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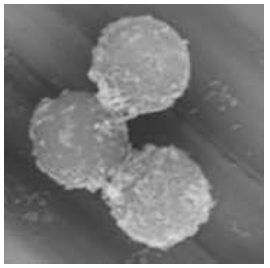
Biotinylated Microbeads and Streptavidin-Coated Magnetic Nanoparticles Binding Study V.2



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We use this protocol and it's working

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Disclaimer

Abstract

This protocol describes the preparation, biotinylation, and conjugation of 2 µm NH_2-coated microparticles with 100 nm and 250 nm streptavidin-coated magnetic nanoparticles. It includes optimized steps for biotinylation, blocking, conjugation, and parallel controls to assess non-specific binding.

Guidelines

- Prepare fresh PBS pH 8.0 buffer before each experiment.
- Perform all binding reactions at room temperature (22–25°C).
- Perform all incubations with gentle rotary agitation unless otherwise specified.
- Use gentle pipetting to avoid bead aggregation.
- Maintain magnetic nanoparticles and streptavidin-coated materials protected from light when possible.
- Always keep the EZ-Link™ reagent on ice and protected from moisture.
- Include negative controls (e.g., beads without biotin or nanoparticles without streptavidin) to evaluate non-specific binding.
- Filter-sterilize all solutions to prevent contamination.

Materials

- Magnetic nanoparticle stock solution
- 2 µm NH_2-coated microparticle stock solution
- Micromod BNF-dextran 100 nm streptavidin-coated nanoparticles
- Phosphate Buffer Saline (PBS), pH 8.0
- PBS-Glycine 100 mM solution
- EZ-Link™ Sulfo-NHS-LC-LC-Biotin linker (Thermo Fisher)
- Bovine Serum Albumin (BSA) 1% in PBS (blocking agent)
- 1.5 mL Eppendorf tubes
- Magnetic separator
- Microtube centrifuge
- Rotary agitator
- Micropipettes and sterile tips
- Laboratory scale

Safety warnings

- The EZ-Link™ reagent is moisture sensitive — minimize exposure to air and keep on ice at all times..
- Excess free biotin or streptavidin-coated nanoparticles may cause high background signals.
- Discard supernatants carefully to avoid loss of beads/nanoparticles
- Do not vortex microbeads after labeling; use gentle inversion or pipetting.

Before start

- Set up a clean workspace.
- Pre-cool centrifuge to 4°C (optional if temperature control is needed).
- Calibrate pipettes.
- Pre-cool refrigerator and freezer for storage steps.
- Prepare magnetic separator and rotary agitator setups.
- Chill PBS-Glycine solution at 4°C.
- Warm PBS to room temperature.

Preparation of 2 µm Beads

1 Transfer 979 µL of PBS (pH 8.0) into a 1.5 mL Eppendorf tube.

2 Add 21 µL of 2 µm bead stock solution and mix gently by pipetting.

3 Centrifuge at 14,000 × g for 4 min.

4 Discard the supernatant.

5 Resuspend the pellet in 1 mL PBS (pH 8.0).

6 Repeat steps 3 - 5 two more times.

Note: Resulting concentration: approx. 2.5 × 10^8 beads/mL.

Preparation of 100 nm Nanoparticles

7 Transfer 900 µL of PBS (pH 8.0) into a separate 1.5 mL Eppendorf tube.

8 Add 41.7 µL of 100 nm nanoparticle stock solution and mix gently.

9 Place the tube in a magnetic separator for 3 min.

10 Carefully discard the supernatant.

11 Repeat steps 9 - 10 two more times.

12 Resuspend the pellet in 100 µL of PBS (pH 8.0).

Note: This ensures a ratio of approximately 1000 nanoparticles per microbead when combined

Preparation of 250 nm Nanoparticles

13 Transfer 489,8 µL of PBS (pH 8.0) into a separate 1.5 mL Eppendorf tube.

14 Add **510.2 µL** µL of 250 nm nanoparticle stock solution and mix gently.

15 Place the tube in a magnetic separator for 3 min.

16 Carefully discard the supernatant.

17 Repeat steps 15 - 16 two more times.

18 Resuspend the pellet in 100 µL of PBS (pH 8.0).

Note: This ensures a ratio of approximately 1000 nanoparticles per microbead when combined

Biotinylation of Beads

19 Directly dissolve 1 mg EZ-Link™ Sulfo-NHS-LC-LC-Biotin in the bead suspension obtained in step 6.

20 Incubate for 30–60 min at room temperature under gentle rotary agitation.

21 Centrifuge at 4,000 × g for 4 min and discard the supernatant.

22 Wash the pellet with 500 µL PBS-Glycine 100 mM.

23 Repeat wash two more times.

24 Resuspend the pellet in 900 µL PBS (pH 8.0).

Blocking Step

25 Add BSA to a final concentration of 1% in the biotinylated bead suspension.

26 Incubate for 30 min at room temperature with gentle rotation.

27 Centrifuge at 4,000 × g for 4 min and resuspend in 900 µL PBS (pH 8.0).

Binding of Nanoparticles to Beads

28 Add 100 µL of the prepared 100 nm or 250 nm nanoparticle suspension (Step 12 and 18) to the 900 µL bead solution.

29 Incubate for 30 – 60 min at room temperature with gentle rotary agitation.

30 Store the final bead-nanoparticle mixture at 4°C until use.

Controls for Non-Specific Binding

31 Process an additional bead suspension without the addition of EZ-Link Biotin (no biotinylation control).

32 Process an additional nanoparticle suspension using non-streptavidin-coated nanoparticles if available.

33 Compare binding levels with experimental samples to assess background binding.