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USER GUIDE

NanoLINK® Streptavidin Magnetic Beads

vector LABORATORIES

Together we breakthrough™

Cat. No. M-1002

Storage 2°–8°C—Do Not Freeze.

Description NanoLINK Streptavidin Magnetic Beads have the highest biotin binding capacity of any commercially available streptavidin microsphere (>12 nmol/mg beads). Based on SoluLINK® bioconjugation technology, NanoLINK Streptavidin Magnetic Beads can be used to immobilize biotinylated biomolecules including antibodies, proteins, dsDNA, and oligonucleotides. A main advantage of NanoLINK Streptavidin Magnetic Beads is the small particle size of approximately 1 µm. This large surface area to mass ratio, in combination with the high binding capacity, reduces the overall particle mass required to immobilize a biotinylated sample, leading to lower non-specific background and reduced costs. This makes NanoLINK Streptavidin Magnetic Beads an affordable solution for automated, high throughput immobilization processes. NanoLINK Streptavidin Magnetic Beads are also ideal for generating single-stranded PCR templates by removal of the unbiotinylated competing PCR strand to dramatically increase hybridization efficiency to complementary targets such as genomic DNA. NanoLINK Streptavidin Magnetic Beads are supplied at 1% solids (10 mg/ml) in nuclease-free water with 0.05% sodium azide.

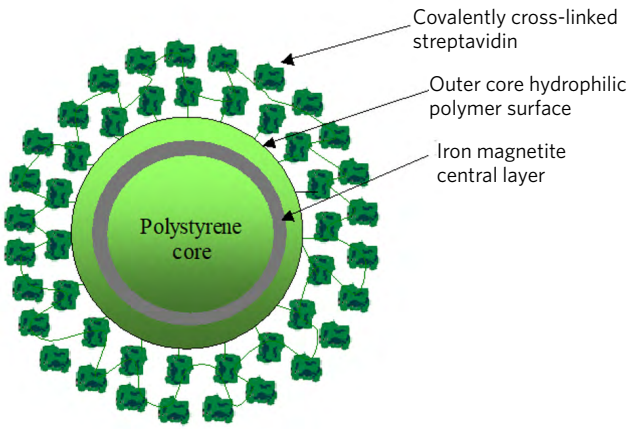

Figure 1. Cross-Section of NanoLINK Streptavidin Magnetic Beads.

Protocol

To determine the quantity of NanoLINK Streptavidin Magnetic Beads required for your application, please refer to Table 1.

Ligand	NanoLINK Bead Binding Capacity	Typical 1 µm Bead Binding Capacity
Free Biotin	>12 nmol/mg	1.3 nmol/mg
Biotinylated Oligo (23-mer)	>2.5 nmol/mg	NA
Biotinylated IgG (4 biotins per IgG)	>1.7 nmol/mg (250 µg/mg)	0.07 nmol/mg (10 µg/mg)

Table 1. NanoLINK Streptavidin Magnetic Bead binding capacity vs. typical bead of similar size. For reference, 5 µl of NanoLINK Streptavidin Magnetic Beads at 10 mg/ml (50 µg) is sufficient to bind 125 pmol of biotinylated oligonucleotide (~80 µg) or a biotinylated PCR product (~40 µg at 500 bp). This volume is also sufficient to immobilize 85 pmol of biotinylated antibody (~12.5 µg).s.

A. Washing procedure for NanoLINK Streptavidin Magnetic Beads

NanoLINK Streptavidin Magnetic Beads should be washed prior to use to remove residual azide and to equilibrate the beads in a buffer appropriate for the intended application. See the specific application sections in this user guide for recommended wash and immobilization buffers.

Note: Formulations for recommended binding and wash buffers referenced in this protocol may be found in the Appendix.

