



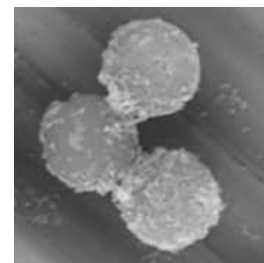
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Biotinylated Microbeads and Streptavidin-Coated Magnetic Nanoparticles Binding Study V.2

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We use this protocol and it's working

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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 μ m Beads

- 1 Transfer 979 μ L of PBS (pH 8.0) into a 1.5 mL Eppendorf tube.
- 2 Add 21 μ L of 2 μ m bead stock solution and mix gently by pipetting.
- 3 Centrifuge at $14,000 \times g$ 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 μ L of PBS (pH 8.0) into a separate 1.5 mL Eppendorf tube.
- 8 Add 41.7 μ L 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 μ L 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** 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 $\times g$ 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 μ L 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 $\times$ g for 4 min and resuspend in 900 μ L PBS (pH 8.0).



Binding of Nanoparticles to Beads

- 28 Add 100 μ L of the prepared 100 nm or 250 nm nanoparticle suspension (Step 12 and 18) to the 900 μ L 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.