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Dynabeads® Oligo $(dT)_{25}$

Catalog nos. 61002, 61005 **Store at 2˚C to 8˚C**

Rev. Date: June 2012 (Rev. 007)

Product Contents

Cat. no.	Volume
61002	2 mL
61005	5 mL

Dynabeads® Oligo $(dT)_{25}$ are supplied as a suspension of approximately 5 mg/mL in PBS pH 7.4 containing 0.02% sodium azide as a preservative.
Caution: Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.

Product Description

Dynabeads® Oligo $(dT)_{25}$ are designed for the rapid isolation of highly purified, intact mRNA from eukaryotic total RNA or directly from crude extracts of cells, animal and plant tissues. The isolated mRNA can be used directly in most downstream applications in molecular biology: RT-PCR, solid-phase cDNA library construction, S1 nuclease analysis, ribonuclease protection assay, primer extension, dot and slot hybridization, *in vitro* translation experiments, RACE, subtractive hybridization, northern analysis, gene cloning, and gene expression analysis. The use of Dynabeads® Oligo $(dT)_{25}$ relies on base-pairing between the poly A tail of messenger RNA and the oligo dT sequences bound to the surface of the beads. After annealing, the vial is placed on a magnet (Dynal MPC™) to concentrate the beads with their bound mRNA at the side of the tube. The supernatant containing unwanted contaminants is discarded. The protocol can be performed in 15 min, without the need to prepare total RNA or perform any other purification steps. The oligo dT bound to the bead surface can be used to capture the mRNA and act as a primer for reverse transcriptase during first strand cDNA synthesis. As the oligo dT is covalently bound to the Dynabeads® surface it is possible to regenerate the Dynabeads® Oligo $(dT)_{25}$ for reuse.

Required Materials

- Magnet (DynaMag™ portfolio). See www.lifetechnologies.com/magnets for recommendations.
- Mixer allowing tilting and rotation of tubes (e.g. HulaMixer® Sample Mixer).
- Sterile and RNase-free test tubes and pipette tips.
- Buffers/solutions (see Table 1).
- Water bath or heating block.

For tissue samples, the following additional materials are required:

- Liquid nitrogen
- Manual tissue grinder
- Syringe and needle

Note: All reagents used should be analytical grade and RNase-free.

Recommended Buffers/ Solutions

All common buffers for mRNA purification and isolation can be used with Dynabeads® Oligo $(dT)_{25}$. To take full advantage of the unique properties of the beads, the buffers described in the following sections are recommended. All buffers should be brought to room temperature prior to use, apart from the 10 mM Tris-HCl which should be kept on ice or at 2°C to 8°C.

General Guidelines

- Always keep the beads in liquid suspension, since dried out beads can lead to reduced isolation efficiency.
- The complete removal of all the buffer during washing is extremely important when working with small volumes.
- If you have cells previously isolated using other Dynabeads® products, remove all the beads from the lysate before before adding Dynabeads® Oligo $(dT)_{25}$.
- We recommend the Dynabeads®-mRNA complex to be used immediately for RT-PCR. If storage is necessary, elute the mRNA from the beads and freeze.

Table 1: Recommended buffers and solutions

Binding Buffer	20 mM Tris-HCl, pH 7.5, 1.0 M LiCl, 2 mM EDTA.
Lysis/Binding Buffer	100 mM Tris-HCl, pH 7.5, 500 mM LiCl, 10 mM EDTA, 1% LiDS, 5 mM dithiothreitol (DTT). If any precipitation is observed, warm the buffer to room temperature and shake until all the components are fully resuspended.
Washing Buffer A	10 mM Tris-HCl, pH 7.5, 0.15 M LiCl, 1 mM EDTA, 0.1% LiDS.
Washing Buffer B	10 mM Tris-HCl, pH 7.5, 0.15 M LiCl, 1 mM EDTA 10 mM Tris-HCl, pH 7.5.
Reconditioning Solution	0.1 M NaOH Storage Buffer Oligo $(dT)_{25}$: 250 mM Tris-HCl, pH 7.5, 20 mM EDTA, 0.1% Tween®-20, 0.02% sodium azide.

