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Dynabeads™ Streptavidin Trial Kit

Catalog Nos. 65801D **Store at 2°C to 8°C**

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Kit contents

Bead type	Volume	Concentration	Buffer	Bead diameter
M-280 Streptavidin	1 mL	10 mg/mL ($6–7 \times 10^8$ beads/mL)	PBS, pH 7.4 0.1% BSA 0.02% Sodium azide	2.8 µm
M-270 Streptavidin	1 mL	10 mg/mL ($6–7 \times 10^8$ beads/mL)	PBS, pH 7.4 0.09% sodium azide	2.8 µm
MyOne™ Streptavidin C1	1 mL	10 mg/mL ($7–12 \times 10^9$ beads/mL)	PBS, pH 7.4 0.01% Tween™ 20 0.09% sodium azide	1.0 µm
MyOne™ Streptavidin T1	1 mL	10 mg/mL ($7–12 \times 10^9$ beads/mL)	PBS, pH 7.4 0.1% BSA 0.02% sodium azide	1.0 µm

Sodium azide is used as a preservative. Phosphate buffered saline (PBS), bovine serum albumin (BSA).

Product description

Dynabeads™ Streptavidin magnetic beads are ideal for numerous applications, including purification of proteins, nucleic acids purification, protein interaction studies, immunoprecipitation, immunoassays, phage display, biopanning, drug screening and cell isolation.

Add Dynabeads™ magnetic beads to a sample containing biotinylated molecules. After a short incubation, the biotinylated molecules bind to the beads. The molecule-bead complex is separated using a magnet. Capture, washing and detection can be optimized for manual or automated use.

Required materials

- DynaMag™ Magnet (See **thermofisher.com/magnets** for recommendations on magnets appropriate for manual or automated protocols)
- Sample 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 **thermofisher.com/handbook**

General guidelines

All Dynabeads™ Streptavidin beads can be used with biotinylated molecules. The suitability of beads for specific applications depend on their characteristics.

Dynabeads™ M-280 Streptavidin and Dynabeads™ MyOne™ Streptavidin T1 beads are typically used for protein and nucleic acids applications.

Dynabeads™ M-270 Streptavidin and Dynabeads™ MyOne™ Streptavidin C1 beads are particularly useful for processing nucleic acid samples with a high concentration if chaotropic salts, immunoassays using small biotinylated antigens, and applications that are incompatible with bovine serum albumin (BSA) (Dynabeads™ MyOne™ beads are not blocked using BSA).

Dynabeads™ MyOne™ have increased binding capacity and lower sedimentation rate compared to standard Dynabeads™ magnetic bead, making them ideal for automated applications, and for isolating larger amounts of biotinylated ligand (e.g., oligonucleotides or peptides) or their specific targets.

- Avoid air bubbles during pipetting.
- Keep the tube on the magnet for up to 2 minuntes to ensure that all the beads are collected on the tube wall.
- For dilute samples, increase the incubation time or divide the sample into several smaller aliquots.

Table 1: Recommended buffers and solutions

For coupling of nucleic acids	For RNA applications	For coupling of proteins and other molecules
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	PBS buffer pH 7.4 Additional buffers (if needed): PBS/BSA (PBS, pH 7.4 containing 0.01% [w/v] BSA) PBST (PBS pH 7.4 containing 0.01% [v/v] Tween™ 20)

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.

For many applications, adding a detergent, such as 0.01–0.1% Tween™ 20 to the washing/binding buffers reduces non-specific binding.

DEPC-treatment:
Add DEPC to a final concentration of 0.1% (1mL/L) to Solution A or B. Shake vigorously, incubate for 1 hour at room temperature. Ready to use after autoclaving.

- Indirect target capture is recommended if 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.

  Perform indirect capture by mixing a biotinylated molecule with the sample to capture the molecule target before adding Dynabeads™ magnetic beads.
- Free biotin in the sample reduces the binding capacity of the beads.
  - For antibodies/proteins, remove unincorporated biotin using a disposable separation column or a spin column.
  - For nucleic acids, perform PCR with limiting concentrations of biotinylated primer, or remove free biotinylated primer by ultrafiltration, microdialysis, or other clean-up protocols (PCR clean-up products are available from **thermofisher.com**).
- 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.

  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.

Protocol

Recommended washing buffers

Application	Washing Buffer	Notes
Nucleic acid	1X B&W Buffer	Dilute 2X B&W Buffer (see Table 1 for recipe) with an equal volume of distilled water.
Antibody/protein	PBS, pH 7.4	

Wash Dynabeads™ magnetic beads

Calculate the amount of beads required based on their binding capacity (see Table 2, page 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 (or at least 1 mL) of Washing Buffer 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 a volume of Washing Buffer equal to 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 capacities for one mg (100 µL) of Dynabeads™ magnetic beads.

