Coating of Amino Particles with Ligands or Proteins Using EDC

Absolute Mag™ amine functionalized magnetic beads are uniform superparamagnetic beads with high density of amino group on the surface. The following information explains generalized protocols for the attachment of ligands to the polystyrene particles. The protocol is to be utilized only as initial conditions. CD encourages the optimization of the coating conditions by changing the buffer, pH or reagents concentration.

Covalent Coupling (one step EDC coupling):

1. Add the following to a 12x75 mL 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
2. Vortex and incubate for two hours at ambient temperature on a rotary mixer or with occasional vortexing or shaking.
3. Centrifuge at 3000x g 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.

Note:

1. For 4.0~4.5 µm magnetic particles, use 0.5 mg of ligands or proteins per 2 mL of 2.5% w/v magnetic particles and 5 mg of EDC.
2. For 1.0~2.0 µm magnetic particles, use 1.0 mg of ligands or proteins per mL of 2.5% w/v magnetic particles and 10 mg of EDC.
3. EDC(1-ethyl-3(-3-dimethylaminopropyl) carbomiimide hydrochloride), Sigma Chemical Cat. No. E7750

Covalent Coupling (two step EDC coupling):

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

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