USER GUIDE

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by life technologies™

Dynabeads® mRNA DIRECT™ Kit

For the isolation of pure mRNA
directly from crude samples

Catalog numbers 61011 and 61012

Revision Date June 2012 (Rev. 009)

For Research Use Only. Not for human or animal therapeutic or diagnostic use.

life technologies™

Contents

Kit Contents and Storage

Storage

Upon receipt, store all components of the Dynabeads® mRNA DIRECT™ Kit at 2°C to 8°C. **Do not freeze** the Dynabeads® Oligo $(dT)_{25}$.

Keep Dynabeads® in liquid suspension during storage and all handling steps, as drying will result in reduced performance. Resuspend thoroughly before use.

Precautions should be taken to ensure that RNase or microbial contamination of the kit components does not occur.

This product contains 0.02% sodium azide as a preservative.

Warning! GENERAL CHEMICAL HANDLING. For every chemical, read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.

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Kit Contents and Storage, continued

Kit contents

The components included in the Dynabeads® mRNA DIRECT™ Kit are listed in the following table.

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.

Sufficient reagents are provided in the kit to perform 20 (Cat. no. 61011) or 40 (Cat. no. 61012) isolations.

Component	Cat. no. 61011	Cat. no. 61012
Dynabeads® Oligo $(dT)_{25}$* (≈5 mg/mL, supplied in PBS pH 7.4)	5 mL	10 mL
Lysis/Binding Buffer 100 mM Tris-HCl, pH 7.5 500 mM LiCl 10 mM EDTA, pH 8 1% LiDS 5 mM dithiothreitol (DTT)	30 mL	60 mL
Washing Buffer A 10 mM Tris-HCl, pH 7.5 0.15 M LiCl 1 mM EDTA 0.1% LiDS	60 mL	120 mL
Washing Buffer B 10 mM Tris-HCl, pH 7.5 0.15 M LiCl 1 mM EDTA	30 mL	60 mL
10 mM Tris-HCl pH 7.5 (Elution Buffer)	15 mL	15 mL

*Contains 0.02% sodium azide as preservative.

Product Use

For Research Use Only. Not for human or animal therapeutic or diagnostic use.

Description of the System

About the Kit

Introduction

This product is designed for simple and rapid isolation of pure, intact polyadenylated (polyA) mRNA directly from the crude lysate of animal and plant cells and tissues.

The isolated mRNA is suitable for use in all downstream applications.

System overview

The isolation protocol relies on base pairing between the polyA 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 polyA tail will not hybridize to the beads and are readily washed away.

RNase inhibiting agents in the Lysis/Binding Buffer together with stringent hybridization and washing conditions ensure the isolation of pure, intact mRNA from crude samples rich in RNase, without the use of strong chaotropic agents.

The protocol is flexible and can easily be scaled up or down to suit all sample sizes. It has successfully been used in the isolation of mRNA from single cells (Karrer, et al., 1995). The high capture efficiency facilitates detection of mRNA by reverse transcriptase (RT)-PCR from highly specialized cells (e.g., isolated from a heterogeneous sample by immunomagnetic separation). In addition, the protocol has been successfully used to isolate mRNA from a wide variety of tissues of mammalian, fish, amphibian, insect, and plant origins (see page 17).

Continued on next page

System overview, continued

For many applications elution of the mRNA from the beads is not required as the beads do not interfere with downstream enzymatic reactions. The bead-bound oligo $(dT)_{25}$ can also function as a primer for RT and synthesis of first-strand cDNA (ref. 60), allowing the construction of solid-phase cDNA libraries and solid-phase RT-PCR.

Binding Capacity

1 mg of Dynabeads® Oligo $(dT)_{25}$ beads (200 µL) binds up to 2 µg of mRNA. A typical mammalian cell contains about 10–30 pg of total RNA, from which 1–5% is mRNA.

Expected Yield

The information in the following table is intended as a rough guide to the expected total RNA content of selected tissues, as well as appropriate bead and buffer volumes.

