Contents

The Dynabeads® mRNA DIRECT™ Kit is for research use only.

1. Introduction

1.1 Intended Use

The **Dynabeads® mRNA DIRECT™** Kit is designed for simple and rapid isolation of pure, intact polyA$^+$ mRNA directly from the crude lysates of animal and plant cells and tissues.

1.2 Product Description

The procedure relies on A-T base pairing. Short sequences of oligo-dT are covalently bound to the surface of the Dynabeads Oligo(dT)$_{25}$. Under optimal conditions the polyA tail of mRNA will hybridize to the bead-bound oligo-dT, allowing a simple and rapid extraction. DNA, proteins, rRNA and small RNAs such as tRNA do not bind to the beads and are discarded, eliminating the need for post extraction DNase treatment etc. RNase inhibiting agent in the Lysis/Binding Buffer together with stringent hybridisation and washing steps ensure the isolation of pure, intact mRNA from crude samples rich in RNase, without the use of strong chaotropic agents.
Dynabeads Oligo(dT)$_{25}$ can bind up to 2 µg of polyA$^+$ mRNA per mg of beads, depending on the tissue or cell type. Since the oligo-dT is covalently bound to the bead surface, the beads can be regenerated and re-used after mRNA elution.
The protocol is flexible and easily scaled up or down to suit specific sample size, and has been used successfully in isolating mRNA from a single cell (Ref. 1). This allows the detection of mRNA by RT-PCR from highly specialized cells (e.g. isolated from a heterogenous sample by LCM or immunomagnetic separation).
The kit protocol has been successfully used to isolate mRNA from a wide variety of tissues of mammalian, fish, amphibian, insect and plant origins (see Appendix 6.III)
The isolated mRNA is suitable for use in all downstream molecular biology applications, e.g. gene cloning and gene expression analysis, S1 nuclease analysis, ribonuclease protection assay, primer extension, subtractive hybridisation, SAGE, RACE etc. For many applications elution of the mRNA from the beads is not required since the beads do not interfere with downstream enzymatic reactions. The bead-bound oligo-dT can also function as a primer for reverse transcription and synthesis of first-strand cDNA, allowing solid-phase RT-PCR and the construction of solid-phase cDNA libraries.

The main advantages of the Dynabeads mRNA DIRECT™ Kit are:
- No pre-purification of total RNA or other extraction steps are necessary.
- The procedure is rapid and simple and can be performed in under 15 minutes.
- Elution of isolated mRNA can be done in very small volumes.
- No need to elute the mRNA from the beads for many applications.
- Enzymatic reactions are not inhibited by the presence of Dynabeads Oligo(dT)$_{25}$.
- The unique properties of Dynabeads (uniformity of size and shape; monodispersity, combined with superparamagnetism) allows for rapid and efficient binding and handling of target.

- The true spherical shape of Dynabeads helps reduse non-specific binding associated with irregular shaped particles.

1.3 Solid-Phase mRNA Applications

Immobilised cDNA libraries for multiple PCR amplifications

One major advantage of using Dynabeads Oligo(dT)$_{25}$ for mRNA isolation is that the oligo-dT capture probe on the beads can function as a primer for first-strand cDNA synthesis, making it possible to create tissue/cell specific cDNA libraries directly on the beads. This solid-phase cDNA is suitable for PCR amplification, and simplifies techniques such as Rapid Amplification of cDNA Ends (5' and 3' RACE) and cDNA cloning (Refs. 1, 2, 3, 4).

The covalent linkage between the cDNA and the bead is extremely stable, allowing detection of different transcripts from the same cDNA library by multiple PCR amplifications. A carefully handled solid-phase cDNA library can be used for at least 10 rounds of amplification (for detection of 10 different transcripts), a major advantage when original sample material is limited e.g single cell investigations.

A solid-phase cDNA library on Dynabeads can be stored at 2-8ºC for several months.

