ambion
by life technologies

Dynabeads® mRNA Purification Kit

Catalog no. 61006 **Store at 2˚C to 8˚C**

Rev. Date: June 2012 (Rev. 005)

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 beads/mL 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®. 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, from which 1–5% is mRNA.

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, 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 from 75 µg of total RNA as starting material. This protocol can be scaled up or down to adjust mRNA yield. Increase or decrease the quantities of the kit reagents proportionally with any changes in total RNA starting sample. Optimization may be needed.

Prepare RNA

1. Adjust the volume of your 75 µg total RNA 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 also step 3 under "Prepare Dynabeads®").
2. Heat to 65°C for 2 min to disrupt secondary structures. Place on ice.

Prepare Dynabeads®

1. Transfer 200 µL (1 mg) of well resuspended Dynabeads® to a micro-centrifuge tube. Place the tube on the magnet for 30 sec, or until all Dynabeads® have migrated to the tube wall.
2. Discard the supernatant, remove the tube from the magnet, and add 100 µL Binding Buffer to calibrate the beads. Put the tube back on the magnet and remove the supernatant. Remove the tube from the magnet.
3. Add 100 µL Binding Buffer to the Dynabeads®. Optimal hybridization conditions are obtained in Binding Buffer added in a 1:1 ratio relative to sample volume. If your total RNA is more dilute than 75 µg/100 µL, then simply add an equal volume of Binding Buffer to the Dynabeads®.

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 min at room temperature to allow mRNA to anneal to the oligo $(dT)_{25}$ on the beads.
2. Place the tube on the magnet until 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. Remove all the supernatant between each washing step with the help of the magnet (this is important when working with small volumes).
4. If elution is required, add the desired amount (10–20 µL, or down to 5 µL) of 10 mM Tris-HCl, pH 7.5. Heat to 65°C to 80°C for 2 min and place the tube immediately on the magnet.
5. Transfer the eluted mRNA to a new RNase-free tube.

Description of Materials

Dynabeads® Oligo $(dT)_{25}$ 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.

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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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