invitrogen™ | DYNAL® invitrogen bead separations

Cat. no. 656.01
656.02

Rev. no. 004

Dynabeads® MyOne™ Streptavidin T1

For research use only.

INDEX

1. PRODUCT DESCRIPTION

Product no.	Volume	Concentration
656.01	2 ml	10 mg/ml*
656.02	10 ml	10 mg/ml*

*corresponds to approximately 7-10 × 10^9 Dynabeads per ml.

Description of Materials

Dynabeads MyOne Streptavidin T1 are uniform, superparamagnetic beads 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. The Dynabeads are dissolved in phosphate buffered saline (PBS) pH 7.4, containing 0.1% BSA and 0.02% NaN_3 as preservatives.

Intended Use

Dynabeads MyOne Streptavidin T1 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.
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.

Principle

With direct capture, 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 complex with a Dynal magnet. The biotinylated molecules are now immobilized on the beads and ready for any downstream applications.
With indirect capture, mix the biotinylated molecule with the sample to capture the target before adding Dynabeads. Indirect target capture can be advantageous when biotinylated molecule/target kinetics are slow, affinity is weak, target concentration is low or biotinylated molecule/target binding requires optimal molecule orientation and true liquid-phase kinetics.

Additional Materials Required

- Magnet for manual or automated protocols. See www.invitrogen.com/magnets for recommendations.
- Mixing device with tilting and rotation, eg. HulaMixer™ Sample Mixer, Cat.no. 159.20D.
- Buffers and Solutions, see table 1 below. For many applications it can be an advantage to add a detergent, e.g. 0.01-0.1% Tween® 20 to the washing/binding buffers to reduce non-specific binding.
- Biotinylated compounds. For advice on biotinylation, section 4.2 of 'The Handbook' (http://probes.invitrogen.com/handbook) gives a guide to available biotinylation reagents.

Table 1: Recommended buffers and solutions

For coupling of nucleic acids	For Dynabeads treatment before RNA manipulations	For coupling of protein or 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 0.1% (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 biotinylated molecule to be immobilized. Beads with immobilized biotinylated molecules are stable in common buffers.

CRITICAL NOTES

- In the protocols we recommend keeping the tube on the magnet for up to 2 mins to ensure that all the beads are collected on the tube wall. For non-viscous samples, separation is often complete in under 1 min, once you can see the beads collected.
- If you do not need to remove preservatives or change buffers you can omit washing of Dynabeads (section 2.1A).
- For diluted sample or large sample volumes, increase the incubation time or isolate in smaller batches using the same beads in each batch.
- Use a mixer to tilt/rotate the tubes so Dynabeads do not settle at the tube bottom.
- Avoid air bubbles during pipetting.
- Free biotin or biotinylated primer in the sample will reduce the binding capacity of the beads.

A disposable separation column or a spin column will remove unincorporated biotin. 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 Invitrogen http://www.invitrogen.com.

2. INSTRUCTIONS FOR USE

2.1 Bead Preparation

1. Resuspend the Dynabeads in the vial carefully before use, i.e.vortex for > 30 seconds or tilt and rotate for 5 minutes.
2. Calculate the amount of beads required based on their binding capacity, see table 2 and section 3, and transfer the beads to a new tube.
3. Wash Dynabeads (see below) to remove preservatives.

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

Free Biotin [pmol]	1100-1700
Biotinylated peptides [pmol]	~ 400
Biotinylated antibody [µg]	up to 20
ds DNA [µg] *)	~ 20
ss oligonucleotides [pmol] *)	~ 400

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

A. General Washing Procedure

4. Place the tube containing the beads on a magnet for 1-2 mins.
5. Remove the supernatant by aspiration with a pipette while the tube is on the magnet.
6. Remove the tube from the magnet.
7. Add washing buffer (see table 1), along the inside of the tube where the beads are collected and resuspend. Use same volume of washing buffer as the initial volume of Dynabeads taken from the vial, or larger.
8. Repeat steps 4 to 7 twice, for a total of 3 washes.