Subtractive Hybridisation

Subtractive hybridisation with Dynabeads Oligo(dT)$_{25}$ is a rapid method that removes mRNAs common to two types of starting materials (solid tissue, cells or cDNA libraries). Re-usable solid-phase cDNA libraries generated on the Dynabead Oligo(dT)$_{25}$ surface provide a very convenient source of subtractor probe. The differentially expressed mRNAs are left in solution for subsequent manipulations like subtractive cloning or differential display (Refs. 3, 5, 6, 7, 8, 9).

1.4 Kit Components and Storage

Dynabeads mRNA DIRECT™ Kit includes Dynabeads Oligo(dT)$_{25}$ and lysis/binding, washing and elution buffers.

Prod. No. 610.11: Provides enough reagents for 20 standard isolations.
Prod. No. 610.12: Provides enough reagents for 40 standard isolations.

The suspension of Dynabeads Oligo(dT)$_{25}$ and the buffers provided are produced and packed under RNase-free conditions. All kit reagents are of analytical grade and are RNase-free.

Table 1. Kit Components

Volume Supplied		Component	Storage
610.11	**610.12**		
5 ml	10 ml	Dynabeads Oligo$(dT)_{25}$ Suspension of 5 mg/ml in PBS pH 7.4 containing 0.02% NaN_3.	2-8ºC
30 ml	60 ml	Lysis/Binding Buffer	2-8ºC *
60 ml	120 ml	Washing Buffer A	2-8ºC *
30 ml	60 ml	Washing Buffer B	2-8ºC *
15 ml	15 ml	10 mM Tris-HCl (Elution Buffer)	2-8ºC

* Some components in these buffers may precipitate during storage at 2-8ºC. Warm to room temperature and mix thoroughly before use to dissolve precipitate.

Store the vials of Dynabeads Oligo$(dT)_{25}$ in an upright position to ensure that the beads are covered with buffer at all times, as drying will reduce their performance. Dried beads should be resuspended in the buffer in which they are supplied and incubated overnight at 4ºC with continous mixing (roller mixer).
Do not freeze the Dynabeads Oligo$(dT)_{25}$.
Properly stored kits are guaranteed stable and RNase free until the expiry date stated on the label.

1.5 Additional Materials Required

- Magnet, Dynal MPC® (Magnetic Particle Concentrator): Dynal MPC®-S, Dynal MPC®-96S or Dynal MPC®-9600.
- RNase-free pipette tips and pipettors
- RNase-free microtubes
- Heat block and /or incubator at 65-80ºC for elution step (if required)
- Roller or end-over-end mixer
- Benchtop microcentrifuge
- Syringe and 21 gauge needle

To isolate mRNA from tissue samples the following is also needed.
- Liquid nitrogen
- Mechanical or manual tissue grinder (Pellet Pestle® Disp w/tube, Prod.No. 749520-0000 from Kimble/Kontes, NJ, USA or equivalent)

2. Sample Guidelines & Recommended Volumes

Dynabeads mRNA DIRECT™ Kit protocols are flexible and can be easily scaled up or down to suit specific sample source and quantity.
The mRNA content of cells and tissues varies greatly depending on the source of the material and RNA expression levels at the time of tissue/cell harvest. The following information is therefore intended only as a rough guide to the expected total RNA content of selected tissues.

Table 2. Estimated total RNA yield from mammalian cells and tissues:

Cell Type & Quantity	Estimated Total RNA Content (1-5% is mRNA)
Single mammalian cell	10 pg
50 mg of muscle tissue	50 – 80 µg
50 mg of liver tissue	400 µg
10^7 cultured fibroblasts	50 – 80 µg
10^7 cultured epithelial cells	100 – 120 µg

Table 3. Recommended volumes of Dynabeads Oligo$(dT)_{25}$ and buffers for use with different amounts of starting material:

