

# Dynabeads mRNA DIRECT™ Micro Kit - Package Insert

This document is a copy of the instructions following the product.

## Handbook

### Dynabeads® mRNA DIRECT™ Micro Kit mRNA isolation for RT-PCR amplification

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## Introduction

The Dynabeads mRNA DIRECT™ Micro Kit provides a fast and simple way to combine poly(A) + RNA isolation and RT-PCR. The kit is designed to isolate highly purified and intact poly(A) + RNA directly from small samples of cells and tissues. The isolated mRNA can be used directly for reverse transcription into cDNA and then to amplify the transcripts by PCR.

The direct isolation is performed in 15 minutes without having to prepare total RNA or perform any other purification steps. The key component in this kit is Dynabeads® Oligo (dT)<sub>25</sub>, which are uniform, superparamagnetic, polystyrene beads with oligo (dT)<sub>25</sub> sequences covalently bound to the surface. The use of the kit relies on the base pairing between the poly (A) + residues of most messenger RNA and the oligo (dT)<sub>25</sub> sequences on the beads. The oligo (dT)<sub>25</sub> bound to the bead surface is used both to capture the mRNA and as a primer for the reverse transcriptase synthesis of the first strand cDNA. The patented Dynabeads® solid-phase cDNA synthesis technology is compatible with the various commercial available cDNA-synthesis kits. The combination of

direct mRNA isolation using Dynabeads Oligo (dT)<sub>25</sub> and one-tube RT-PCR offers a convenient system for fast and reliable RT-PCR detection.

The Dynabeads mRNA DIRECT™ Micro Kit provides enough reagents for 100 mRNA isolations from up to 2.5 x 10<sup>4</sup> mononuclear cells, up to 1 x 10<sup>4</sup> cultured cells or up to 5 mg tissue depending on the tissue used. High quality mRNA purification is conveniently performed in a single tube. The isolated mRNA can be used directly in the RT-PCR amplification without eluting the mRNA from the beads.

Dynabeads mRNA DIRECT™ Micro Kit includes Dynabeads Oligo (dT)<sub>25</sub>, Lysis/Binding Buffer, Washing Buffers and 10 mM Tris-HCl.

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### 1. Product Description and Performance

Dynabeads mRNA DIRECT™ Micro Kit is based on the unique Dynabeads biomagnetic separation technology and is designed for the simple and rapid isolation of poly (A)<sup>+</sup> RNA from small samples of tissue and cells, to be used directly for RT-PCR amplification.

The oligo (dT)<sub>25</sub> bound to the bead surface is both used to capture the mRNA and as a primer for the reverse transcriptase to synthesise the first strand cDNA.

The direct poly (A)<sup>+</sup> RNA isolation is performed in 15 minutes without having to perform any preliminary total RNA purification steps.

#### 1.1 Kit components

- Dynabeads Oligo (dT)<sub>25</sub>:

Dynabeads Oligo (dT)<sub>25</sub> are supplied in the kit as a suspension of approximately 5 mg/ml in phosphate-buffered saline (PBS) pH 7.4, containing

0.02% NaN<sub>3</sub> as a preservative:

137mM NaCl

2.7 mM KCl

4.3 mM Na<sub>2</sub>HPO<sub>4</sub> x 7H<sub>2</sub>O

1.4 mM KH<sub>2</sub>PO<sub>4</sub>

0.02% NaN<sub>3</sub>

2 x 1 ml supplied in the kit.

- Lysis/Binding Buffer:

100 mM Tris-HCl, pH 7.5

500 mM LiCl

10 mM EDTA, pH 8.0

1% LiDS

5 mM dithiothreitol (DTT)

15 ml supplied in the kit.

- Washing Buffer A:

10 mM Tris-HCl, pH 7.5

0.15 M LiCl

1 mM EDTA

0.1% LiDS

30 ml supplied in the kit.

- Washing Buffer B:

10 mM Tris-HCl, pH 7.5

0.15 M LiCl

1 mM EDTA

30 ml supplied in the kit.