B. Washing Procedure if Using Dynabeads for RNA Manipulation

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

9. Wash the beads twice in Solution A for 2 mins. Use same volume of solution A as the initial volume of Dynabeads taken from the vial, or larger.
10. Wash the beads once in Solution B. Use the same volume of beads as in step 9.
11. Resuspend the beads in Solution B.

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

2.2 Immobilization Protocols

2.2.1 Immobilization of Antibodies/Proteins

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

Any biotinylated molecule can be immobilized using a similar protocol.

2.2.2 Immobilization of Nucleic Acids

1. Resuspend beads in 2 × 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 H_2O to dilute the NaCl concentration in the 2 × B&W Buffer from 2M to 1 M for optimal binding.
3. Incubate for 15 mins at room temperature using gentle rotation. Incubation time depends on the nucleic acid length: short oligonucleotides (<30 bases) require max. 10 mins. DNA fragments up to 1 kb require 15 mins.
4. Separate the biotinylated DNA/RNA coated beads with a magnet for 2-3 mins.
5. Wash 2–3 times with a 1 × B&W Buffer.
6. Resuspend to the desired concentration. Resuspend the beads with the immobilized DNA/RNA fragment in a buffer with low salt concentration, suitable for downstream applications. Binding is now complete.

2.3 Release of Immobilized Biotinylated Molecules

The biotin-streptavidin bond is broken by harsh conditions. 5 mins incubation at 65°C or 2 mins 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 mins in 0.1% SDS for 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 aqueous solution at temperature above 70°C (ref 1).

2.4 Immunoassay Strategies

Due to their high surface area per weight, uniformity, excellent batch reproducibility and ease of adaptation to automated processes, Dynabeads have become the solid phase of choice for developing immunoassays (http://www.invitrogen.com/IVD).

2.5 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.

3. TECHNICAL INFORMATION

Binding capacity

Both the size of the molecule to be immobilized and the biotinylation procedure will affect the binding capacity. Large as well as small biotinylated molecules can be immobilized. 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.

For an overview of our streptavidin coated Dynabeads, please visit www.invitrogen.com/streptavidin.

4. REFERENCE

1. Holmberg et al. (2005) The biotin-Streptavidin interaction can be reversibly broken using water at elevated temperatures. Electrophoresis 26, 501-510.

For a list of selected references where Dynabeads Streptavidin have been used, please visit www.invitrogen.com/streptavidin-references.

5. GENERAL INFORMATION

Invitrogen Dynal® AS complies with the Quality System Standards ISO 9001:2000 and ISO 13485:2003.

5.1 Storage and Stability

If stored unopened at 2-8°C, the Dynabeads are stable until the expiration date stated on the label. Store the vial upright to keep beads in liquid suspension, as drying of the beads will result in reduced performance. Do not freeze the product. Thoroughly resuspend the Dynabeads in the vial prior to use. Dynabeads Streptavidin are not supplied in RNase free solution. Avoid bacterial contamination of the beads.

5.2 Warnings & Limitations

This product is for research use only. Not intended for any animal or human therapeutic or diagnostic use unless otherwise stated. Sodium azide is toxic if ingested. Avoid pipetting by mouth. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. When disposing through plumbing drains, flush with large volumes of water to prevent azide buildup.
Certificate of Analysis /Compliance is available upon request.
Material Safety Data Sheet (MSDS) is available at http://www.invitrogen.com.

5.3 Trademarks & Patents

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5.6 Warranty

The products are warranted to the original purchaser only to conform to the quantity and contents stated on the vial and outer labels for the duration of the stated shelf life. Invitrogen Dynal's obligation and the purchaser's exclusive remedy under this warranty is limited either to replacement, at Invitrogen Dynal's expense, of any products which shall be defective in manufacture, and which shall be returned to Invitrogen Dynal, transportation prepaid, or at Invitrogen Dynal's option, refund of the purchase price.
Claims for merchandise damaged in transit must be submitted to the carrier.
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