Component	Maxi	Standard	Mini	Micro
Plant Tissue	100-400 mg	20-100 mg	4-20 mg	≤ 4 mg
Animal Tissue	50-200 mg	10-50 mg	2-10 mg	≤ 2 mg
Cultured Cells	4-20 x 10^6	1-4 x 10^6	0.15-1 x 10^6	≤ 150,000
Dynabeads Oligo$(dT)_{25}$	1 ml	250 µl	50 µl	10 µl
Lysis/Binding Buffer	5 ml	1250 µl	300 µl	300 µl
Washing Buffer A	10 ml	1-2 ml	600 µl	600 µl
Washing Buffer B	5 ml	1-1.5 ml	300 µl	300 µl
Tris-HCl (Elution is optional)	50-100 µl	10-25 µl	10 µl	10 µl

For isolation from micro-scale samples, use the Dynabeads® mRNA DIRECT™ Micro Kit (Prod. No. 610.21).
For isolation from total RNA samples, use the Dynabeads® mRNA Purification Kit (Prod. No. 610.06).

3. Initial Checklist & Technical Tips

You are advised to read this section before starting the mRNA isolation protocol.

Checklist

- All buffers except the 10 mM Tris-HCl (Elution Buffer) should be brought to room temperature prior to use. The 10 mM Tris-HCl should be stored on ice or at 2-8ºC prior to use.
- Ensure that the Dynabeads Oligo$(dT)_{25}$ have been fully resuspended before use. Resuspend by brief vortexing or pipetting.
- Check the Lysis/Binding Buffer and Washing Buffers A and B for precipitate. If any precipitation is observed, continue warming to room temperature and mix thoroughly.
- If performing solid-phase applications with the isolated mRNA, prepare the enzyme mix prior to mRNA isolation. Store the mix on ice. Prepare extra reaction buffer (without enzyme) for one final wash prior to performing the enzymatic reaction.

Prevent mRNA Degradation

- Work in a designated "ribonuclease-free zone".
- Wear disposable gloves and change them frequently.
- Work quickly and keep everything cold during preparation of starting material and the lysis step.
- A rapid lysis in Lysis/Binding Buffer is critical for obtaining undegraded mRNA. Thawing of frozen material prior to the lysis step must not occur.
- Use sterile disposable plasticware wherever possible.
- Laboratory glassware should be heat baked at 250ºC for at least 4 hours.
- Treat water, glassware and salt solutions with diethyl pyrocarbonate (DEPC).
- Use sterile, RNase-free microtubes and pipettors.
- RNase inhibitors may be added to the protocol at any step. This is normally redundant. However, if storage of the eluted mRNA is required, addition of an RNase inhibitor at the elution step might be useful.
- Keep the eluted mRNA on ice.

Technical Tips

- The bead to volume ratio is important for optimal binding of mRNA to the Dynabeads Oligo$(dT)_{25}$, especially when scaling up or down the protocol. As a general rule, optimal binding occurs at a 1:1 to 1:2 beads:buffer ratio (i.e. 100 µg Dynabeads Oligo$(dT)_{25}$ in 100–200 µl Lysis/Binding Buffer.
- Complete resuspension of the beads/mRNA complex during washing and complete removal of the washing buffers at each step will prevent carry over of LiDS and other salts to your downstream reaction. Transferring the beads/mRNA complex to new tubes at each washing step will help prevent carry over of LiDS etc to downstream enzymatic reactions. LiDS is a strong inhibitor of enzymatic reactions.

- When isolating mRNA from specific cells isolated by immunomagnetic separation (IMS), make sure that all IMS-Dynabeads are removed from the lysate before adding Dynabeads Oligo(dT)$_{25}$.
- The complete removal of supernatant from the Dynabeads Oligo(dT)$_{25}$ is extremely important when working with very small samples. The use of carrier RNA (e.g tRNA) will result in a more efficient and specific binding of polyA$^+$ mRNA.
- If spectrophotometric determination of mRNA concentration is desired, the eluted mRNA should be placed on the magnet while aliquots are removed since trace amounts of beads will interfere with the absorbance readings.

