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Dynabeads™ M-270 Streptavidin

Catalog nos. 65305, 65306 **Store at 2°C to 8°C**

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Product Contents

Cat. no.	Volume	Concentration
65305	2 mL	10 mg /mL
65306	10 mL	10 mg /mL

Dynabeads™ M-270 Streptavidin contains 10 mg ($6–7 \times 10^8$) Dynabeads™/mL, in 0.0065 M phosphate buffer pH 7.4, with 0.14 M NaCl and 0.02% sodium azide as a preservative. **Caution**: Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.

Product Description

Dynabeads™ M-270 Streptavidin are ideal for nucleic acid applications, specifically with samples with a high chaotropic salt concentration, immunoassays involving small biotinylated antigens and applications that are not compatible with bovine serum albumin (BSA) (these beads are not blocked with BSA).

Add Dynabeads™ magnetic beads to a sample containing biotinylated molecules, e.g. peptides or oligonucleotides. During a short incubation, the biotinylated molecule binds to the beads. Separate the molecule-bead complex with a magnet. Capture, washing, and detection can be optimized for manual or automated use. With indirect target capture, mix the biotinylated molecule with the sample to capture the molecule-target complex before adding Dynabeads™ magnetic beads. Indirect target capture can be advantageous when molecule-target kinetics are slow, affinity is weak, molecule concentration is low, or molecule-target binding requires optimal molecule orientation and true liquid-phase kinetics.

Required Materials

- DynaMag™ Magnet (See www.lifetechnologies.com/magnets for recommendations).
- Mixer allowing tilting and rotation of tubes (e.g. HulaMixer® Sample Mixer).
- Buffers and Solutions, see Table 1.
- For biotinylation details, download the Molecular Probes® Handbook from www.lifetechnologies.com/handbook.

General Guidelines

- Keep the tube on the magnet for 2 min to ensure that all the beads are collected on the tube wall.
- For diluted samples, increase the incubation time or divide the sample into several smaller aliquots.
- Avoid air bubbles during pipetting.
- Free biotin in the sample reduces the binding capacity of the beads. A disposable separation column or a spin column can be used to remove unincorporated biotin.
- For some applications, it can be an advantage to add a detergent such as 0.01–0.1% Tween®-20 to the washing/binding buffers to reduce non-specific binding.
- Run the PCR with limiting concentrations of biotinylated primer, or remove free biotinylated primer by ultrafiltration, micro-dialysis, or other clean-up protocols. PCR Clean Up products are available from www.lifetechnologies.com.
- Use a mixer to tilt/rotate the tubes so Dynabeads™ magnetic beads do not settle at the bottom of the tube.

Table 1 Recommended buffers and solutions

For coupling of nucleic acids	For Dynabeads™ treatment before RNA manipulations
Binding and washing (B&W) Buffer (2X): 10 mM Tris-HCl (pH 7.5) 1 mM EDTA 2 M NaCl	**Solution A:** DEPC-treated 0.1 M NaOH DEPC-treated 0.05 M NaCl **Solution B:** DEPC-treated 0.1 M NaCl

The salt concentration and pH (typically 5–9) of the chosen binding/washing buffers can be varied depending on the type of molecule to be immobilized. Beads with immobilized molecules are stable in common buffers.

Both the size of the molecule to be immobilized and the biotinylation procedure will affect the binding capacity. 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.

Protocol

Recommended Washing Buffers

- Nucleic acid applications: 1X B&W Buffer (see Table 1 for recipe). Dilute to 1X B&W Buffer with distilled water.
- Antibody/protein applications: PBS, pH 7.4.

Wash Dynabeads™ Magnetic Beads

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

1. Resuspend the Dynabeads™ magnetic beads in the vial (i.e. vortex for >30 sec, or tilt and rotate for 5 min).
2. Transfer the desired volume of Dynabeads™ magnetic beads to a tube.
3. Add an equal volume of Buffer, or at least 1 mL and resuspend.
4. Place the tube on a magnet for 1 min and discard the supernatant.
5. Remove the tube from the magnet and resuspend the washed magnetic beads in the same volume of Buffer as the initial volume of Dynabeads™ magnetic beads taken from the vial (step 2).
6. Repeat steps 4–5 twice, for a total of 3 washes.

