Coating of Carboxyl Particles with Avidin Using EDC

Absolute Mag™ carboxyl functionalized magnetic beads are uniform superparamagnetic beads with high density of carboxyl group on the surface. The beads are used to specifically conjugate primary amine containing ligands with low non-specific binding. Briefly, the magnetic beads are activated using EDC followed by conjugation to amine groups that are present on the target protein/ligands.

Covalent Coupling (one step EDC coupling):

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
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 Avidin per 2 mL of 2.5% w/v magnetic particles and 5 mg of EDC.
2. 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.

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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