1. Resuspend NanoLINK Streptavidin Magnetic Beads in their original vial using a vortex mixer to mix vigorously for 1–2 minutes. If a bath sonicator is available, the beads may also be sonicated for 1–2 minutes to ensure a monodisperse solution.
2. Transfer the desired volume of resuspended NanoLINK Streptavidin Magnetic Beads to a new tube. At least 2 µl (20 µg) is recommended per sample for manual procedures to observe the bead pellet more easily.
3. Add sufficient wash buffer to bring the final volume to at least 250 µl, then vortex to resuspend and wash the beads.

   Note: A minimum volume of 250 µl wash buffer is recommended. For larger volumes of beads, use approximately 1 ml of buffer per 4 mg of beads, or other volume compatible with the workflow.
4. Place the tube on a magnet for 2 minutes to pellet the beads, then carefully remove and discard the supernatant without disturbing the bead pellet using a P-200 pipet.
5. Repeat steps 3 and 4 one additional time.
6. Remove the tube from the magnet and resuspend the washed beads in a suitable volume of wash buffer (a bead concentration of 2–4 mg/ml is suitable for most applications).
7. NanoLINK Streptavidin Magnetic Beads are now ready for immobilization of biotinylated biomolecule.

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B. Immobilization of biotinylated PCR products

1. Calculate the mass of NanoLINK Streptavidin Magnetic Beads required to bind the desired mass of purified biotinylated PCR product and transfer this amount to an appropriately sized tube. Table 1 may be used as a guide for these calculations.

   Note: The presence of biotinylated primers will compete with biotinylated PCR product for binding to streptavidin beads. Kits are available for purification of PCR products to remove excess primers. Follow the manufacturer's instructions to purify the PCR product prior to immobilization. If purification is not desired, use an excess of NanoLINK Streptavidin Magnetic Beads (approximately 2-fold) to account for binding of the biotinylated primers.

2. Wash the beads using Nucleic Acid Binding and Wash Buffer as described in step A, if not already performed.
3. Add the purified PCR product (free of excess biotinylated primers) to the washed NanoLINK Streptavidin Magnetic Beads.
4. Vortex gently to mix.
5. Incubate for 30 minutes at room temperature on a platform shaker to keep the beads fully suspended during the binding process. For maximum capture efficiency, do not allow the beads to settle during binding.

   Note: For biotinylated oligonucleotides and DNA fragments <2 kb, a 30-minute incubation is sufficient. For larger amplicons (≥ 5 kb), binding at 40°C for 60 minutes may be required. Inefficient biotinylation of the amplicon or the presence of biotinylated primers will lead to reduced capture efficiency.

6. After immobilization, place the tube on a magnet for 2 minutes to pellet the beads, then carefully remove the unbound supernatant without disturbing the bead pellet using a P-200 pipet. Save the supernatant for analysis, if desired.

   Note: The optical density of the supernatant can be used to estimate the amount of unbound DNA remaining (e.g., for double-stranded DNA 1 OD_{260} = 50 µg/ml, and for single-stranded DNA 1 OD_{260} = 33 µg/ml).

7. Wash the NanoLINK Streptavidin Magnetic Beads/immobilized amplicon three times using an appropriate volume of Nucleic Acid Binding and Wash Buffer.
8. After the third and final wash, carefully remove and discard the supernatant without disturbing the bead pellet using a P-200 pipet, then proceed immediately to section C.

C. Dissociation of un-biotinylated PCR strand from immobilized PCR product

1. Resuspend the DNA-coated NanoLINK Streptavidin Magnetic Beads in at least 50 µl of freshly prepared 100 mM sodium hydroxide, or 50 µl of 100 mM sodium hydroxide per 50 µg of beads used to capture the PCR product, whichever is higher.

   Note: 100 mM NaOH should be prepared daily from a 10N NaOH stock solution using ultrapure water.

2. Incubate the beads in 100 mM NaOH at room temperature for 1 minute with shaking.
3. Place the tube back on the magnet for 1 minute, then transfer the supernatant to a new labeled tube. This supernatant contains the non-biotinylated DNA strand.
4. Immediately neutralize the non-biotinylated strand by adding 5.2 µl of 1 M acetic acid for every 50 µl of 100 mM NaOH used. Confirm the pH of the neutralized solution by spotting 1 µl on 0–14 pH paper. After neutralization, store the solution at 4°C or below for later use.