4. Protocols

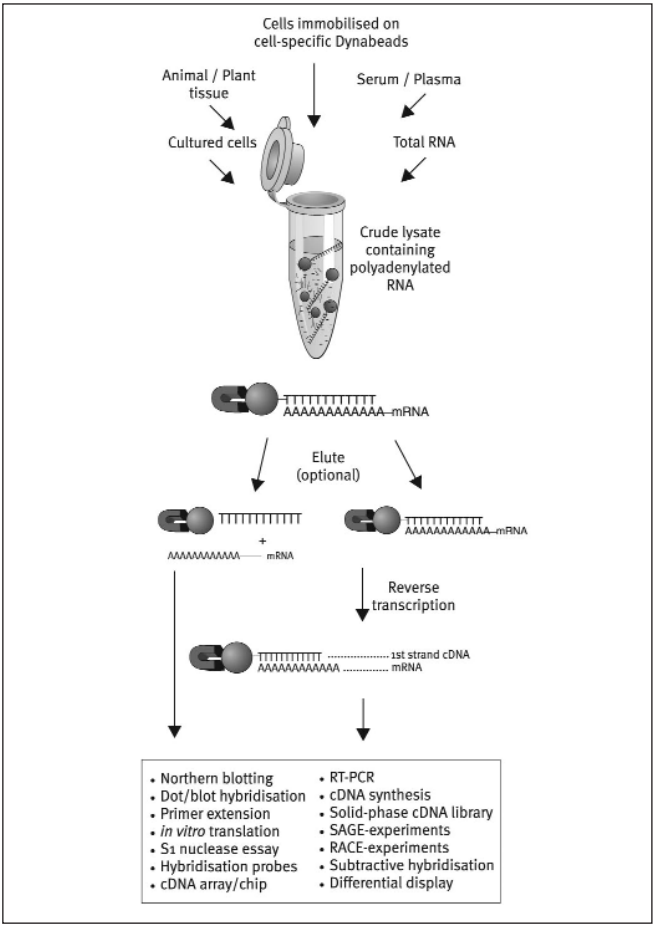

Fig 1: Outline of the protocol for isolating mRNA from a crude starting sample using Dynabeads Oligo(dT)$_{25}$. The isolated mRNA is suitable for use in all downstream molecular biology applications.

You are advised to read section 3 before starting the mRNA isolation protocol.
For specific volumes used throughout the protocol, see section 2, table 3.

4.1 Preparation of Dynabeads Oligo(dT)$_{25}$

1. Resuspend Dynabeads Oligo(dT)$_{25}$ thoroughly before use.
2. Transfer the desired volume of beads from the stock tube to a RNase-free 1.5 ml microcentrifuge tube and place the tube on a magnet (e.g. Dynal MPC-S).
3. After 30 seconds (or when the suspension is clear) remove the supernatant.
4. Remove the vial from the magnet (or slide out the magnetic bar of the Dynal MPC-S) and wash the beads by resuspending in an equivalent volume of fresh Lysis/Binding Buffer.
5. When the sample lysate is ready for combination with the beads, place the tube on the magnet and remove the supernatant after 30 seconds (or when the suspension is clear).

Note: Do not remove the supernatant from the beads until immediately before adding the sample lysate, as drying of the Dynabeads Oligo(dT)$_{25}$ may lower their capacity.