- 10mM Tris-HCl:

10 mM Tris-HCl, pH 7.5

15 ml supplied in the kit.

**Note:** All reagents used are of analytical grade and RNase-free.

**Note:** If some of the buffers show precipitation, warm to room temperature and mix the buffer until the precipitate is dissolved.

## 1.2 Dynabeads Oligo (dT)<sub>25</sub> characteristics

Diameter:  $\mu$ 2.8 m (C.V max 5%)

Density:  $\sim$ 1.3 g/cm<sup>3</sup>

Magnetic mass susceptibility:  $16 \pm 3 \times 10^{-5} \text{ m}^3/\text{kg}$

Surface area: 4-8 m<sup>2</sup>/g

## 1.3 Storage and stability

Dynabeads mRNA DIRECT™ Micro Kit should be stored at 2-8°C. The components in the kit are guaranteed stable until the expiry date stated on the label when stored unopened at 2-8°C.

Dynabeads Oligo (dT)<sub>25</sub> are stable in a pH range of 4-13.

Dynabeads Oligo (dT)<sub>25</sub> are supplied as a suspension of approximately 5 mg/ml in phosphate-buffered saline (PBS) pH 7.4 containing 0.02% NaN<sub>3</sub> as a preservative.

**Note:** The Dynabeads Oligo (dT)<sub>25</sub> may be frozen in the buffer they are supplied in. Repeated freezing and thawing should be avoided. Do not store or freeze the Dynabeads in distilled water.

The suspension of Dynabeads Oligo (dT)<sub>25</sub> and the buffers provided in the Dynabeads mRNA DIRECT™ Micro Kit are produced and quality controlled to be ribonuclease free and thoroughly tested for optimal performance. Before use, resuspend Dynabeads Oligo (dT)<sub>25</sub> thoroughly by shaking the vial gently to obtain a homogeneous dispersion of beads in solution.

**Note:** Keep the vials of Dynabeads Oligo (dT)<sub>25</sub> in an upright position to ensure that the beads are covered with buffer, as drying will reduce their performance. Dried Dynabeads Oligo (dT)<sub>25</sub> should be resuspended in the buffer they are supplied in by placing the vial on a Dynal Mixer or equivalent overnight (4°C) to ensure continuous mixing. This treatment will completely restore the function of Dynabeads Oligo (dT)<sub>25</sub>.

## 1.4 Additional materials needed

- Magnetic Particle Concentrator. (Recommended Dynal MPC®; Dynal MPC®-E, Dynal MPC®-E-1, Dynal MPC®-P-12, Dynal MPC®-96 or Dynal MPC®-9600)
- Sterile, RNase-free microtubes of the Eppendorf type
- Sterile, RNase-free pipette tips
- Dynal Mixer or equivalent

When working with tissue samples, the following additional materials are needed:

- Liquid nitrogen
- Manual tissue grinder. We recommend to use the Pellet Pestle® Disp w/tube (prod. no. 749520-0000), Kontes Biotechnology, NJ, USA or equivalent
- Syringe and needle (see section 4)

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

## 1.5 Product performance

The Dynabeads mRNA DIRECT™ Micro Kit provides enough reagents for 100 microscale mRNA isolations directly from up to  $2.5 \times 10^4$  mononuclear cells, up to  $1 \times 10^4$  cultured cells or up to 5 mg tissue (depending on the tissue type) for direct use in RT-PCR. The isolation is performed in 15 minutes without the need for an intermediate total RNA isolation step or any other purification step. A strong RNase inhibiting agent together with stringent hybridization and washing buffers ensure the isolation of intact, high purity mRNA even from crude samples rich in RNases. Enzymatic downstream applications are not inhibited by the presence of the Dynabeads Oligo (dT)<sub>25</sub>.

