

Dynabeads® MyOne™ Streptavidin Trial Kit

Catalog no. 65801D

Store at 2 to 8°C

Rev. Date: October 2011 (Rev. 002)

Kit Contents

Dynabeads®	Volume (mL)	Concentration mg/mL	Supplied in	Bead diameter µm
M-280 Streptavidin	1	10 (6–7 × 10 ⁹ beads/mL)	PBS, pH 7.4 / 0.1% BSA / 0.02% Sodium azide	2.8
M-270 Streptavidin	1	10 (6–7 × 10 ⁹ beads/mL)	PBS, pH 7.4, / 0.09% Sodium azide	2.8
MyOne™ Streptavidin C1	1	10 (7–12 × 10 ⁹ beads/mL)	PBS, pH 7.4 / 0.01% Tween®-20 / 0.09% Sodium azide	1.0
MyOne™ Streptavidin T1	1	10 (7–12 × 10 ⁹ beads/mL)	PBS, pH 7.4 / 0.1% BSA / 0.02% Sodium azide	1.0

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

Product Description

Dynabeads® Streptavidin 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® to a sample containing biotinylated molecules. During a short incubation, the biotinylated molecule will bind 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 capture, the biotinylated molecule is mixed with the sample to capture the molecule-target complex before adding Dynabeads®. 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

- Magnet (DynaMag™) See www.lifetechnologies.com/magnets for recommendations.
- Mixing device with tilting and rotation, 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

All Dynabeads® Streptavidin can be used with biotinylated molecules. Some beads are more suitable for particular applications due to their characteristics. Dynabeads® M-280 Streptavidin and Dynabeads® MyOne™ Streptavidin T1 are commonly used for protein and nucleic acids applications. Dynabeads® M-270 Streptavidin and Dynabeads® MyOne™ Streptavidin C1 are preferred for nucleic acid diagnostics, specifically with samples with a high chaotropic salt concentration, immunoassays involving small biotinylated antigens and applications that are not compatible with BSA (these beads are not blocked with BSA). MyOne™ Dynabeads® offer increased binding capacity and slower sedimentation rate, making them ideal for automated applications and for when larger amounts of biotinylated ligand, or their specific target, need to be isolated.

- 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 aliquote the sample into several smaller volumes.

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.

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.

- 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.

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.

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

Table 2: Typical binding capacities for one mg (100 µL) of Dynabeads®.

Dynabeads®	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® 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 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® according to “Wash Dynabeads®” (Page 1) 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 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. 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.

Immunoassay Strategies

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

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 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.

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