4.2 Preparation of Lysate from Solid Plant/Animal Tissues.

1. Aliquote (weigh) the animal or plant tissue while frozen, to avoid mRNA degradation. Ideally the tissue should be weighed and aliquoted before freezing. Do not exceed the specified amount of tissue, as using too much tissue will reduce the mRNA yield and purity.
2. Grind frozen tissue in liquid nitrogen. Work quickly.
3. Transfer the frozen powder to the appropriate volume of Lysis/Binding Buffer and homogenize until complete lysis is obtained (approx. 1-2 min). A rapid lysis in the Lysis/Binding Buffer is critical for obtaining undegraded mRNA.
4. If the raw extract is noticeably viscous, force the lysate 3-5 times through a 21 gauge needle using a 1-2 ml syringe to shear the DNA. Use force.
5. Spin the lysate for 30-60 seconds in a microcentrifuge to remove debris (and to reduce foaming after shearing). The lysate can be frozen and stored at –80°C for later use.
6. Transfer the lysate to the tube containing the prepared Dynabeads Oligo(dT)$_{25}$ (in section 4.1 above, step 5) and continue with the mRNA extraction as described in section 4.4 below.

4.3 Preparation of Lysate from Cultured Cells/Cell Suspensions.

1. 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 or at -80°C for later use.
2. Add the appropriate volume of 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 release of DNA during lysis results in a viscous solution which confirms complete lysis.

3. For samples containing over 500,000 cells a DNA-shear step is advised. Force the lysate 3-5 times through a 21 gauge needle using a 1-2 ml syringe to shear the DNA. Reduced viscosity should be visible. Repeated shearing causes foaming of the lysate due to detergent in the buffer, however, this should not effect the mRNA yield. The foam can be reduced by a 30 second centrifugation. The lysate can be frozen and stored at -80ºC for later use.
4. Transfer the lysate to the tube containing the prepared Dynabeads Oligo$(dT)_{25}$ in (section 4.1 above, step 5) and continue with the mRNA extraction as described in section 4.4.

4.4 Direct mRNA Isolation Protocol

1. Remove the Lysis/Binding Buffer from the pre-washed Dynabeads Oligo$(dT)_{25}$ by using the magnet (section 4.A. above, step 5).
2. Transfer the microtube from the magnet to another rack and add the sample lysate.
3. Mix the beads with the sample lysate and incubate with continuous mixing (rotating or roller mixer) for 3-5 min. at room temperature to allow the polyA-tail of the mRNA to anneal to the oligo-dT on the beads. Increase the incubation time if the solution is viscous.
4. Place the vial on the magnet for 2 min. and remove the supernatant. If the solution is noticeably viscous, increase the time to approx. 10 min.
5. Wash the beads/mRNA complex two times with the appropriate volume of Washing Buffer A at room temperature. Use the magnet to separate the beads from the solution between each washing step.
6. Wash the beads/mRNA complex once with the appropriate volume of Washing Buffer B at room temperature. Use the magnet to separate the beads from the solution.

Note: Mix the beads/mRNA complex thoroughly in the washing buffers either by a short gentle vortex or pipetting. Ensure that the buffer is removed completely between washing steps.

7. If the isolated mRNA is to be used in enzymatic downstream applications, one extra wash in Washing Buffer B plus a final wash in the enzymatic buffer to be used should be performed. E.g. reverse transcription first-strand synthesis buffer (without the enzyme).
8. If elution of mRNA from the beads is desired, add an appropriate volume of 10 mM Tris-HCl (Elution Buffer) and incubate at 65-80ºC for 2 min. Place the tube immediately on the magnet and transfer the supernatant containing the mRNA to a new RNase-free tube. Place the mRNA immediately on ice.

Note: For Northern blotting the mRNA can be eluted directly in the loading buffer.

4.5 Re-use of Dynabeads Oligo(dT)$_{25}$ for Large Scale Isolations:

Multiple isolations from the same sample can be performed by re-using Dynabeads Oligo(dT)$_{25}$ after mRNA elution without regeneration. Simply follow the protocol described in section 4.4, after elution of mRNA the beads are washed once in Lysis/Binding Buffer (section 4.1 above), a new volume of sample lysate is added to the beads and the isolation continued. Alternatively, the beads can be re-applied to the same sample lysate until all the mRNA has been captured.