Biotinylated target	M-280 Streptavidin	M-270 Streptavidin	MyOne™ Streptavidin C1	MyOne™ Streptavidin T1
Free Biotin (pmol)	650–900	≥950	≥2500	1100–1700
Biotinylated peptides (pmol)	~200	~200	~400	~400
Biotinylated antibody (µg)	~10	~10	~20	~20
ds DNA (µg) *	~10	~10	~20	~20
ss oligonucleotides (pmol)*	~200	~200	~500	~400

*** 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 beads **are not** supplied in RNase-free solutions. Prepare the beads for RNA applications according to the following steps:

1. Wash the Dynabeads™ magnetic beads as directed in "Wash Dynabeads™ magnetic beads" (see page 1).
2. Wash the beads twice in Solution A for 2 min. Use a volume of Solution A equal to, or larger than the initial volume of Dynabeads™ magnetic beads originally taken from the vial.
3. Wash the beads once in Solution B. Use a volume of Solution B equal to the volume used for Solution A.
4. Resuspend the beads in Solution B.
5. Coat the beads with the biotinylated molecule of your choice.

Immobilization protocol

The following instructions describe a general protocol for immobilizing biotinylated molecules on the surface of Dynabeads™ MyOne™ Streptavidin C1 magnetic beads. For examples of immobilization protocols for specific applications, see "Immobilize nucleic acids" or "Immobilize antibodies/proteins".

1. Wash the Dynabeads™ magnetic beads as directed in "Wash Dynabeads™ magnetic beads" (see page 1).
2. Add the biotinylated molecule to the washed Dynabeads™ magnetic beads.
3. Incubate for 15–30 min at room temperature with gentle rotation of the tube.
4. Place the tube in a magnet for 2–3 min and discard the supernatant.
5. Wash the coated beads 3–4 times in washing buffer.
6. Resuspend to desired concentration in a suitable buffer for your downstream use.

Immobilize nucleic acids

1. Resuspend washed Dynabeads™ magnetic beads in 2X B&W Buffer to a final concentration of 5 µg/µL (twice original volume).
2. Add an equal volume of biotinylated DNA or RNA (in distilled water). Optimal binding occurs when the NaCl concentration is reduced from 2 M to 1 M.
3. Incubate for 15 min at room temperature using gentle rotation. Incubation time depends on nucleic acid length: short oligonucleotides (<30 bases) require a maximum of 10 min. DNA fragments up to 1 kb require 15 min.
4. Separate the biotinylated DNA or RNA coated beads with a magnet for 2–3 min.
5. Wash the coated beads 2–3 times with 1X B&W Buffer.
6. Resuspend to the desired concentration. Binding is now complete. Resuspend the beads with the immobilized nucleic acid fragment in a suitable buffer with low salt concentration for downstream applications.

Immobilize antibodies/proteins

1. Incubate the washed Dynabeads™ magnetic 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.

- Incubate beads in 10 mM EDTA, pH 8.2 with 95% formamide at 65°C for 5 min, or 90°C for 2 min. >96% of immobilized biotinylated DNA is typically dissociated.
- Boil the beads for 5 min in 0.1% SDS to dissociate biotinylated proteins.
- It has been reported that a short incubation in non-ionic water at >70°C can break the biotin-streptavidin interaction.

Note: proteins are denatured by such treatments. Do not re-use Dynabeads™ Streptavidin beads after releasing immobilized biotinylated molecules.

Automation

Magnetic separation and handling using Dynabeads™ magnetic beads can easily be automated on a wide variety of liquid handling platforms. Dynabeads™ MyOne™ Streptavidin beads share similar properties to Dynabeads™ M-280 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 **thermofisher.com/automation**.

Description of materials

Dynabeads™ Streptavidin beads are uniform, superparamagnetic beads with a streptavidin monolayer covalently coupled to the hydrophilic bead surface. This layer ensures negligible streptavidin leakage while the lack of excess adsorbed streptavidin ensures batch consistency and reproducibility of results. These beads give simple and stable binding of biotinylated molecules such as small molecules, peptides, proteins, antibodies, sugars, lectins, oligonucleotides, DNA/RNA, etc.

Related products

Product	Cat. No.
Dynabeads™ M-280 Streptavidin	11205D
Dynabeads™ M-270 Streptavidin	65305
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.

Important licensing information

These products may be covered by one or more Limited Use Label Licenses. By use of these products, you accept the terms and conditions of all applicable Limited Use Label Licenses.

Manufactured by Thermo Fisher Scientific Baltics UAB, V.A. Graiciuno 8, LT-02241 Vilnius, Lithuania. Thermo Fisher Scientific Baltics UAB complies with Quality System Standards ISO 9001 and ISO 13485.

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