Dynabeads™ Streptavidin for Target Enrichment

Catalog Numbers 65605D, 65606D, and 65607D

Pub. No. MAN0028561 Rev. A.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

Dynabeads™ Streptavidin for Target Enrichment are uniform, 1.0 µm diameter superparamagnetic beads with a streptavidin monolayer covalently coupled to the hydrophobic bead surface. This layer ensures negligible streptavidin leakage while the lack of excess adsorbed streptavidin ensures batch consistency and reproducibility of results.

Dynabeads™ Streptavidin for Target Enrichment can be used for several applications involving streptavidin-biotin binding interactions but is ideal for enrichment of biotinylated target Nucleic Acid (NA) sequences made during the library prep as a part of the next generation sequencing (NGS) workflow. Additionally, the beads have increased binding capacity and lower sedimentation rate compared to the larger (2.8 µm) Dynabeads™ magnetic beads, making them ideal for automated applications.

Contents and storage

Product	Cat. No.	Amount	Storage
Dynabeads™ Streptavidin for Target Enrichment[1]	65605D	2 mL	Store at 2–8°C. Do not freeze.
	65606D	10 mL	
	65607D	50 mL	

[1] Dynabeads™ Streptavidin for Target Enrichment contain 10 mg/mL of magnetic beads (~7–10 × 10^9 beads/mL) in phosphate buffered saline (PBS), pH 7.4 with 0.1% bovine serum albumin (BSA), and 0.02% sodium azide as a preservative.

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Catalog numbers that appear as links open the web pages for those products.

Item	Source
DynaMag™ Magnet	thermofisher.com/magnets
Sample mixer (e.g. HulaMixer™ Sample Mixer)[1]	15920D

[1] Sample mixer needs to allow tilting and rotation of tubes

Procedural guidelines

- Avoid making air bubbles when pipetting.
- Use a mixer to tilt/rotate the tubes so Dynabeads™ magnetic beads do not settle at the tube bottom.
- When placed on the magnet, keep the tube for up to 2 minutes 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.
- Indirect sequence-specific nucleic acid capture is recommended if streptavidin molecule- target DNA kinetics are slow, affinity is weak, target molecule concentration is low, or molecule-target binding requires optimal molecule orientation and true liquid-phase kinetics.
- Perform indirect capture by mixing a biotinylated ‘bait’ oligo with the DNA sample to capture the target sequence before adding Dynabeads™ magnetic beads.

- 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.
- Magnetic separation and handling using Dynabeads™ magnetic beads can easily be automated on a wide variety of liquid handling platforms and any of the Thermo Fisher™ KingFisher™ Sample Purification Systems instruments. Selected protocols are available at **thermofisher.com/automation**

Workflow

The Dynabeads™ Streptavidin for Target Enrichment beads are intended for enrichment of the target sequences hybridized on the biotinylated probe that is immobilized to the streptavidin-coated magnetic beads (shown in red). The hybridization step can either be performed prior to the bead-based target enrichment step (indirect hybrid capture technique) or after the biotinylated probe is bound to the Dynabeads™ (direct hybrid capture technique).

Prepare buffers

Prepare buffers as shown below.

Table 1 Prepare buffers

Buffer	Component(s)
2X Binding and Washing (B&W) Buffer	• 10 mM Tris-HCl, pH 7.4 • 1 mM EDTA • 2 M NaCl
1X B&W Buffer	Dilute 2X B&W Buffer with equal volume of nuclease-free water (1:1 ratio)
(Optional) Storage Buffer	50 mM Tris HCl, pH 7.4

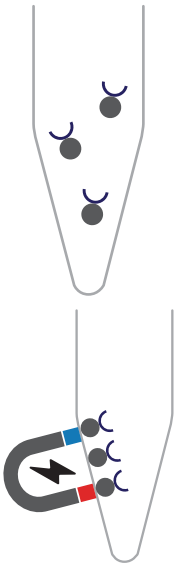
Note: The salt concentration and pH (typically 5–9) of the chosen binding/washing buffers can be varied depending on the type of molecule to be immobilized. Beads with immobilized molecules are stable in common buffers. For many applications, adding a detergent, such as 0.01–0.1% Tween™ 20 to the washing/binding buffers reduces non-specific binding.

Dynabeads™ Streptavidin for Target Enrichment

Figure 1 Dynabeads™ superparamagnetic bead with a streptavidin monolayer

Figure 2 Biotinylated probe

1 Wash magnetic beads



The beads should be washed thoroughly prior to use to remove any excess streptavidin in the solution.

1.1. Resuspend the Dynabeads™ Streptavidin for Target Enrichment completely by vortexing, then leave on a roller mixer for ≥ 20 minutes.

1.2. Transfer the desired volume of bead suspension to a new tube.

1.3. Place the tube on a magnet for 1–2 minutes, then carefully discard the supernatant.

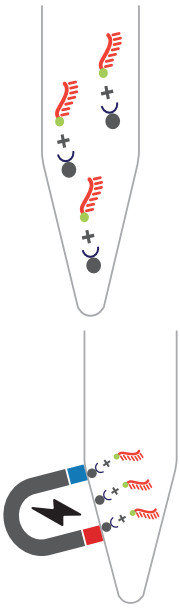
1.4. Add an equal volume of 1X B&W Buffer, then resuspend the beads by vortexing.