1 ml of Dynabeads Oligo (dT)<sub>25</sub> has a capacity to isolate 6 - 12  $\mu$ g of poly(A)<sup>+</sup> RNA depending on the tissue or cell type. 20  $\mu$ l of Dynabeads Oligo (dT)<sub>25</sub> are used per microscale isolation.  $1 \times 10^4$  cultured cells contain 0.3 - 25 ng mRNA depending on the cell type and state.

## 2. Isolation Protocol

### 2.1 Schematic diagram

## 2.2 Isolation of mRNA from cells for RT-PCR

We advise you to read Initial Check-list and Technical Tips (section 3) before starting your mRNA protocol.

**Note:** All buffers except the 10 mM Tris-HCl, should be brought to room temperature prior to use.

To prevent degradation of RNA by RNase contamination: USE GLOVES and CHANGE GLOVES FREQUENTLY.

### A. Preparation of Dynabeads Oligo (dT)<sub>25</sub>

1. Resuspend the Dynabeads Oligo (dT)<sub>25</sub> thoroughly before use.
2. Transfer Dynabeads Oligo (dT)<sub>25</sub> needed for all samples (using 20 µl Dynabeads Oligo (dT)<sub>25</sub> per mRNA isolation) from the stock tube suspension, to an RNase-free 1.5 ml microcentrifuge tube.
3. Place the sample tube in a magnetic particle concentrator (Dyna MPC, e.g. Dynal MPC-E).
4. After 30 seconds (or when the suspension is clear) remove the supernatant.
5. Remove the sample tube from the magnet and pre-wash Dynabeads Oligo (dT)<sub>25</sub> by resuspending in Lysis/Binding Buffer to the original volume by pipetting.
6. Place the tube on the magnet and remove supernatant. **Note:** Do not leave the Dynabeads Oligo (dT)<sub>25</sub> dry for long periods, as this may lower their capacity.
7. Remove the sample tube from the magnet and resuspend the beads in Lysis/binding buffer to the original volume and aliquot 20 µl to each microcentrifuge tube.

### B. Preparation of lysate from cultured cells and cell suspensions

1. This protocol is recommended for use with up to  $1 \times 10^4$  cultured cells or up to  $2.5 \times 10^4$  mononuclear cells.
2. Wash the cell suspension in phosphate-buffered saline (PBS) prior to preparing a cell pellet by centrifugation. The cell pellet can be used immediately, or frozen in liquid nitrogen and stored at  $-80^\circ\text{C}$  for later use. Add 100 µl Lysis/Binding Buffer to either a frozen cell pellet or to a fresh cell pellet. Perform a repeated passage of the solution through a pipette tip to obtain complete lysis. The lysate may be frozen ( $-80^\circ\text{C}$ ) and stored for later use.
3. The lysate is combined with Dynabeads Oligo (dT)<sub>25</sub> in the mRNA isolation protocol below, step 2.

### C. Protocol for mRNA isolation for RT-PCR amplification

1. Prepare the Dynabeads Oligo (dT)<sub>25</sub> and the lysate as described in section 2.2. A and B.
2. Transfer the clear lysate to the tube containing 20 µl pre-washed Dynabeads Oligo (dT)<sub>25</sub>.
3. Mix by pipetting up and down a few times.
4. Place the tube on a Dynal Mixer or roller for 5 min. at room temperature to allow the mRNA to anneal to the Dynabeads. It is important that the solution is in a continuous rotation movement.
5. Place the sample tube on the magnet and discard the supernatant
6. Remove the sample tube from the magnet and resuspend the Dynabeads-mRNA complex in 100 µl Washing Buffer A by careful pipetting.
7. Place the sample tube on the magnet and discard the supernatant.
8. Repeat steps 6 - 7 once.
9. Resuspend the Dynabeads-mRNA complex in 100 µl Washing Buffer B.
10. Transfer the suspension to a new microcentrifuge tube.
11. Place the new sample tube on the magnet and discard the supernatant.
12. Resuspend the Dynabeads-mRNA complex in 100 µl Washing Buffer B.