Note: Buffer volumes supplied with the kit (Prod. No. 610.11 and 610.12) may not be sufficient for large scale mRNA isolations.

4.6. Elimination of rRNA Contamination

In some cases trace amounts of ribosomal RNA have been observed in the mRNA samples. For many applications such as Northern blotting and RT-PCR, trace amounts of rRNA contamination will not interfere with the analysis or interpretation of the results. However, for other applications, like cDNA library construction and microarray analysis, the possibility of rRNA contamination should be avoided.
Ribosomal RNA is effectively eliminated by re-extracting the mRNA from the eluate. Re-use of the same Dynabeads Oligo(dT)$_{25}$ used for the original isolation is recommended. If new beads are used, it is recommended that the beads are washed in 50 mM Sodium-pyrophosphate before the isolation of mRNA.

1. Follow the isolation protocol (section 4.4 above). Elute the mRNA in 10 mM Tris-HCl (Elution Buffer). Do not discard the beads.
2. Wash the beads two times in Washing Buffer B. Remove the supernatant completely from the washed beads.
3. Dilute the eluted mRNA in 4 times its volume of Lysis/Binding Buffer. (E.g. if the mRNA is eluted in 20 µl, add 80 µl of Lysis/Binding Buffer.)
4. Add the diluted mRNA to the washed beads and incubate with mixing at room temperature for 3-5 min.
5. Continue with the isolation protocol (section 4.4, starting at step 4.)

5. Troubleshooting

Problem:	The beads have become clumped during the incubation step with sample lysate
Cause:	The DNA in the sample lysate has not been completely sheared
Remedy:	Try pipetting the bead solution several times through a 1ml pipitte tip to break up the clumps. In future sample preparations increase the force and/or the number of passages through the needle in the shear step

Problem:	mRNA is contaminated with DNA
Cause:	i) Incomplete DNA shearing ii) Incomplete removal of sample lysate after hybridisation step and subsequent carry over to wash and elution steps. iii) Inefficient washing. iv) Incomplete removal of wash buffers v) Too high sample to beads ratio
Remedy:	i) Increase the force and /or the number of passages through the needle in the sample shear step. ii) Completely remove the sample lysate after hybridisation. iii) Make sure beads/mRNA complex is fully resuspended in washing buffers. iv) Completely remove the sample/washing buffers before continuing to the next step. v) Dilute the sample lysate or increase the amount of beads vi) Re-extract the mRNA from the eluate

Problem:	mRNA yield is lower than expected
Cause:	i) Inefficient elution of mRNA from the beads ii) Beads-to-sample ratio is too low iii) Cells/tissue incompletely lysed
Remedy:	i) Increase the elution volume/time/temperature or perform the elution step two times pooling the eluate. ii) Increase the amount of beads iii) Repeat the homogenisation step

Problem:	After reverse transcription the beads/cDNA complex is clumped and sticking to the tubes and pipette tips
Cause:	Non-specific electrostatic interactions between the cDNA molecules and the plastic materials of the tubes/pipette tips
Remedy:	i) Adding BSA (0.2-1.0% final concentration) to the reverse transcription mix before performing the cDNA synthesis will reduce clumping of the beads and result in a more efficient cDNA synthesis. Note: use highest possible quality of BSA ii) Where appropriate, 0.05% Tween-20 can be added to the reaction buffers iii) Alternatively, dilute the beads/cDNA solution (after reverse transcription) with an equal volume of the 1x reverse transcription reaction buffer containing 0.05% Tween-20, mix by pipetting and transfer the suspension to a new tube. Any beads remaining stuck to the walls of the tube can be removed by washing with a fresh aliquot of buffer containing Tween-20. These beads are then pooled with the original bead-suspension, Place the pooled bead-suspension on a magnet and remove the supernatant, then wash 2-3 times with the buffer containing Tween-20. The solid-phase cDNA library should be stored in an appropriate buffer containing 0.05% Tween-20.