   Note: If necessary, add small incremental volumes (e.g., 0.5 µl) of either 100 mM NaOH or 1 M acetic acid to achieve neutrality. Always confirm the pH of the solution by applying a 1 µl aliquot of the neutralized sample onto pH paper. A pH in the range of 7–9 is preferable for DNA.

5. Immediately wash the beads coated with the immobilized biotinylated strand three times with Nucleic Acid Binding and Wash Buffer.
6. Resuspend the NanoLINK Streptavidin Magnetic Beads (coated with the immobilized biotinylated strand) in Nucleic Acid Binding and Wash Buffer. Leave the beads in this solution at 4°C until used in other downstream applications, such as genomic DNA capture. Do not freeze the beads, as this will lead to shearing.

D. Hybridization of genomic DNA to NanoLINK Streptavidin Magnetic Beads

1. Place NanoLINK Streptavidin Magnetic Beads with immobilized biotinylated capture strand on a magnet for 2 minutes, then carefully remove the supernatant without disturbing the bead pellet using a P-200 pipet.
2. Wash the beads once with 0.25 ml of pre-hybridization buffer and carefully remove the supernatant.
3. Add 50–100 µl of pre-hybridization buffer containing heat-denatured blocking DNA to the beads and incubate on a heated shaker at 45°C for 2 hours.
4. After incubation, carefully remove the pre-hybridization blocking buffer using a magnet and add the solution containing heat-denatured genomic DNA.

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5. Gently but thoroughly mix, then place the tube back on the heated shaker. Allow the hybridization reaction to incubate for several hours to overnight.
6. Place the beads on a magnet for 2 minutes, then carefully remove the supernatant without disturbing the bead pellet.
7. Wash the beads with 0.25 ml of Stringency Wash Buffer I on a heated mixer at 45°C for 5 minutes.
8. Place the beads back on the magnet for 2 minutes, then carefully remove and discard the supernatant.
9. Repeat steps 7 and 8 using Stringency Wash Buffer II. Discard the supernatant.
10. Release hybridized genomic DNA by heating the beads in 50–100 µl of molecular biology grade water at 95°C for 5 minutes.
11. Quickly place the beads on a magnet for 1 minute, then carefully transfer the supernatant containing genomic DNA to a labeled storage tube.

E. Immobilization of biotinylated antibody

1. Refer to Table 1 to determine the mass of NanoLINK Streptavidin Magnetic Beads required to capture and immobilize the desired mass of biotinylated-IgG or other biotinylated protein.

   Note: 100 µl of NanoLINK Streptavidin Magnetic Beads at 10 mg/ml (1.0 mg) is sufficient to bind 1.7 nmol of biotinylated antibody (~250 µg) or other biotinylated protein of similar molecular weight. When using antibody-coated beads in complex samples (e.g., cell lysate), pre-blocking will reduce non-specific binding of cellular proteins to the bead surface. See section F for the recommended blocking solution and procedure.
2. Transfer the required volume of stock or pre-blocked NanoLINK Streptavidin Magnetic Beads into a 1.5 ml microcentrifuge tube.
3. Place the tube on a magnet for 2 minutes to pellet the beads, then carefully remove and discard the supernatant without disturbing the bead pellet using a P-200 pipet.
4. Wash the beads with 0.25 ml of 1X Antibody Binding and Wash Buffer. The volume of wash buffer may be increased for larger samples (e.g., 1 ml of wash buffer per 4 mg of beads).
5. Place the beads back on the magnet for 2 minutes, then carefully remove and discard the supernatant.
6. Repeat steps 4 and 5 one additional time.
7. Add 0.125 ml of 2X Antibody Binding and Wash Buffer, and 0.125 ml of biotinylated IgG sample to the NanoLINK Streptavidin Magnetic Beads. These volumes may be increased proportionally for larger sample volumes.
8. Mix the NanoLINK Streptavidin Magnetic Beads well, then incubate on a platform shaker at room temperature for 30 minutes to immobilize the biotinylated antibody.
9. After the incubation is complete, place the tube on the magnet for 2 minutes, then carefully remove the supernatant without disturbing the bead pellet.
10. Wash the NanoLINK Streptavidin Magnetic Beads twice using 0.25 ml of 1X Antibody Binding and Wash Buffer.
11. The beads with immobilized antibody are now ready for use.

