounts bound to the polymer chains. Depending upon manufacturing conditions and particle size, the surface charge density of sulfate groups ranges from about one charge group for every 200 Å² of particle surface down to one group for every 2000 Å² of surface.

The pKa of the sulfate group is <2; consequently, the particles are stable in acidic media. Particle suspensions are stable up to almost 0.30 M univalent electrolyte concentrations and therefore may be used in media of physiological ionic strength. However, they are hydrophobic particles and will undergo aggregation in the presence of low concentrations of divalent or trivalent cations unless stabilized by a hydrophilic coating. Sulfate latex beads are suitable for calibration of particle size analysis equipment and appropriate for diagnostic test systems which rely upon physical adsorption of antigens or antibodies.

Materials

1. Protein or Antibody of choice, in MES Buffer (*See Note 1*)
2. IDC UltraClean™ Amidine Latex beads
3. MES buffer,0.025 M, pH 6 (See Note 1)
4. Micro BCA Protein Determination Kit from Pierce
5. Wash Buffer (Phosphate-Buffered Saline (PBS), 0.1 M, pH 7.2)
6. Storage Buffer (Phosphate-Buffered Saline (PBS), 0.1 M, pH 7.2,0.1% glycine; 0.1% NaN3) (*See Note 2*)

NOTES:

1. A buffer should be chosen which has a pH value close to the isoelectric point of the protein. This will maximize the protein density on the particle surface. Best results are obtained in terms of reactivity towards antigen if the pH is a little way away from the isoelectric point as this will

avoid too compacted a conformation of the antibody. Do not use buffer systems with multivalent anions (phosphate or borate) prior to coating the microspheres as these ion species will compromise the colloidal stability of the latex.

2. Glycine is used in the storage buffer to fill any reactive sites on the microsphere surface which have not been covered by the protein. This is to reduce non-specific binding. Bovine Serum Albumin (BSA) may be used for the same purpose if desired. The NaN_3 is present as a biocide. If the latex is kept sterile, NaN_3, which is not compatible with cell or tissue culture, can be omitted.

Determining Antibody and Latex Quantities

The protocol is for 1 µm Amidine Latex Beads at a concentration of 1% solids. However, this reaction can easily be scaled up or down to fit individual needs. The following equation can be used to calculate the optimal amount antibody or protein.

Example 1: If you want to label 5 mg of antibody using 1 µm particles, you will need 100 mg of particles.

$$\text{Weight of Ab} = \frac{\text{Weight of Ab for 1 µm particle}}{\text{particle diameter in µm}}$$

Example 2: For 100 mg of 0.3 µm amidine particles you would need:

$$16.7\text{ mg} = \frac{5}{0.3}$$

Example 3: for 100 mg of 4.3 µm amidine particles you would need:

$$1.2\text{ mg} = \frac{5}{4.3}$$

Latex Preparation

1. Pipette 2.5 ml (40 mg/ml) latex microspheres and dilute with 10 ml MES buffer.
2. Centrifuge the mixture to sediment the particles: ~3,000g for ~20 min.
3. Remove supernatant and re-disperse the pellet in 10 ml MES buffer.
4. Centrifuge again and remove the supernatant from the particles.

5. Re-suspend pellet in 5 ml MES buffer, being sure to completely suspend microsphere particles.

The latex suspension is now at approx. 2 % solids (i.e. ~20 mg/ml).

Protocol

In this example the antibody is prepared in MES at 1mg/ml. To 5 ml of the antibody solution in MES buffer (i.e. 5 mg of Ab) add 5 ml of the latex (this is ~200% of that required for a monolayer). This order of addition will ensure the best coating of the particles with the least possibility of aggregation.

1. Incubate latex/protein mixture with gentle mixing at room temperature overnight.
2. Centrifuge to separate the protein-labeled latex particles from unbound protein.
3. Remove and retain supernatant for protein determination. (See NOTE)
4. Resuspend the pellet in 10 ml PBS.
5. Centrifuge again to sediment the particles.
6. Repeat steps 5-6 twice more for a total of 3 washes.
7. Re-suspend the final latex in 10 ml Storage Buffer giving a final concentration of 1% solids.

Store at 4°C until used. **DO NOT FREEZE.**

NOTE:

At this step, the supernatant can be reserved and a protein determination made. Any residual

protein in the supernatant can be subtracted from the original amount added. The remainder is coated on the particles. The only method for protein determination compatible with latex microspheres is the Micro BCA Protein Determination Kit from Pierce.

Note: The method above refers to **ThermoFisher.**

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