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Dynabeads® M-270 Streptavidin

For research use only

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1. PRODUCT DESCRIPTION

1.1 Intended Use

Dynabeads M-270 Streptavidin are ideal 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).

1.2 Principle

Add Dynabeads to a sample containing biotinylated molecules such as peptides, oligonucleotides etc. During a short incubation, the biotinylated molecule will bind to the beads. Separate the molecule-bead complex with a Dynal magnet. 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 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.

1.3 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 hydrophilic bead surface. This layer ensures negligible streptavidin leakage while the lack of excess adsorbed Streptavidin ensures batch consistency and reproducibility of results.

Materials Supplied

Dynabeads M-270 Streptavidin are supplied as a suspension containing 10 mg (6-7 x 10^8) Dynabeads per ml, dissolved in phosphate buffered saline (PBS) pH 7.4, containing 0.09% NaN_3 as preservatives. Available in three volumes:

2 ml (Cat.no. 653.05)
10 ml (Cat. no. 653.06)
100 ml (Cat. no. 653.07).

Additional Materials Required

- Magnet for manual or automated protocols. See www.invitrogen.com/magnets-selection for recommendations.
- Mixing device with tilting and rotation.
- Buffers and Solutions (see Table 1).
- Biotinylated compounds. For advice on biotinylation, see http://www.invitrogen.com dynal. 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	For Dynabeads treatment acids 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.

2. PROTOCOLS

▲ 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.
- For diluted sample 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 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 http://www.invitrogen.com.

2.1 Immobilization Procedure

2.1.1 Bead Preparation

1. Resuspend the beads in the original vial by rotation or vortexing.
2. Calculate the amount of beads required based on their binding capacity, see table 3, and transfer the beads to a new tube.
3. Wash Dynabeads (see 2.1.2) to remove preservatives.

Recommended washing buffers:

– nucleic acid applications: 1x B&W Buffer
– antibody/protein applications: PBS, pH 7.4

Note: 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.

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

Free Biotin [pmol]	> 950
Biotinylated peptides [pmol]	~ 200
Biotinylated antibody [µg]	up to 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.

2.1.2 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 along the inside of the tube where the beads are collected and Resuspend (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.

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 the same volume of beads as recommended in step 7.
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.

2.1.3 General Immobilization Protocol

Wash the Dynabeads according to section 2.1.2 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 mins 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.

2.1.4 Immobilization of 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 H_2O to dilute the NaCl concentration in the 2x 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 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.

2.2 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 protein dissociation.

Please note that proteins will be denatured by such treatment and Dynabeads Streptavidin cannot be re-used.

It has also been reported that the biotin-streptavidin interaction can be broken by a short incubation in nonionic water at a temperature above 70°C (ref 1).

2.3 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).

2.4 Automation

Magnetic separation and handling using Dynabeads 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.invitrogen.com/automation.

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.

4. OTHER STREPTAVIDIN DYNABEADS

Bead type	Available product formats
Dynabeads® M-280 Streptavidin	2 ml (Cat. No. 112.05D) 10 ml (Cat. No. 112.06D) 100 ml (Cat. No. 602.10)
Dynabeads® MyOne™ Streptavidin C1	2 ml (Cat. No. 650.01) 10 ml (Cat. No. 650.02) 100 ml (Cat. No. 650.03)
Dynabeads® MyOne™ Streptavidin T1	2 ml (Cat. No. 656.01) 10 ml (Cat. No. 656.02) 100 ml (Cat. No. 656.03)
Dynabeads® kilobaseBINDER™	Kit (Cat. No. 601.01)

(For biotinylated DNA fragments > 2 kb)

5. 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 M-270 have been used, please visit http://www.invitrogen.com/streptavidin.

6. GENERAL INFORMATION

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

6.1 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.

6.2 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.

6.3 Trademarks & Patents

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6.6 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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