13. Place the sample tube on the magnet and discard the supernatant.
14. Remove the sample tube from the magnet and resuspend in 100 µl of ice-cold 10mM Tris-HCl.
15. Place the sample tube on ice prior to RT-PCR amplification. We recommend preparing the RT-PCR mix before starting the mRNA isolation protocol.
16. Immediately prior to adding the RT-PCR mix, place the sample tube on the magnet and discard the supernatant.
17. For one-tube RT-PCR, resuspend the Dynabeads-mRNA complex in 50 µl RT-PCR mix and transfer to PCR-tube. For two-step RT-PCR, resuspend the Dynabeads-mRNA complex in the RT-reaction mix according to the manufacturers recommendation.
18. The cDNA synthesis should be performed as recommended by the manufacturer of the reverse transcriptase. When using a thermostable reverse transcriptase and the oligo (dT)<sub>25</sub> as primer for first strand cDNA synthesis, an initial step of incubation at 50°C for 5 minutes is necessary before proceeding at recommended temperature. (For more information see section 5.)
19. When the RT-PCR is completed, transfer 10 µl of each RT-PCR reaction to a new tube and add 5 µl of gel loading buffer to each tube. Load the samples on a pre-made agarose gel.

### **2.3 Isolation of mRNA from tissues for RT-PCR**

#### **A. Preparation of lysate from plant and animal tissues**

1. Grind frozen tissue sample (up to 5 mg depending on the tissue type) in an Eppendorf tube, using a manual tissue grinder (see section 1.4). Work quickly. Aliquot (weigh) the animal or plant tissue while frozen, to avoid mRNA degradation. Use the specified amount of tissue, since an excess of tissue will reduce the mRNA yield and purity.
2. Keep the sample frozen by dipping the sample tube in liquid nitrogen.
3. Add 100 µl Lysis/Binding Buffer and thaw sample while continuing to grind until complete lysis is obtained (approx. 1-2 min). A rapid lysis in the Lysis/binding buffer is critical for obtaining undegraded mRNA. If the raw extract is noticeably viscous a shearing step might be beneficial (see section 4).
4. Spin the lysate for 30-60 seconds in a microcentrifuge to remove debris. The lysate can be frozen and stored at -80°C for later use.

#### **B. mRNA isolation for RT-PCR amplification**

The 100 µl lysate is combined with the 20 µl pre-washed Dynabeads Oligo (dT)<sub>25</sub> (from section 2.2 A). Continue with the protocol for mRNA isolation for RT-PCR amplification (section 2.2 C, step 3).

### **2.4 Tumour cell enrichment followed by mRNA isolation and RT-PCR**

The Dynabeads<sup>®</sup> Epithelial Enrich (Prod. No. 161.01/02) is designed for the optimised enrichment of epithelial tumour cells from blood or MNC. 5 ml anticoagulated whole blood samples or MNC at a concentration of 1-2 x 10<sup>7</sup> cells/ml are mixed with the Dynabeads coated with the monoclonal antibody BerEP4 against the human epithelial antigen (HEA). Epithelial cells bind to the beads in a 30 minute incubation. The Dynal<sup>®</sup> CK19 Nested Primer Set (Prod. No. 112.96) is designed to specifically amplify the CK19 gene with no pseudogene amplification, thereby avoiding false positive results. The isolated mRNA can also be used in RT-PCR for other genes. Please contact Dynal Biotech Technical Service for more information or see package insert for Dynabeads Epithelial Enrich (Prod. No. 161.01/02) and Dynal CK19 Nested Primer Set (Prod. No. 112.96).

#### **A. Preparation of lysate from tumour cells isolated from whole blood**

1. Follow the protocol for positive isolation of tumour cells from whole blood using Dynabeads Epithelial Enrich
2. After adding the final Washing solution, the bead suspension is transferred to a microcentrifuge tube. The Dynabeads-cell complex is kept on ice for immediate mRNA isolation and RT-PCR.