Problem:	Unable to detect specific mRNA molecules.
Cause:	i) The beads-to-sample ratio is too low ii) Inappropriate sample volume iii) Hybridisation time too short
Remedy:	i) Increase the amount of beads ii) Reduce the sample volume/increase sample concentration iii) Increase the hybridisation incubation time to 10-15 min.
Comment	Reaction kinetics show that short mRNA molecules are the first to bind to the beads, followed by the longer molecules. Therefore, in the presence of a large excess of mRNA or short incubation time, binding to the beads may be biased towards short molecules, and many of the rare, larger mRNA molecules will remain undetected in the sample lysate. A similar bias may occur if the sample volume-to-bead ratio is too high, or if the sample is too dilute. Using the appropriate bead-to-sample ratio (volume and concentration), mRNA molecules over 15 kb in length have been detected using real-time PCR, after a standard 5 min. hybridisation incubation.

6. Appendix

I. Buffer Components

Lysis/Binding Buffer:
100 mM Tris-HCl, pH 7.5
500 mM LiCl
10 mM EDTA, pH 8
1% LiDS
5 mM dithiothreitol (DTT)

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 (Elution Buffer):
10 mM Tris-HCl, pH 7.5

II. Dynabeads Oligo(dT)$_{25}$ Characteristics

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
Concentration: 5 mg/ml

Dynabeads Oligo(dT)$_{25}$ are stable in a pH range of 4-13.
Do not freeze the Dynabeads Oligo(dT)$_{25}$

III. Sample Types from Which mRNA has Been Isolated Using Dynabeads Oligo(dT)$_{25}$

Using the Dynabeads mRNA DIRECT™ Kit, mRNA has been successfully prepared from various solid tissues, cells and viruses (see listed tables below). Isolation of cell-specific mRNA is done by combining immunomagnetic separation (IMS) and mRNA isolation. Specific cell types are rapidlyisolated from human whole blood using cell-specific Dynabeads. The cells are lysed while still attached to the Dynabeads and mRNA recovered from the supernatant for subsequent RT-PCR detection. Since many nucleated blood cells have a low content of mRNA, this represents a powerful approach to gene regulation studies. Combined Dynabeads immunomagnetic cell isolation and downstream mRNA isolation has been used to study leucocytes, as well as subsets of blood cells (e.g. CD4$^+$ and CD8$^+$ T-cells, CD19$^+$ B-cells and CD14$^+$ monocytes) and can be used for other immunomagnetically isolated cells such as metastatic cancer cells isolated from blood or bone-marrow (10).

Note: Dynabeads Oligo(dT)$_{25}$ can also be used for the isolation of poly A+ RNA from total RNA preparations as well as for isolation of viral polyA RNA from cells, serum or plasma.

Table 1: mRNA DIRECT from animal tissues

Tissue	Species	References
Adipose tissue	Pig	11
Adrenals	Rat	4
Brain	Mouse, trout	12,4
Brain (cerebral cortex, preoptic area)	Rat	13,14,15
Cartilage	Human	16
organ of Corti and spiral ganglion	Guineapig, rat	17
Eggs	Trout	4
Gut (paraffine embedded)	Human	18
Heart	Rat	13,19*
Hypothalamus	Rat	15
Kidney	Rat	13
Kidney (glomerular preparations)	Human	20
Liver, paraffin-embedded	Human	18
Liver	Rat, trout, Xenopus	13,19*,4

Lung, paraffin-embedded	Human	18
Lung	Rat	13
Muscle	Rat, trout	19*,4
Ovaries	Trout, Xenopus	4
Pancreas	Rat	19*
Paraffin-embedded lung, liver, gut	Human	18
Paraffin-embedded keratinocytes	Human	21
Pituitary	Rat	22
Pronephros	Trout	4
Spleen	Rat	4,13
Trematode	Schistosoma mansoni	23
Whole insect	Drosophila	4

* Lysis buffer with 4 M urea and 1% SDS.

