Procedure

Sera-Mag™ SpeedBeads Amine-Blocked Magnetic Particles

Sera-Mag™ SpeedBeads Amine Blocked Magnetic Particles (code number 19152104011150, 1 mL) are uniform, colloidally stable, mono-dispersed, non-porous super-paramagnetic spheres made by a proprietary core-shell method. The core is a carboxylate-modified particle made by free radical emulsion polymerization of styrene and acid monomer. Magnetite (Fe_3O_4) is coated onto this core particle and then encapsulated with propriety polymers. The final surface is blocked using a proprietary method to help prevent nonspecific binding of proteins.

Typical applications include:

- Cell separation
- Protein purification
- Positive separation

Important information before using Sera-Mag™ SpeedBeads Amine Blocked Magnetic Particles

- Refer to respective MSDS for reagents used and follow appropriate handling precautions.
- Coupling efficiency and particle performance is protein specific. As a result, this procedure should be optimized to produce best results.
- Each wash consists of magnetic separation, aspiration of supernatant, addition of buffer, and vortexing for 15 s or a time sufficient to resuspend pellet. A wash step begins with each addition of buffer. An optional sonication step in the end may be desirable to achieve a uniform suspension.
- Dilute the protein stock solution (typically 1–20 mg/mL) before addition to the particles.
- Performing a protein titration (via Micro-BCA) or binding isotherm is a good first experiment.
- Optimize the coupling buffers by choosing Buffer A, one of the two options of Buffer B, and one of the two options of Buffer C.

Materials needed

Coupling Reagents

Buffer A: 10 mM Pyridine, pH 6.0

Buffer B: 10 mM Pyridine, pH 6.0 or
50 mM Bicarbonate buffer, pH 10.0

Buffer C: 0.1 M Ethanolamine, pH 8.0 or
0.1 M Glycine, pH 8.0

Storage Solutions

Option 1: 0.05 % Sodium azide

Option 2: Buffer of choice

Coupling procedure for Sera-Mag™ Blocked Amine Magnetic Particles

1. Place desired amount of particles into a suitable container.
2. Magnetically separate and wash the particles two times with Buffer A (Each wash consists of magnetic separation, aspiration of supernatant, addition of buffer, and vortexing for 15 s or a time sufficient to resuspend pellet. A wash step begins with each addition of buffer).
3. Resuspend particles to 2.5% solids with Buffer A.
4. Add 10 µl of 25% glutaraldehyde solution per mg of particle (should bring particle concentration to 2% solids, and glutaraldehyde concentration to 5% during activation).
5. Mix (roll or mechanically stir) for 3 hours.
6. Wash particles four times with Buffer B.
7. Resuspend final pellet with Buffer B to a concentration of 2% solids.
8. Add protein stock solution to achieve desired input loading (20 µg/mg to 100 µg/mg typical input range).

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9. Dilute with Buffer B to achieve a final particle concentration of 1% solids.

10. Mix (roll or mechanically stir) overnight.

11. Magnetically separate and remove supernatant.

12. Resuspend pellet to 2% solids with Buffer B.

13. Prepare a 2.5% sodium cyanoborohydride stock solution in Buffer B.

14. Add sodium cyanoborohydride stock solution and additional Buffer B to produce 1% particle solid and 1% sodium cyanoborohydride final.

15. Mix by rolling or mechanically stirring for 1 hour.

16. Add 250 µl of Buffer C per 1 mL of reaction volume (i.e. add 2.5 mL of Buffer C to a 10 mL reaction).

17. Continue to mix (roll or mechanically stir) for 1 hour.

18. Wash four times with storage solution.

19. Resuspend to final desired particle concentration with storage solution.

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