3. Immediately before use, place the sample tube on the magnet for 2-3 minutes and remove supernatant.
4. Lyse the rosetted cells by resuspending the Dynabeads-cell complex in 100  $\mu$ l Lysis/Binding Buffer.
5. Mix by pipetting up and down two to three times.

## **B. Preparation of lysate from tumour cells isolated from MNC**

1. Follow the protocol of Dynabeads Epithelial Enrich for positive isolation of tumour cells from MNC.
2. After adding the final Washing solution, the bead suspension is transferred to a microcentrifuge tube. The Dynabeads-cell complex is kept on ice for immediate mRNA isolation and RT-PCR.
3. Immediately before use, place the sample tube on the magnet for 2-3 minutes and remove supernatant.
4. Lyse the rosetted cells by resuspending the Dynabeads/cell complex in 100  $\mu$ l Lysis/Binding Buffer.
5. Mix by pipetting up and down two to three times.

## **C. mRNA isolation for RT-PCR amplification**

1. Place the sample tube with the lysed cells on the magnet for 5 minutes.
2. Transfer the supernatant (clear lysate) to a microcentrifuge tube containing 20  $\mu$ l pre-washed Dynabeads Oligo (dT)<sub>25</sub> (from section 2.2 A).
3. Continue with the protocol for mRNA isolation for RT-PCR amplification (section 2.2 C, step 2).

## **3. Initial Check-list and Technical Tips for Dynabeads« mRNA DIRECT™ Micro kit**

You are strongly advised to read this section before starting your mRNA isolation protocol

### **3.1 Check-list**

1. All buffers except the 10 mM Tris-HCl, should be brought to room temperature prior to use. The 10 mM Tris-HCl should be stored on ice or 2-8°C prior to use.
2. Ensure that the Dynabeads Oligo (dT)<sub>25</sub> have been fully resuspended before use. Resuspend by brief vortexing or pipetting.
3. Check that your lysis buffer has not precipitated. If any precipitation is observed, please warm to room temperature and shake to full resuspension.
4. Prepare your RT-PCR mix before the mRNA isolation and keep on ice.
5. When working with cells isolated by immunomagnetic separation (IMS), make sure that all IMS-Dynabeads are removed from the lysate before adding Dynabeads Oligo (dT)<sub>25</sub> (section 2.4 C, step 1 and 2).
6. A rapid lysis in Lysis/binding buffer is critical for obtaining undegraded mRNA. Thawing of frozen material prior to the lysis step must be avoided.

### **3.2 Technical Tips**

- Keep the vials of Dynabeads Oligo (dT)<sub>25</sub> in an upright position to ensure that the beads are covered with buffer, as drying will reduce their performance. Dried Dynabeads Oligo (dT)<sub>25</sub> should be resuspended in the buffer they are supplied in by placing the vial on a Dynal Mixer or equivalent overnight (4°C) to ensure continuous mixing. This treatment will restore complete functionality of Dynabeads Oligo (dT)<sub>25</sub>.
- Wear disposable gloves and change them frequently.
- Use sterile, RNase-free microtubes and pipette tips.

- RNase inhibitors may be added to the protocol at any step.

**Note:** The addition of an RNase inhibitor is normally redundant.

- We recommend to immediately use the mRNA-Dynabeads complex for RT-PCR. If storage is needed, elute off the mRNA from the beads and freeze. When storing mRNA, it is critical that no RNase is present in your sample.
- If elution of mRNA is necessary, add 10-20 µl 10 mM Tris-HCl and incubate at 80-90°C for 2 minutes. Place the tube on the magnet and immediately transfer the supernatant to a new microcentrifuge tube. The eluate may be used directly for reverse transcription or frozen for later use (-80°C).
- If needed the concentration of mRNA can be measured by measuring the absorbance of eluted mRNA at 260 nm. The solution must be free of Dynabeads Oligo (dT)<sub>25</sub> as the beads will interfere with the spectrophotometric readings.
- For isolation from larger sample volume, use the Dynabeads mRNA DIRECT™ kit.