Table 2: mRNA DIRECT from plant tissues

Tissue	Species	References
Whole plants	Arabidopsis	24,25,26,27,23
Bud	Tobacco	28
Epidermal leaf cell (single cells)	Tomato	1
Embryos	Maize	29
Flowers	Maize,tobacco	30
Guard cell in leaf (single cells)	Tomato	1
Leaves	Barley	31,4,32
	Brassica oleracea	33
	Maize	29,27
	Potato	34
	Tobacco	28
	Tomato	1
Ovules	Maize	29
Roots	Barley	31
	Brassica oleracea	33
	Spruce	6
	Maize	29
Seed aleurone	Barley	31,4,35,36,32
Seed endosperm	Barley	35,36,32
Seed embryos	Barley	31,4,35,36,32
Seedlings	Maize, tobacco	29,28
Single leave cells	Tomato	1
Stem	Tobacco	28
Stigma	Brassica oleracea	33,37
Stolon tips	Potato	34

Table 3: mRNA DIRECT from different types of cells

Cell type/cell line	Origin	References
Chondrocytes	Human	16
Cervical cancer cells; HeLa	Human	38,39
Colon carcinoma cell line; COLO320	Human	40
Fibroblast cell line; ST-1 and SKB-1	Human	41,9
Fibroblasts; D551	Human	8,9
Fibroblasts; RTG-2	Trout	4
Endothelial cells, umbilical cord	Human	8,9
Hepatocyte cell line; HepG2	Human	8,39
Keratinocytes	Human	42,21
Langerhans cells	Human	42
Lymphoblast B-cell lines (Reh, Daudi, HL-60, IM9)	Human	8,4,39,43
Mamma carcinoma cells; MCF7	Human	38,39
Mamma carcinoma cells; T47D	Human	40
Monocytes	Human	44
Pancreas, insulinoma Rinm5F cells	Rat	45
Peripheral blood mononuclear cells (PBMC)	Human	46
Peritoneal exoduate cells	Human	42
Placental cell line;AMA	Human	38,39
T-cells/T-cell clones	Human	2,47,48,49,50
Yeast (Saccharomyces cerevisiae, Hansenula polymorpha	in soil samples	51
Yeast (Saccharomyces cerevisiae)	culture	52

* GTC lysis buffer.

Table 4: Direct isolation of viral poly A+ RNA with Dynabeads Oligo(dT)$_{25}$

Starting material	Virus	References
Cells in broncho-alveolar washes	HIV-1	47[1)]
Cerebrospinal fluid	HIV-1	53[1)]
Cell line	HTLV-I/II	54[2)]
Peripheral blood mononuclear cells (PBMC)	HIV-1	47[1)]
Plasma	HIV-1, HIV-2	54[1) 2)]
Serum	HIV-1	55[1)],56[1)],54[1) 2)]
T-lymphocytes cell line, CD4$^+$	HIV-1	47[1)]

[1)] Lysis/binding buffer: 1 M LiCl, 2% SDS, 2xTE, 50 µg tRNA, Vanadyl ribonucleosyl complexes.
[2)] Lysis/binding buffer: 4 M GTC, 0.5% sarkosyl, 1% DTT, 0.5 M LiCl, 0.1 M Tris pH8.

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Prod. No.	Product description
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7.1 Storage/Stability

This product is stable until the expiry date stated on the label when stored unopened at 2-8ºC. Store opened vials at 2-8ºC and avoid bacterial contamination.
Keep Dynabeads in liquid suspension during storage and all handling steps, as drying will result in reduced performance. Resuspend well before use.

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This product contains 0.02% sodium azide as a preservative, which is cytotoxic. **Avoid pipetting by mouth!** 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. Certificate of Analysis (CoA) is available upon request.
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