invitrogen™ | DYNAL® invitrogen bead separations

Cat. no. 610.06

Rev. no. 004

Dynabeads® mRNA Purification Kit

For research use only

Index:

1. KIT CONTENT
2. PRODUCT DESCRIPTION
3. INSTRUCTIONS FOR USE
4. GENERAL INFORMATION

1. KIT CONTENT

Dynabeads mRNA Purification Kit, Product number 610.06	
Dynabeads Oligo $(dT)_{25}$*	1 × 2 ml
Binding Buffer	4 ml
Washing Buffer B	4 ml
Elution Buffer	4 ml

*Approximately 5 mg/ml, supplied in PBS pH 7.4, containing 0.02% NaN_3 as a preservative.

2. PRODUCT DESCRIPTION

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

2.1 Principle of Isolation

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.

2.2 Physical Characteristics of Dynabeads Oligo $(dT)_{25}$

Diameter: 2.8 µm ± 0.2 µm (C.V. max 5%)
Surface area: 3–7 m^2/g
Density: approx. 1.6 g/cm^3
Magnetic mass susceptibility: 20 ± 25 × 10–6 m^3/kg

2.3 Buffers

Binding Buffer **4 ml**
20 mM Tris-HCl (pH 7.5)
1.0 M LiCl
2 mM EDTA

Washing Buffer B **4 ml**
10 mM Tris-HCl (pH 7.5)
0.15 M LiCl
1 mM EDTA

10 mM Tris-HCl (pH 7.5) **4 ml**

2.4 Additional Material Required

- Magnet: See www.invitrogen.com/magnets-selection for magnet recommendations.
- Mixer allowing both tilting and rotation.
- Sterile, RNase free microcentrifuge tubes.
- Sterile, RNase free pipette tips.

3. INSTRUCTIONS FOR USE

3.1 Technical Advice

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

3.2 Purification of mRNA from total RNA

The protocol below describes mRNA isolation from 75 µg of total RNA as starting material.

Preparation of 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 *'Preparation of Dynabeads').*
2. Heat to 65°C for 2 minutes to disrupt secondary structures. Place on ice.

Preparation of Dynabeads

1. Transfer 200 µl (1 mg) of well resuspended Dynabeads to a microcentrifuge tube. Place the tube on the magnet for 30 seconds, or until all Dynabeads have migrated to the tube wall.
2. Pipette off 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.

Isolation of 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 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 desired amount (10–20 µl, or down to 5 µl) of 10 mM Tris-HCl, pH 7.5. Heat to 65–80°C for 2 minutes and place the tube immediately on the magnet. Transfer the eluted mRNA to a new RNase-free tube.

Note: 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.

3.3 Regeneration and Reuse of Dynabeads Oligo $(dT)_{25}$

The oligo $(dT)_{25}$ sequences are covalently attached to the bead surface. Whilst this enables a single step hybridisation, the covalent nature of the binding also allows regeneration of the Dynabeads Oligo $(dT)_{25}$. The beads may be reused a total of four times without loss of yield. The reusable properties of these Dynabeads provides a cost effective choice for the user. When mRNA is to be isolated from the same crude extract, the Dynabeads Oligo $(dT)_{25}$ can be reused without regeneration. By reusing the Dynabeads on the same sample (without regeneration), large amounts of mRNA can be isolated. Simply follow the mRNA isolation procedure. After elution of the mRNA, wash the Dynabeads once in Binding Buffer before adding the sample and a new capture of mRNA is performed. This can be repeated several times until no mRNA is left in the sample.

Note: The buffers in this kit is not sufficient for re-use of the Dynabeads, and they will have to be made. The Binding Buffer in this kit is supplied as a 2 × concentrate. This is to obtain optimal hybridisation condition when mixing 1:1 with the sample. When used to wash the Dynabeads for reuse, adjust the Binding Buffer to 1 × concentration by adding an equal volume of DEPC-treated water.

Note: Buffers such as Tris cannot be DEPC treated as Tris inactivates DEPC. Solutions should be DEPC-treated and autoclaved before adding Tris. Subsequently after adding Tris, autoclave the solution again. DEPC is a suspected carcinogen – handle with care. Wear gloves.

3.4 Regeneration Protocol

Use this protocol if you need to avoid cross contamination of mRNA between samples.

1. Resuspend used Dynabeads (original volume 200 µl) in 200 µl Reconditioning Solution and transfer suspension to a new RNase-free tube.
2. Incubate at 65°C for 2 minutes.
3. Place tube in magnetic field for at least 30 seconds and remove supernatant.
4. Wash twice in Reconditioning Solution, by repeating steps 1 and 3 twice.
5. Resuspend Dynabeads in 200 µl Storage Buffer Oligo $(dT)_{25}$.
6. Place tube on the magnet for at least 30 seconds and remove supernatant.
7. Repeat steps 5 and 6 three times, or until pH of supernatant is below 8.0.
8. Resuspend Dynabeads in desired volume of Storage Buffer Oligo $(dT)_{25}$. The Dynabeads are now reconditioned and ready for another mRNA isolation. Store Dynabeads in Storage buffer Oligo $(dT)_{25}$ at 2–8°C.

