invitrogen

Dynabeads™ mRNA Purification Kit

Catalog No. 61006 **Store at 2°C to 8°C**

Publication No. MAN0015808 **Rev. Date:** 18 March 2016 (Rev. A.0)

Kit contents

Kit contents	Volume
Dynabeads™ Oligo $(dT)_{25}$	2 mL
Binding Buffer	5 mL
Washing Buffer B	5 mL
10 mM Tris-HCl	5 mL

Dynabeads™ Oligo $(dT)_{25}$ contains 5 mg/mL of beads in phosphate buffered saline (PBS), pH 7.4, with 0.05% Tween™ and 0.02% sodium azide as a preservative.
Caution: Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. See "Description of materials" for Buffer content.

Product description

This product is designed for rapid isolation of highly purified and intact mRNA from total RNA.

The isolation protocol relies on base-pairing between the poly A residues at the 3′ end of most mRNA, and the oligo $(dT)_{25}$ residues covalently coupled to the surface of the Dynabeads™ magnetic beads. Other RNA species lacking a poly A tail will not hybridize to the beads and are readily washed away.

1 mg of beads (~200 µL) will isolate up to 2 µg of mRNA, depending on the sample. A typical mammalian cell contains about 10–30 pg of total RNA, of which 1–5% is mRNA.

Required materials

- DynaMag™ Magnet (See **thermofisher.com/magnets** for recommendations)
- Sample mixer allowing tilting and rotation of tubes (e.g. HulaMixer™ Sample Mixer)
- Sterile RNase-free microcentrifuge tubes
- Sterile RNase-free pipette tips

General guidelines

- Work RNAse-free and wear gloves.
- Keep the Dynabeads™ Oligo $(dT)_{25}$ in liquid suspension during storage and handling steps. Resuspend well before use.
- All common buffers for mRNA purification and isolation can be used with Dynabeads™ Oligo $(dT)_{25}$.

Protocol

The following protocol describes mRNA isolation using 75 µg of total RNA as starting material. This protocol can be scaled up or down by increasing or decreasing reagent volumes proportionally with any changes in the amount of the total RNA starting material. Optimization may be needed.

Prepare RNA

1. Adjust the volume of the total RNA sample (75 µg) to 100 µL with distilled DEPC-treated water, or with 10 mM Tris-HCl, pH 7.5. Omit this step if only a small adjustment is needed (see step 3 of "Prepare Dynabeads™ magnetic beads").
2. Heat the sample to 65°C for 2 minutes to disrupt secondary structures.
3. Place sample on ice.

Prepare Dynabeads™ magnetic beads

1. Transfer 200 µL (1 mg) of resuspended Dynabeads™ magnetic beads to a microcentrifuge tube. Place the tube on the magnet for 30 seconds, or until all the Dynabeads™ magnetic beads have adhered to the tube wall.
2. Discard the supernatant, remove the tube from the magnet, and add 100 µL of Binding Buffer to equilibriate the beads. Place the tube on the magnet and remove the supernatant. Remove the tube from the magnet.
3. Add 100 µL Binding Buffer to the Dynabeads™ magnetic beads. Optimal hybridization conditions require a 1:1 ratio of Binding Buffer to sample volume. If the total RNA is more dilute than 75 µg/100 µL, then add a volume of Binding Buffer equal to the sample volume to the Dynabeads™ magnetic beads.

Isolate mRNA

1. Add the total RNA to the Dynabeads™/Binding Buffer suspension. Mix thoroughly, and rotate on a roller or mixer for 3–5 minutes at room temperature to allow the mRNA to anneal to the oligo $(dT)_{25}$ on the beads.
2. Place the tube on the magnet until the solution is clear. Remove the supernatant.
3. Remove the tube from the magnet and wash the mRNA-bead complex twice with 200 µL Washing Buffer B. Use the magnet to remove all traces of supernatant between each washing step (this is important when working with small volumes).
4. (*Optional*) Add 10–20 µL (or down to 5 µL) of 10 mM Tris-HCl, pH 7.5 to elute the mRNA.
5. Heat the sample at 65°C to 80°C for 2 minutes and place the tube immediately on the magnet.
6. Transfer the eluted mRNA to a new RNase-free tube.

Description of Materials

Dynabeads™ Oligo $(dT)_{25}$ beads are uniform, superparamagnetic polysterene beads (2.8 µm diameter) covalently coupled with Oligo $(dT)_{25}$ sequences. Binding Buffer contains 20 mM Tris-HCl (pH 7.5), 1.0 M LiCl, and 2 mM EDTA. Washing Buffer B contains 10 mM Tris-HCl (pH 7.5), 0.15 M LiCl, and 1 mM EDTA. The 10 mM Tris-HCl solution has a pH of 7.5.

Related Products

Product	Cat. No.
DynaMag™-Spin	12320D
DynaMag™-2	12321D
DynaMag™-5	12303D
HulaMixer™ Sample Mixer	15920D
Dynabeads™ Oligo $(dT)_{25}$	61002

REF on labels is the symbol for catalog number.

Important licensing information

These products may be covered by one or more Limited Use Label Licenses. By use of these products, you accept the terms and conditions of all applicable Limited Use Label Licenses.

Manufactured by Thermo Fisher Scientific Baltics UAB, V.A. Graiciuno 8, LT-02241 Vilnius, Lithuania. Thermo Fisher Scientific Baltics UAB complies with Quality System Standards ISO 9001 and ISO: 13485.

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