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Dynabeads® kilobaseBINDER™ Kit

Catalog no. 60101 **Store at 2 to 8°C**

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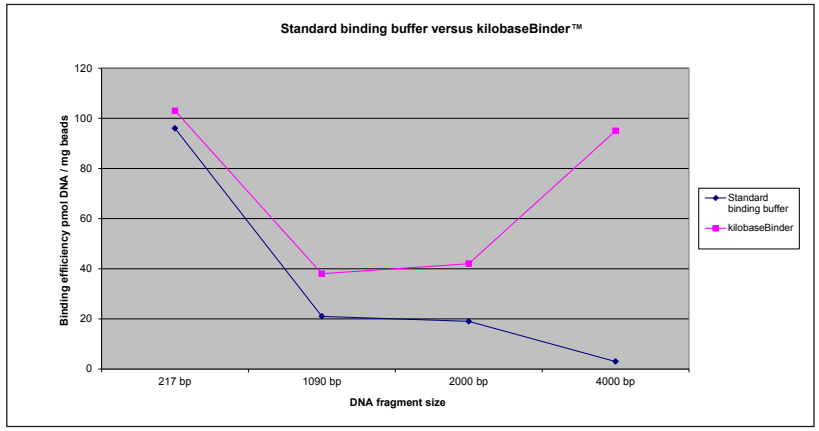

Figure 1: When working with traditional Binding buffers, the binding capacity is drastically decreased as the biotinylated DNA fragments increase in size. In contrast, the kilobaseBINDER™ Binding Solution drastically improves the DNA binding capacity when working with biotinylated DNA fragments >2 kb.

Kit Contents

Kit components	Volume
Dynabeads® M-280 Streptavidin	1 mL
Binding Solution (bottle 1)	15 mL
Washing Solution (bottle 2)	15 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 a preservative. The Washing Solution contains 10 mM Tris-HCl (pH 7.5), 1 mM EDTA, and 2.0 M NaCl. The Binding Solution composition is proprietary.

Product Description

The Dynabeads® kilobaseBINDER™ Kit is designed for immobilization of long biotinylated double-stranded DNA-fragments. The Dynabeads® M-280 Streptavidin acts as the magnetic solid-phase for simple and efficient separation of biotinylated targets (e.g. DNA/RNA, proteins, immunoglobulins, cells). Biotinylated targets bound to the beads are pulled to the tube wall by applying a magnet, and the supernatant is pipetted off.

Downstream manipulations and handling of the bead-bound DNA are easy due to the strength and stability of the biotin/streptavidin interaction and the magnetic properties of the Dynabeads®.

The Binding Solution included in the kit enhances immobilization of long (>2 kb) biotinylated DNA fragments to the beads several folds, giving an increased binding efficiency (see Figure 1).

Downstream Applications

- Pure template isolation for sequencing procedures/DNA footprinting.
- Chromatin isolation for cell biology applications.
- Intracellular transport studies of mRNA and mRNA processing (by immobilization of whole genes followed by *in vitro* transcription).
- Other functional applications may include transcription and replication studies or studies on protein-DNA interactions.

Required Materials

- Magnet (DynaMag™) See www.lifetechnologies.com/magnets for magnet recommendations.
- Mixing device with tilting and rotation, e.g. HulaMixer® Sample Mixer.

General Guidelines

- All biotin reagents should contain a spacer arm, at least 6 C-atoms in length, to reduce steric hindrance.
- Free biotin in your sample reduces the binding capacity of the Streptavidin-coupled Dynabeads®. A disposable column or a separation centrator titer device will remove unincorporated biotin from your sample.
- It is important to specifically end-label the DNA fragment with biotin to obtain an oriented immobilization to avoid random incorporation and cross-reactions of the DNA with streptavidin.
- Maximum binding capacity is 70 pmoles of 4 kb biotinylated DNA/mg Dynabeads®.

Alternative Biotinylation Procedures

- End labelling through a fill-in reaction with a biotinylated deoxyribonucleotide using the Klenow fragment of DNA polymerase I.
- End-labelling using PCR with a biotinylated primer.

Protocol

Immobilization Procedure

1. Thoroughly resuspend the Dynabeads® in the vial (vortex >30 sec or tilt and rotate for 5 min).
2. Transfer 5 µL (50 µg) resuspended beads to a 1.5 mL microcentrifuge tube. Place the tube on the magnet for 2 min.
3. Carefully remove and discard the supernatant while the tube remains on the magnet. Avoid touching the bead pellet with the pipette tip.
4. Remove the tube from the magnet. Add 20 µL Binding Solution along the inside wall of the tube where the beads are collected and gently resuspend by pipetting.
   Note: the solution may be viscous. Avoid foaming.
5. Place the tube on the magnet for 2 min and remove the supernatant.

6. Resuspend the beads in 20 µL Binding Solution.
7. Add 20 µL of a solution containing the biotinylated DNA-fragments to the resuspended beads. Mix carefully to avoid foaming of the solution.
8. Incubate the tube at room temperature for 3 hours on a roller to keep the beads in suspension.
9. Place the tube on the magnet and remove the supernatant as in step 3, above.
10. Wash the Dynabeads®/DNA-complex twice in 40 µL Washing Solution and once in distilled water or Tris-HCl pH 8.0.
11. Resuspend the Dynabeads®/DNA-complex in the appropriate buffer for your downstream application.

Note: Some applications might need an additional washing step in the appropriate downstream buffer to "condition" the Dynabeads®/DNA-complex.

Release Immobilized Biotinylated Molecules

Breaking the biotin-streptavidin bond requires harsh conditions. As an example, 5 min incubation at 65°C or 2 min at 90°C in 10 mM EDTA pH 8.2 with 95% formamide typically dissociates >96% of immobilized biotinylated DNA. Alternatively, the Dynabeads® with immobilized target molecules may be boiled for 5 min in 0.1% SDS.

Description of Material

The kit contains Dynabeads® M-280 Streptavidin (uniform, superparamagnetic, polymer beads with a monolayer of streptavidin covalently attached to the hydrophobic bead surface), a Binding Solution, and a Washing Solution.

The recombinant streptavidin employed is a protein (approx. 52 kDa) made up of four identical subunits, each containing a high-affinity binding site for biotin (KD = 10–15 M). It has the same biotin binding properties as avidin, but less non-specific binding is observed. The interaction between streptavidin and biotin is formed very rapidly and, once formed, unaffected by wide extremes of pH, temperature, organic solvents, and other denaturing agents. The streptavidin monolayer is covalently coupled to the beads, ensuring negligible leakage. The absence of excess physically-adsorbed streptavidin also ensures low batch-to-batch variations and optimal reproducibility for your application or assay.

Related Products

Product	Cat. no.
Dynabeads® M-280 Streptavidin	11205D
Dynabeads® M-270 Streptavidin	65305
Dynabeads® MyOne™ Streptavidin C1	65001
DynaMag™-2	12321D
HulaMixer® Sample Mixer	15920D

REF on labels is the symbol for catalog number.

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Manufactured by Life Technologies AS, Norway. Life Technologies AS complies with the Quality System Standards ISO 9001:2008 and ISO 13485:2003.

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