- RNases are very stable, active enzymes and generally require no cofactors to function. RNase inhibitors may be added to the protocol at any step, although this is normally not necessary. If storage of the eluted mRNA is required, addition of an RNase inhibitor at the elution step is recommended.

Technical Advice

Preparation of mRNA for Downstream Applications

For northern analysis, the mRNA can be eluted directly into a loading buffer containing formamide and loaded directly onto the gel. If the mRNA is to be used in downstream enzymatic applications (cDNA synthesis, *in vitro* translations experiments, RT-PCR), detergents should be omitted in the final washing steps and the elution step. Enzymatic downstream applications are not inhibited by the presence of the beads. It is possible to construct solid-phase cDNA libraries specific for a particular cell type or tissue directly on the bead-surface. The covalently linked oligo dT sequence is used to capture the mRNA and as a primer for the reverse transcriptase to synthesize the first strand cDNA. This results in a covalently linked first-strand cDNA library.

Avoiding Contamination

To obtain good preparations of eukaryotic mRNA, it is necessary to minimize the activity of RNases by creating a ribonuclease-free environment. The following precautions should be taken to avoid contamination:

Contamination by personnel

Wear disposable gloves at all times during the procedure. Gloves remain RNase-free only if they do not come into contact with "dirty" glassware and surfaces. Change gloves frequently when working with RNA.

Solutions

Any water and salt solutions used in RNA preparation should be RNase-free, i.e. by treatment with diethylpyrocarbonate (DEPC). Wherever possible, the solutions should be treated with 0.1% DEPC for at least 1 hour at 37°C and then heated to 100°C for 15 min or autoclaved for 15 min to remove any traces of DEPC. Tris Buffers cannot be DEPC-treated, as Tris inactivates DEPC. Solutions should be DEPC-treated and autoclaved before adding Tris. After addition of Tris, the solution should be autoclaved again. DEPC is a suspected carcinogen and should be handled with great care. Sterile, disposable plasticware is essentially free of RNases and can be used for the preparation and storage of RNA without pre-treatment. General laboratory plasticware should be rinsed with chloroform.

Protocol

Wash Dynabeads®

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

Prepare Lysate from Animal Tissues, Plants, and Cells

The protocol is recommended for a sample size of 20–50 mg animal tissue, 100 mg plant tissue or 1–4 × 10^6cells, but can be scaled up or down to suit specific sample size requirements.

Prepare lysate from solid animal and plant tissues

1. Aliquot the amount of animal or plant tissue while it is frozen. Use the specified amount of tissue, as an excess of tissue will reduce the mRNA yield and purity.
2. Grind frozen tissue in liquid nitrogen. Ensure tissue remains frozen at all times to avoid RNA degradation.
3. Transfer the frozen powder to a homogenizer containing 1 mL Lysis/Binding Buffer and homogenize for 1–2 min until the tissue has completely lysed. A rapid lysis in Lysis/Binding Buffer will prevent degradation of mRNA. If the raw extract is noticeably viscous a shear step might be helpful (see step 3 of "Prepare Lysate from Cultured Cells and Cell Suspensions").
4. Centrifuge the lysate for 30–60 sec in a microcentrifuge to remove debris. The lysate is now ready for mRNA isolation (see "Isolation of mRNA from Crude Lysate") or can be frozen and stored at –80°C for later use.

Prepare Lysate from Cultured Cells and Cell Suspensions

1. Wash the cell suspension in phosphate-buffered saline (PBS) and centrifuge to obtain a cell pellet. The cell pellet can be used immediately, or frozen in liquid nitrogen and stored at -80°C for later use. A stored cell pellet should be used directly from frozen.
2. Add 1.0 mL Lysis/Binding Buffer to the cell pellet (1–4 × 10^6 cells). Pipet up and down a couple of times to ensure complete lysis. The release of DNA during lysis results in a viscous solution which confirms complete lysis.
3. Reduce the viscosity by a DNA-shear step. The lysate is passed three times through a 21 gauge needle using a 1–2 mL syringe. Repeated shearing may cause the lysate to foam. Foaming should however not affect the mRNA yield. The foam can be reduced by a 30 sec centrifugation.
4. The lysate is now ready for mRNA isolation (see "Isolation of mRNA from Crude Lysate") or can be frozen and stored at –80°C for later use.