## 4. Troubleshooting

### 4.1 Viscosity reduction by DNA-shearing

If the raw extract is noticeably viscous due to released DNA, a DNA-shear step should be included in your protocol by passage through a syringe. Use force to shear the DNA properly. In addition, be aware that repeated shearing causes foaming of the lysate due to a detergent in the buffer. However, this should not affect the mRNA yield. The foam can be reduced by a 30 second centrifugation.

### 4.2 DNA contamination

Direct mRNA isolation methods have a potential risk of DNA contamination. However, with the Dynabeads mRNA DIRECT™ Micro kit small amounts of cells and tissue are used and consequently this is minimised. Nevertheless, if high viscosity is observed, it is important to reduce this either by diluting the sample or by DNA-shearing as described above. There are several possibilities for controls to ensure the detection of cDNA and not genomic DNA:

- Choose the primers in neighbouring exons to obtain different amplicon sizes for cDNA and genomic DNA.
- Include a negative control with no reverse transcriptase added.
- Use genomic DNA specific primers to detect any contamination.
- Use RNase-free DNase to treat the mRNA sample before cDNA synthesis to get rid of any DNA contamination (ref.1).

## 5. Downstream Applications

### 5.1 Solid-phase cDNA synthesis and RT-PCR

Enzymatic downstream applications are not inhibited by the presence of Dynabeads Oligo (dT)<sub>25</sub> making it possible to use the isolated mRNA directly for solid-phase cDNA synthesis and RT-PCR (Ref. 2,3,4,5,6,7). All reverse transcriptases which have been tested have been found to function in solid-phase cDNA synthesis (AMV, M-MLV, SuperScript™, rTth, Retrotherm™, Thermoscript™).

The oligo (dT)<sub>25</sub> sequence on the Dynabeads is not only used to capture the poly (A)<sup>+</sup> mRNA, but is also used as a primer for the subsequent reverse transcriptase synthesis of first strand cDNA. The resulting first strand cDNA is covalently linked to the Dynabeads surface and may be used for cDNA amplification. The Dynabeads solid-phase technology is compatible with most cDNA-synthesis kits commercially available. cDNA synthesis and the PCR can be performed sequentially in one tube, i.e. in the same reaction buffer. The combination of direct mRNA isolation using Dynabeads Oligo (dT)<sub>25</sub> and one-tube RT-PCR offers a convenient system for fast and reliable RT-PCR detection.

The cDNA synthesis should be performed as recommended by the manufacturer of the reverse transcriptase. When using thermostable reverse transcriptase and the oligo (dT)<sub>25</sub> as primer for first strand cDNA synthesis, an initial step of incubation at 50°C for 5 minutes is necessary before proceeding at recommended temperature. The PCR is not inhibited by the presence of the Dynabeads.

### 5.2 Construction of immobilised cDNA libraries for multiple RT-PCR amplifications

A reusable solid-phase cDNA library can be made as the first strand cDNA synthesised (using

the oligo (dT)<sub>25</sub> as primer) is covalently-linked to the Dynabeads (Ref. 2,5,6,7,8,9). The solid-phase first strand cDNA library can be reused for multiple PCR amplifications of specific transcripts. The different transcripts are amplified by adding different specific primer sets in successive PCR reactions using the solid-phase first strand cDNA library as template. Some of the advantages of using Dynabeads for construction of immobilised cDNA libraries are :

- One extraction allows the amplification of several gene transcripts.
- Enables simple and rapid buffer changes required to optimise the conditions for specific enzymes.

### 5.3 PCR amplification from a reusable solid-phase cDNA library

1. Add PCR-mix with primers and Taq polymerase, and resuspend the cDNA-Dynabeads properly.
2. Cycle twice to generate enough template for further amplification with a 5 min. extension at 72°C.
3. Melt the strands at 94°C for 2 min, place on magnet and transfer the supernatant with the amplification product to a new PCR tube. Continue the cycling reaction.
4. Wash the cDNA-Dynabeads twice in 10 mM Tris-HCl or 1 x PCR buffer and reuse them by adding a new PCR-mix for amplification of a different transcript. For storage of the cDNA library use TE-buffer or equivalent
5. Amplified PCR-products are analysed by agarose electrophoresis.

