Streptavidin Beads

Diameter: 1 μm (Core-shell GMA particle)
Concentration: 10 mg/ml
Ligand: Streptavidin
Type Magnetization: Superparamagnetism
Store at 2-8°C（up to 6 months）in Storage Buffer (25 mM tris-HCl, 0.15 M NaCl, 0.05% tween20, 0.05% NaN3, pH 7.2). Do not freeze the reagent.

Binding capacity

Free Biotin	> 2500 pmol
Biotinylated peptides	~ 400 pmol
Biotinylated antibody	up to 20 pmol
ds DNA	~ 20μg
ss oligonucleotides	~ 500 pmol

Recommended buffers and solutions

For coupling of Nucleic Acids	Binding and washing (B&W)Buffer (2x): 10 mM Tris-HCl (pH7.5) 1 mM EDTA 2M NaCl
For beads treatment before RNA manipulations	Solution A: DEPC-treated 0.1 M NaOH DEPC-treated 0.05 M NaCl Solution B: DEPC-treated 0.1 M NaCl
For coupling of protein or other molecules	PBS buffer pH 7.4 or PBST (PBS, pH 7.4 containing 0.1% (v/v) Tween 20)

How to use this product

Critical Notes

In the protocols we recommend keeping the tube on the magnet for up to 2 mins to ensure that all the beads are collected on the tube wall. For non-viscous samples, separation is often complete in under 1 min, once you can see the beads collected.

For diluted samples increase the incubation time or isolate in smaller batches using the same beads in each batch.

Use a mixer to tilt/rotate the tubes so beads do not settle at the tube bottom.

Avoid air bubbles during pipetting.

Free biotin in the sample will reduce the binding capacity of the beads. A disposable separation column or a spin column will remove unincorporated biotin.

Run the PCR with limiting concentrations of biotinylated primer, or remove free biotinylated primer by ultrafiltration, microdialysis or other clean-up protocols.

Both the size of the molecule to be immobilized and the biotinylation procedure will affect the binding、capacity. Large as well as small biotinylated、molecules can be immobilized. The capacity for biotinylated molecules depends on steric availability and charge interaction between bead and molecule and between molecules. There are two or three biotin binding sites available for each streptavidin molecule on the surface of the bead after immobilization.

Optimize the quantity of beads used for each individual application by titration.

Use up to two-fold excess of the binding capacity of the biotinylated molecule to saturate streptavidin.

Binding efficiency can be determined by comparing molecule concentration before and after coupling.

Immobilization Procedure

■ Bead Preparation

1) Resuspend the beads in the original vial.

2) Calculate the amount of beads required based on their binding capacity and transfer the beads to a new tube.

3) Wash beads to remove preservatives. nucleic acid applications: 1x B&W Buffer antibody/protein applications: PBS, pH 7.4

■ Washing Procedure

4) Place the tube containing the beads on a magnet for 1-2 mins.

5) Remove the supernatant by aspiration with a pipette while the tube is on the magnet.

6) Remove the tube from the magnet.

7) Add washing buffer along the inside of the tube where the beads are collected and Resuspend (same volume of washing buffer as the initial volume of beads taken from the vial or larger).

8) Repeat steps 4 to 7 twice, for a total of 3 washes.

If using beads for RNA Manipulation: As beads Streptavidin are NOT supplied in RNase-free solutions, perform the following steps after washing for RNA applications:

9) Wash the beads twice in Solution A for 2 mins. Use the same volume of beads as recommended in step 7.

10) Wash the beads once in Solution B. Use the same volume of beads as in step 9.

11) Resuspend the beads in Solution B.

The beads are now ready to be coated with the biotinylated molecule of your choice.

■ General Immobilization Protocol

Wash the beads according to section above before use.

1) Add the biotinylated molecule to the washed beads.

2) Incubate for 15-30 min at room temperature with gentle rotation of the tube.

3) Place the tube in a magnet for 2-3 mins and discard the supernatant.

4) Wash the beads 3-4 times in washing buffer.

5) Resuspend to desired concentration in a suitable buffer for your downstream use. Here are some examples of immobilization protocols for specific applications.

■ Immobilization of Nucleic Acids

1) Resuspend beads in 2x B&W Buffer to a final concentration of 5 μg/μl (twice original volume).

2) To immobilize, add an equal volume of the biotinylated DNA/RNA in H2O to dilute the NaCl concentration in the 2x B&W Buffer from 2M to 1 M for optimal binding.

3) Incubate for 15 mins at room temperature using gentle rotation. Incubation time depends on the nucleic acid length: short oligonucleotides (< 30 bases) require max. 10 mins. DNA fragments up to 1 kb require 15 mins.

4) Separate the biotinylated DNA/RNA coated beads with a magnet for 2-3 mins.

5) Wash 2–3 times with a 1x B&W Buffer.

6) Resuspend to the desired concentration. Binding is now complete. Resuspend the beads with the immobilized DNA/RNA fragment in a buffer with low salt concentration, suitable for downstream applications.

■ Release of Immobilized Biotinylated Molecules

The biotin-streptavidin bond is broken by harsh conditions. 5 mins incubation at 65°C or 2 mins at 90°C in 10 mM EDTA pH 8.2 with 95% formamide will typically dissociate >96% of immobilized biotinylated DNA. Alternatively, boil the sample for 5 mins in 0.1% SDS for protein dissociation.

Please note that proteins will be denatured by such treatment and beads Streptavidin cannot be re-used. It has also been reported that the biotin-streptavidin interaction can be broken by a short incubation in nonionic water at a temperature above 70°C.