1.5. Place the tube on a magnet for 1–2 minutes, then carefully discard the supernatant.

1.6. For a total of three washes, repeat steps step 1.4 and step 1.5 twice.

1.7. Proceed to “Immobilize biotinylated probes”.

2 Immobilize biotinylated probes



This protocol is based on the direct hybridization technique where Dynabeads™ magnetic beads are binding to the DNA target probe before the hybridization step and thus present throughout the hybridization.

If you are using the indirect technique, the final hybrids are immobilized directly on the beads. The conditions need to be optimized depending on the application.

2.1. Resuspend the pre-washed beads in 2X B&W Buffer to a final concentration of 5 µg/µL.

Note: The volume will be twice the original volume.

2.2. Add an equal volume of biotinylated probe (in nuclease-free water).

2.3. Incubate for 15 minutes at room temperature using gentle rotation.

2.4. Separate the biotinylated DNA coated beads with a magnet for 1–2 minutes.

2.5. Wash the coated beads 2–3 times with equal volume of 1X B&W Buffer as the total volume in step 2.2.

2.6. Resuspend to the desired concentration. Continue with the hybridization process.

Note: If the beads need to be stored for a shorter period prior to the hybridization process, resuspend the beads in the Storage Buffer (10 µg/µL) and store the beads at 2–8°C.

General hybridization guidelines for Dynabeads™ Streptavidin for Target Enrichment

To maximize rate of annealing, hybridization should be done in buffers such as:

- Bait probe <200 bp: 6X SSPE / SSC
- Bait probe >200 pb: 1-3X SSPE / SSC

Hybridization time of DNA probes to targets will vary greatly depending on the type of hybridization reaction being performed.

Dynabeads™ binding capacity

- The bead volume required per test needs to be calculated based on the binding capacity (see Table 2).

Table 2 Typical binding capacity per mg (100 µL) of beads

Biotinylated target	Binding/mg (100 µL) beads
Free Biotin (pmol)	950–1500
ds DNA (µg)[1]	~20
ss oligonucleotides (pmol)[2]	~500

[1] Oligonucleotides and DNA fragments

[2] Input amount of biotinylated ss-oligonucleotides (~20 nt) in the immobilization step is 500 pmol/mg beads. 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.

Binding capacity of Dynabeads™ Streptavidin for Target Enrichment

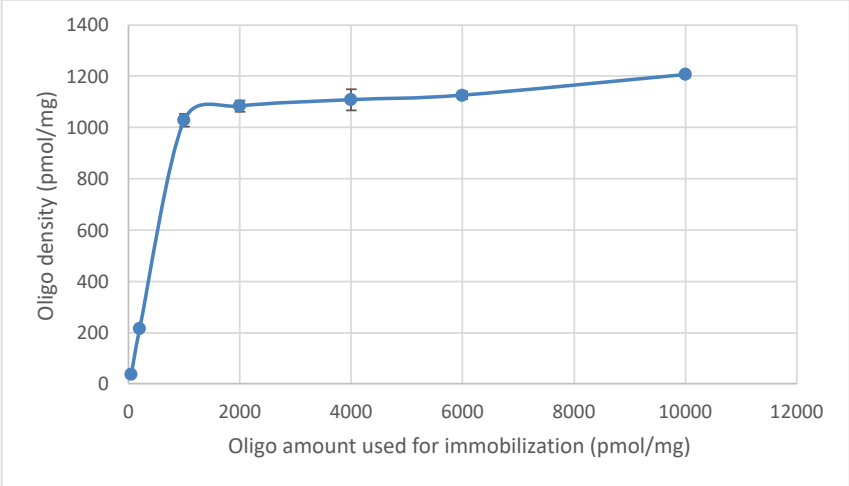


Figure 3 Immobilization of biotinylated ss-oligonucleotides

Titration study of binding capacity of Dynabeads™ Streptavidin for Target Enrichment beads using different input amount of ss-oligonucleotides (20 nt). The recommended oligo density (pmol/mg beads) falls in the middle of the linear response, 500 pmol/mg bead. 100% binding efficiency up to 1000 pm/mg (maximum capacity where the beads are saturated).

Related products

Unless otherwise indicated, all materials are available through thermofisher.com. "MLS" indicates that the material is available from fisherscientific.com or another major laboratory supplier.

Catalog numbers that appear as links open the web pages for those 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™ kilobaseBINDER™ Kit[1]	60101
UltraPure™ SSPE, 20X	15591043
UltraPure™ SSC, 20X	15557044
DynaMag™-2 Magnet	12321D
DynaMag™-96 Side	12331D
DynaMag™-96 Side Skirted	12027
DynaMag™-96 Bottom	12332D
HulaMixer™ Sample Mixer	15920D
KingFisher™ Sample Purification Systems	–[2]

[1] For biotinylated DNA fragments >2 kb.
[2] Visit www.thermofisher.com/kingfisher for instrument details.

Thermo Fisher Scientific Baltics UAB |
V.A. Graiciuno 8, LT-02241 |
Vilnius, Lithuania

For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

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Revision	Date	Description
A.0	9 January 2023	New document for Dynabeads™ Streptavidin for Target Enrichment.