F. Protein blocking procedure (for 1 mg of beads)

1. Transfer 100 µl of resuspended NanoLINK Streptavidin Magnetic Beads (at 10 mg/ml) to a 1.5 ml microcentrifuge tube.
2. Place the tube on a magnet for 2 minutes to pellet the beads, then carefully remove and discard the supernatant without disturbing the bead pellet using a P-200 pipet.
3. Add 1 ml of Blocker™ Casein in TBS and resuspend the NanoLINK Streptavidin Magnetic Beads with vigorous vortexing. Bath sonication for 1–2 minutes may also be used to ensure a monodisperse bead suspension.
4. Place the tube on a platform shaker for 30 minutes.
5. Place the tube on a magnet for 2 minutes, then carefully remove and discard the blocking solution without disturbing the pellet using a P-200 pipet.
6. Wash the beads four times with 1 ml of 1X Antibody Binding and Wash Buffer. Ensure the beads are monodisperse between washes by vigorously vortexing. Sonication may also be used, if available.
7. After the final wash, resuspend the blocked NanoLINK Streptavidin Magnetic Beads at 10 mg/ml using 100 µl of 1X Antibody Binding and Wash Buffer.
8. The blocked NanoLINK Streptavidin Magnetic Beads are now ready for use.

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Appendix:

Use the following Nucleic Acid Binding and Wash Buffers for immobilization and washing of biotinylated oligonucleotides, PCR products, or other biotinylated nucleic acids.

Nucleic Acid Binding and Wash Buffer

50 mM Tris-HCl, 150 mM NaCl, 0.05% Tween® 20, pH 8.0

Nucleic Acid Binding and Wash Buffer with Blocking DNA

50 mM Tris-HCl, 150 mM NaCl, 100 µg/ml denatured herring sperm DNA, 0.05% Tween 20, pH 8.0

Use the following buffers for genomic DNA capture and washing.

Pre-Hybridization Buffer

3X SSC Buffer, 100 µg/ml denatured herring sperm DNA, 0.05% Tween 20, pH 7.0

Note: 20X Saline Sodium Citrate (SSC) buffer = 3.0 M NaCl, 0.3 M sodium citrate, pH 7.0

Hybridization Buffer

3X SSC + 0.05% Tween 20, pH 7.0

Stringency Wash Buffer I

2X SSC + 0.05% Tween 20, pH 7.0

Stringency Wash Buffer II

0.5X SSC + 0.05% Tween 20, pH 7.0

Use the following Antibody Binding and Wash Buffers for immobilization and washing of biotinylated antibodies and other proteins.

1X Antibody Binding and Wash Buffer

50 mM Tris-HCl, 150 mM NaCl, 0.05% Tween 20, pH 8.0

2X Antibody Binding and Wash Buffer

100 mM Tris-HCl, 300 mM NaCl, 0.1% Tween 20, pH 8.0

Bead Blocking Solution

Blocker Casein in TBS is recommended for blocking NanoLINK Streptavidin Magnetic Beads. Filter the casein solution through a 0.45 µm syringe filter before use for best results.

Note: Use only Hammarsten-grade casein for blocking NanoLINK Streptavidin Magnetic Beads since other sources of casein (e.g., nonfat milk) may contain endogenous biotin.

Storage

The digoxigenin-labeled antibody should be stored at 2–8°C. A bacteriostatic agent such as 0.05% sodium azide or 0.01% thimerosal may be added to prevent microbial growth and extend shelf-life.

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