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Dynabeads™ M-280 Streptavidin

Catalog nos. 11205D, 11206D, 60210 **Store at 2 to 8°C**

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Product Contents

Cat. no.	Volume
11205D	2 mL
11206D	10 mL
60210	100 mL

Dynabeads™ M-280 Streptavidin contains 10 mg (~6–7 × 10^8) Dynabeads™/mL in phosphate buffered saline (PBS) pH 7.4, with 0.1% bovine serum albumin (BSA) and 0.02% sodium azide as preservatives.

Product Description

Dynabeads™ M-280 Streptavidin are ideal for numerous applications, including purification of proteins and nucleic acids, protein interaction studies, immunoprecipitation, immunoassays, phage display, biopanning, drug screening and cell isolation.

Add Dynabeads™ to a sample containing biotinylated molecules, e.g. peptides, proteins, antibodies, sugars, lectins, oligonucleotides, DNA/RNA. During a short incubation, the biotinylated molecule will bind to the beads. Separate the molecule-bead capture, washing, and detection can be optimized for manual or automated use. With indirect capture, mix the biotinylated molecule with the sample to capture the molecule-target complex before adding Dynabeads™. Indirect target capture is an advantage 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

- Magnet (DynaMag™): See www.lifetechnologies.com/magnets for magnet recommendations.
- Mixing device with tilting and rotation (e.g. HulaMixer® Sample Mixer).
- Buffers and Solutions (see Table 1).
- Biotinylated compounds. For advice on biotinylation, see www.lifetechnologies.com/Dynabeads.
- 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 isolate in smaller batches using the same beads in each batch.
- 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.
- 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, microdialysis or other clean-up protocols. PCR Clean Up products are available from www.lifetechnologies.com.

Table 1: Recommended buffers and solutions

For coupling of nucleic acids	For Dynabeads™ treatment before RNA manipulations	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 These buffers can also be used for your application 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.

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™

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™ in the vial (i.e. vortex for >30 sec, or tilt and rotate for 5 min).
2. Transfer the desired volume of Dynabeads™ to a tube.
3. Add an equal volume of Washing buffer, or at least 1 mL, and mix (vortex for 5 sec, or keep on a roller for at least 5 min).
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 Dynabeads™ in the same volume of washing buffer as the initial volume of Dynabeads™ taken from the vial (step 2).

Table 2: Typical binding capacities for one mg of Dynabeads™.

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™ for RNA Manipulation

As Dynabeads™ Streptavidin are *not* supplied in RNase-free solutions, perform the following steps after washing for RNA applications:

1. Wash the beads twice in Solution A for 2 min. Use the same volume of Solution A as the initial volume of Dynabeads™ taken from the vial or larger.
2. Wash the beads once in Solution B. Use the same volume of beads as in step 5.
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™ according to "Wash Dynabeads™" section before use.

1. Add the biotinylated molecule to the washed Dynabeads™.
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 2 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. 5 min incubation at 65°C or 2 min 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 min in 0.1% SDS for protein dissociation. Please note that proteins will be denatured by such treatment and Dynabeads™ Streptavidin can not be re-used. It has also been reported that the biotin-streptavidin interaction can be broken by a short incubation in non-ionic water at a temperature above 70°C.

Automation

Magnetic separation and handling using Dynabeads™ can easily be automated on a wide variety of liquid handling platforms. Dynabeads™ MyOne™ Streptavidin T1 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 www.lifetechnologies.com/automation.

Description of Materials

Dynabeads™ 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-270 Streptavidin	65305
Dynabeads™ MyOne™ Streptavidin C1	65001
Dynabeads™ MyOne™ Streptavidin T1	65601
Dynabeads™ Kit kilobaseBINDER™	60101
DynaMag™-2	12321D
HulaMixer® Sample Mixer	15920D

REF on labels is the symbol for catalog number.

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