Isolate mRNA from Crude Lysate

1. Remove the solution from the washed Dynabeads® Oligo $(dT)_{25}$ (see "Wash Dynabeads®") and add the lysate (from section "Prepare lysate from solid animal and plant tissues" or "Prepare lysate from cultural cells and cell suspensions").
2. Mix beads and lysate. Allow binding by rotating on a mixer for 3-5 min at room temperature, increase the annealing time if the solution is viscous. During this step the mRNA anneals to the oligo dT sequence.
3. Place the vial on the magnet for 2 min and remove the supernatant.
4. Wash the beads twice in room temperature using the magnet: Wash once with 1 mL Washing Buffer A, and once with 1 mL Washing Buffer B. Resuspend the beads thoroughly in the Washing Buffers to remove possible contaminants, and remove the supernatant completely between the washing steps.
5. Perform ***one*** of the following:
   a. If the bead-bound isolated mRNA is to be used in enzymatic downstream applications (e.g. solid-phase cDNA synthesis), wash one extra time with Washing Buffer B (500 µL) followed by one wash with the enzymatic buffer used in the downstream application.
   b. To elute mRNA from the beads, remove the Washing Buffer B and add 10–20 µL 10 mM Tris-HCl. Incubate at 75°C to 80°C for 2 min, then place the tube on the magnet and quickly transfer the supernatant containing the mRNA to a new RNase-free tube. The final yield may vary somewhat between tissues/cells depending on mRNA abundance.

Purify mRNA from Total RNA

In the next example, mRNA is purified from 75 µg of total RNA starting material.

1. Adjust the volume of the 75 µg total RNA sample to 100 µL with distilled DEPC-treated water or with 10 mM Tris-HCl pH 7.5.
2. Add 100 µL of Binding Buffer. If total RNA is more dilute than 75 µg/100 µL, then simply add an equal volume of Binding Buffer to the beads.
3. Heat to 65°C for 2 min to disrupt secondary structures. Immediately place on ice.
4. Add the 200 µL of total RNA to the 100 µL washed beads (see "Wash Dynabeads®" section). For every 75 µg total RNA, use 1 mg beads which are washed and resuspended in 100 µL of Binding Buffer.
5. Mix thoroughly and allow binding by rotating continuously on a mixer for 5 min at room temperature.
6. Place the tube on the magnet for 1-2 min and carefully remove all the supernatant.
7. Remove the tube from the magnet and add 200 µL Washing Buffer B. Mix by pipetting carefully a couple of times.
8. Apply to the magnet for 1 min and remove the supernatant.
9. Repeat steps 7–8 once.
10. Perform ***one*** of the following:
    a. If the bead-bound isolated mRNA does not need to be eluted off the beads, wash one more time with the same buffer that will be used in the downstream application.
    b. To elute mRNA from the beads, remove the Washing Buffer B and add 10–20 µL 10 mM Tris-HCl. Incubate at 75°C to 80°C for 2 min, then place the tube on the magnet and quickly transfer the supernatant containing the mRNA to a new RNase-free tube.

Description of Materials

Dynabeads® Oligo $(dT)_{25}$ are uniform superparamagnetic, monodisperse polymer particles with oligo dT sequences covalently coupled to the bead surface.

Binding Capacity

Up to 2 µg poly(A)$^+$ RNA can be isolated per 200 µL (1 mg) of beads, depending on the tissue or cell type and the expression level of the mRNA. A typical mammalian cell contains about 10–30 pg of RNA of which 1–5% is mRNA. The total capacity per mL of beads is approx. 10 µg mRNA. If the same beads are reused for a total of 5 mRNA isolations (four regeneration cycles) the total capacity of 1 mL beads is up to 50 µg of mRNA.

Related Products

Product	Cat. no.
DynaMag™-2	12321D
DynaMag™-5	12303D
HulaMixer® Sample Mixer	15920D
Phosphate Buffered Saline	14190
Dynabeads® mRNA DIRECT™ Kit	61011
Dynabeads® mRNA DIRECT™ Micro Kit	61021
Dynabeads® mRNA Purification Kit	61006

REF on labels is the symbol for catalog number.

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