Buffers for regeneration (not supplied):

Reconditioning Solution:
0.1 M NaOH

Storage Buffer Oligo $(dT)_{25}$:
250 mM Tris-HCl (pH 7.5)
20 mM EDTA
0.1% Tween20
0.02% Sodium azide (NaN_3)

Note: Do not mix regenerated Dynabeads Oligo $(dT)_{25}$ with original stock suspension.

4. GENERAL INFORMATION

Invitrogen Dynal AS complies with the Quality System Standards ISO 9001:2000 and ISO 13485:2003.

4.1 Storage/Stability

This product is stable until the expiry date stated on the label, when stored unopened at 2–8°C.

Store opened vials at 2–8°C and avoid bacterial contamination. Do not store the Dynabeads Oligo $(dT)_{25}$ in distilled water.

4.2 Technical Support

Contact details for your local Invitrogen technical support can be found at http://www.invitrogen.com/contact.

Certificate of Analysis/Compliance is available upon request.

The latest revision of the package insert/instruction for use is available on www.invitrogen.com.

4.3 Warnings and Limitations

The Dynabeads mRNA Purification Kit is for research use only. The product is not for use in human diagnostic or therapeutic procedures.

The Dynabeads Oligo $(dT)_{25}$ contains 0.02% sodium azide (NaN_3) as a preservative. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. When disposing through plumbing drains, flush with large volumes of water to prevent azide build-up. Preservatives such as sodium azide are toxic if ingested. Avoid pipetting by mouth.

Standard methods for preventing contamination by RNases during the preparation of mRNA must be taken. Take precautions to prevent RNase contamination of opened vials.

Material Safety Data Sheet is available from www.invitrogen.com.

Intellectual Property Disclaimer

Invitrogen Dynal will not be responsible for violations or patent infringements that may occur with the use of our products.

4.4 Warranty

The products are warranted to the original purchaser only to conform to the quantity and contents stated on the vial and outer labels for the duration of the stated shelf life. Invitrogen Dynal's obligation and the purchaser's exclusive remedy under this warranty is limited either to replacement, at Invitrogen Dynal's expense, of any products which shall be defective in manufacture, and which shall be returned to Invitrogen Dynal, transportation prepaid, or at Invitrogen Dynal's option, refund of the purchase price.

Claims for merchandise damaged in transit must be submitted to the carrier.

This warranty shall not apply to any products which shall have been altered outside Invitrogen Dynal, nor shall it apply to any products which have been subjected to misuse or mishandling.

ALL OTHER WARRANTIES, EXPRESSED, IMPLIED OR STATUTORY, ARE HEREBY SPECIFICALLY EXCLUDED, INCLUDING BUT NOT LIMITED TO WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. Invitrogen Dynal's maximum liability is limited in all events to the price of the products sold by Invitrogen Dynal. IN NO EVENT SHALL INVITROGEN DYNAL BE LIABLE FOR ANY SPECIAL, INCIDENTAL OR CONSEQUENTIAL DAMAGES. Some states do not allow limits on warranties, or on remedies for breach in certain transactions. In such states, the limits set forth above may not apply.

4.5 Trademarks and Patents

Dynal and Dynabeads are either registered trademarks or trademarks of Invitrogen Dynal AS, Oslo, Norway. Any registration or trademark symbols used herein denote the registration status of trademarks in the United States. Trademarks may or may not be registered in other countries.

Limited Use Label License

No. 5: Invitrogen Technology – The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes. The buyer may transfer information or materials made through the use of this product to a scientific collaborator, provided that such transfer is not for any Commercial Purpose, and that such collaborator agrees in writing (a) not to transfer such materials to any third party, and (b) to use such transferred materials and/or information solely for research and not for Commercial Purposes. Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research. Invitrogen Corporation will not assert a claim against the buyer of infringement of patents owned or controlled by Invitrogen Corporation which cover this product based upon the manufacture, use or sale of a therapeutic, clinical diagnostic, vaccine or prophylactic product developed in research by the buyer in which this product or its components was employed, provided that neither this product nor any of its components was used in the manufacture of such product. If the purchaser is not willing to accept the limitations of this limited use statement, Invitrogen is willing to accept return of the product with a full refund. For information on purchasing a license to this product for purposes other than research, contact

Licensing Department,
Invitrogen Corporation,
1600 Faraday Avenue, Carlsbad,
California 92008.
Phone (760) 603-7200.
Fax (760) 602-6500.
Email: outlicensing@invitrogen.com

Invitrogen Dynal is a part of the Invitrogen Group.

Contact details for your local Invitrogen sales office/technical support can be found at http://www.invitrogen.com/contact

SPEC-06216