Covalent Protein Coupling Protocol

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

1. To the latex suspension prepared above, add the following:
   (1) 50 mg/ml EDAC in MES (i.e. 100 mg of EDAC in 2 ml MES). Note: this solution should be freshly prepared.
   (2) 5 ml (i.e., 5 mg of Ab) Ab solution (this is ~200% of that required for a monolayer)
2. Incubate latex/protein mixture with gentle mixing at room temperature for 3 to 4 hours.
3. Centrifuge to separate the protein-labeled latex particles from unbound protein.
4. Remove and retain supernatant for protein determination*.
5. Resuspend the pellet in 10 ml PBS.
6. Centrifuge again to sediment the particles.

7. Repeat steps 5-6 twice more for a total of 3 washes.
8. 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: The method above refers to **ThermoFisher**.