**Note:** If it is not necessary to reuse the cDNA-Dynabeads, just run the PCR with the beads present through the cycling reactions as described.

**Note:** Subsequent solid-phase sequencing of the amplified cDNA is possible if one biotinylated primer and one non-biotinylated primer is used in the PCR reaction. First, remove any Dynabeads Oligo (dT)<sub>25</sub>. Perform a binding of the biotinylated PCR-product to Dynabeads<sup>®</sup> M-280 Streptavidin. Proceed with making a single-stranded sequencing template.

For more information, see Dynal Biotech Technical Handbook, Biomagnetic Techniques in Molecular Biology, or contact Dynal Biotech Technical Service.

### 6. Other Dynabeads<sup>®</sup> Oligo (dT)<sub>25</sub> Products

Prod. No. Product description

610.11/12 Dynabeads<sup>®</sup> mRNA DIRECT™ Kit

For direct isolation of poly (A)<sup>+</sup> RNA from cells, animal and plant tissue.

610.06 Dynabeads<sup>®</sup> mRNA Purification Kit

For poly (A)<sup>+</sup> RNA purification from total RNA.

610.02 Dynabeads<sup>®</sup> Oligo (dT)<sub>25</sub>, (2 x 1 ml)

610.05 Dynabeads<sup>®</sup> Oligo (dT)<sub>25</sub>, (5 x 1 ml)

610.31 Dynabeads<sup>®</sup> mRNA DIRECT™ Buffer Set

### 7. References

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For more references, please contact Dynal Biotech Technical Service.

#### **8. Warnings and Limitations**

The Dynabeads<sup>®</sup> mRNA DIRECT<sup>™</sup> Micro Kit is guaranteed stable until the expiry date stated on the label when stored unopened at 2-8°C. The Dynabeads mRNA DIRECT<sup>™</sup> Micro Kit is for research use only. The phosphate-buffered saline (PBS) contains 0.02% sodium azide (NaN<sub>3</sub>) as a preservative. Sodium azide may react with lead and copper drain-pipes to form highly explosive metal azides. When disposing through drains, flush with large volumes of water to prevent azide build-up. Sodium azide is toxic if ingested. RNase contamination should be prevented by standard procedures during the preparation of starting material and during the experiment. Avoid pipetting by mouth.

Prior to use, the Dynabeads Oligo (dT)<sub>25</sub> should be washed once in Lysis/binding buffer as described in the Dynabeads mRNA DIRECT<sup>™</sup> Micro Kit protocol. Remember to resuspend the Dynabeads Oligo (dT)<sub>25</sub> well to obtain a homogenous dispersion of Dynabeads in solution. The Dynal MPC should not be kept in close contact with magnetic tapes, computer discs or other magnetic storage systems, as they can be damaged by the strong magnetic field. The Dynal MPC's have been designed for use with Dynal Biotech's Dynabeads products and their application in molecular biology. The Dynabeads and Dynal MPC products are covered by various patents. These products may not be repackaged, reformulated or resold in any form without written consent of Dynal Biotech ASA, Oslo, Norway.

#### **9. Warranty**

The products are warranted to the original purchaser only to conform to the specifications indicated on the unit label for a period of one year from the date of purchase. Dynal Biotech's obligation and the purchaser's exclusive remedy under this warranty is limited either to replacement, at Dynal Biotech's expense, of any products which shall be defective in manufacture, and which shall be returned to Dynal Biotech, transportation prepaid, or at Dynal Biotech's opinion, refund of the purchase price. It shall be the responsibility of the purchaser to pack returned item(s) in a manner to avoid shipping damage to the unit. Claims for merchandise damaged in transit must be submitted to the carrier.

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