Table 2 Typical binding capacity for 1 mg (100 µL) of Dynabeads™ magnetic beads

Biotinylated target	Binding/mg
Free Biotin (pmol)	650–900
Biotinylated peptides (pmol)	~200
Biotinylated antibody (µg)	~10
ds DNA (µg) *	~10
ss oligonucleotides (pmol) *	~200

*** Oligonucleotides and DNA fragments**
For oligonucleotides, capacity is inversely related to molecule size (number of bases). Reduced binding capacity for large DNA fragments may be due to steric hindrance.

Dynabeads™ Magnetic Beads for RNA Manipulation

Dynabeads™ Streptavidin are ***not*** supplied in RNase-free solutions. When using Dynabeads™ magnetic beads for RNA applications, perform the following steps after washing:

1. Wash the beads twice in Solution A for 2 min. Use the same volume (or greater) of Solution A as the initial volume of Dynabeads™ magnetic beads taken from the vial.
2. Wash the beads once in Solution B. Use the same volume as with Solution A.
3. Resuspend the beads in Solution B.

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

Immobilization Protocol

Wash the Dynabeads™ magnetic beads according to "Wash Dynabeads™ Magnetic Beads" section before use.

1. Add the biotinylated molecule to the washed Dynabeads™ magnetic 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 min and discard the supernatant.
4. Wash the coated 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.

Immobilize 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 distilled water to dilute the NaCl concentration in the 2X B&W Buffer from 2 M to 1 M for optimal binding.
3. Incubate for 15 min at room temperature using gentle rotation. Incubation time depends on the nucleic acid length: short oligonucleotides (<30 bases) require max. 10 min. DNA fragments up to 1 kb require 15 min.
4. Separate the biotinylated DNA/RNA coated beads with a magnet for 2–3 min.
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.

Immobilize Antibodies/Proteins

1. Incubate the beads and biotinylated antibodies in PBS for 30 min at room temperature using gentle rotation.
2. Separate the antibody-coated beads with a magnet for 2–3 min.
3. Wash the coated beads 4–5 times in PBS containing 0.1% BSA.
4. Resuspend to the desired concentration for your application.

Release Immobilized Biotinylated Molecules

The biotin-streptavidin bond is broken by harsh conditions. A 5 min incubation at 65°C or 2 min at 90°C in 10 mM EDTA pH 8.2 with 95% formamide typically dissociates >96% of immobilized biotinylated DNA. Alternatively, boil the sample for 5 min in 0.1% SDS for protein dissociation. Note that proteins will be denatured by such treatment and Dynabeads™ Streptavidin can not be re-used. The biotin-streptavidin interaction can also be broken by a short incubation in non-ionic water at a temperature above 70°C.

Immunoassay Strategies

Due to their high surface area per weight, uniformity, excellent batch reproducibility and easy adaptation to automated processes, Dynabeads™ magnetic beads have become the solid phase of choice for immunoassays (www.lifetechnologies.com) *in vitro* diagnostics.

Automation

Magnetic separation and handling using Dynabeads™ magnetic beads can easily be automated on a wide variety of liquid handling platforms. Dynabeads™ MyOne™ Streptavidin C1 share similar properties to Dynabeads™ M-270 Streptavidin but are smaller, making them ideal for automation applications due to their small size, low sedimentation rate and high magnetic mobility. Selected protocols are available at www.lifetechnologies.com/automation.

Description of Materials

Dynabeads™ M-270 Streptavidin are uniform, superparamagnetic beads of 2.8 µm in diameter with a streptavidin monolayer covalently coupled to the surface. This layer ensures negligible streptavidin leakage while the lack of excess adsorbed streptavidin ensures batch consistency and reproducibility of results.

Related Products

Product	Cat. no.
Dynabeads™ M-280 Streptavidin	11205D
Dynabeads™ MyOne™ Streptavidin C1	65001
Dynabeads™ MyOne™ Streptavidin T1	65601
Dynabeads™ Kit kilobaseBINDER™*	60101
DynaMag™-2	12321D
HulaMixer® Sample Mixer	15920D

* For biotinylated DNA fragments >2 kb.

REF on labels is the symbol for catalog number.

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