Cell Types & Quantity	Estimated Total RNA content (1–5% is mRNA)
Single Mammalian Cell	10–30 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

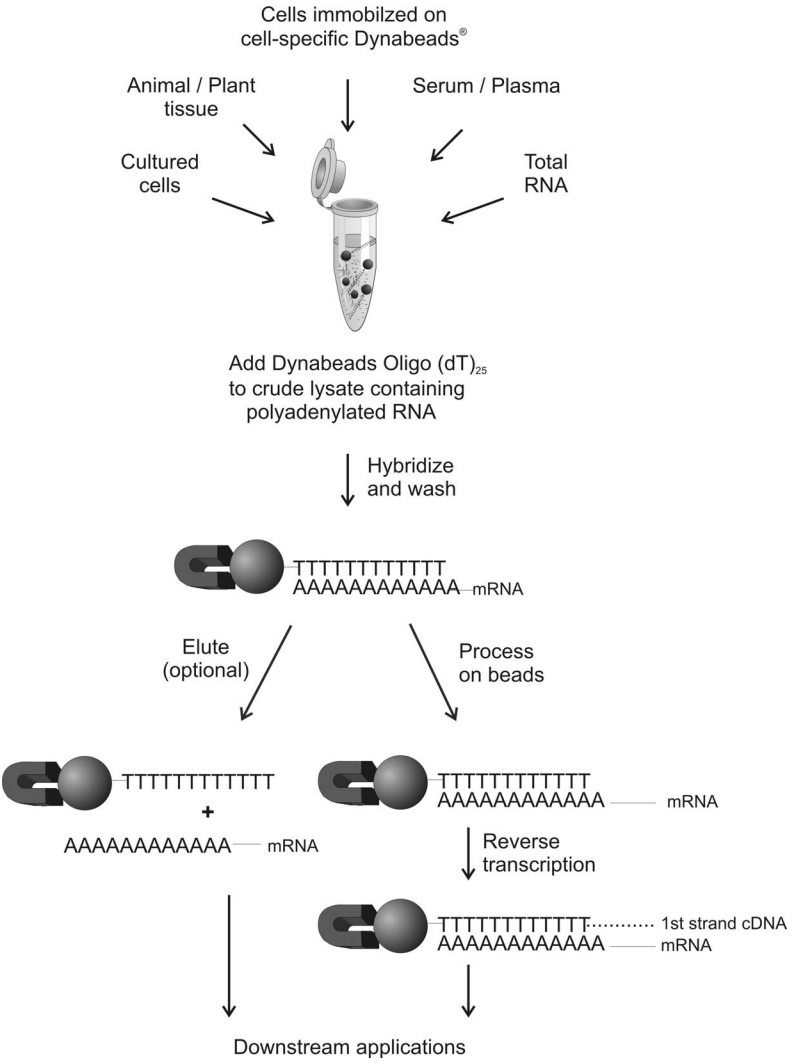
Sample Bias

When the appropriate bead-to-sample ratio (volume and concentration) is used there is no bias in hybridization based on mRNA size. However, with a large excess of mRNA or short incubation time, binding to the beads may be biased towards the short molecules. A similar bias may occur if the sample volume-to-bead ratio is too high.

Experimental Overview

mRNA Isolation Workflow

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.



Methods

General Guidelines

General Guidelines

- Keep Dynabeads® Oligo $(dT)_{25}$ in liquid suspension during storage and all handling steps. Resuspend thoroughly before use.
- Work RNase free and wear gloves.
- Precipitate may form in the buffers. Dissolve precipitate before use by warming to room temperature and mixing thoroughly.
- Bring Lysis/Binding Buffer and Washing Buffers A and B to room temperature prior to use.
- 10mM Tris-HCl pH 7.5 (Elution Buffer) should be stored on ice or at 2°C to 8°C prior to use.
- Thorough resuspension of the beads/mRNA complex during washing and complete removal of the washing buffer at each step will prevent carryover of LiDS and other salts to the downstream reaction. Transferring the beads/ mRNA complex to new tubes before the last washing step will further reduce LiDS carry over. LiDS is a strong inhibitor of enzymatic reactions.

Sample Preparation

Introduction

mRNA content of cells and tissues vary depending on the source of the material and RNA expression levels at the time of tissue/cell harvest. Dynabeads® mRNA DIRECT™ Kit protocols can be scaled up or down to suit specific sample source and quantity.

Required Materials

Components required but not supplied:

- RNase-free micropipettors, disposable pipette tips (aerosol resistant preferred) and micro-centrifuge tubes
- DynaMag™-2 magnet, see **Accessory Products** (page **16**) for ordering information or visit **www.lifetechnologies.com/magnets** for magnet recommendations
- Mixer allowing both tilting and rotation
- Water bath or heating block (for optional elution)
- Microcentrifuge capable of achieving >10,000 × *g*

Components required for isolation of mRNA from tissue samples:

- Liquid Nitrogen
- Mechanical or manual tissue grinder
- Syringe and 21 gauge needle

Components supplied with the kit:

- Dynabeads® Oligo $(dT)_{25}$
- Lysis/Binding Buffer
- Washing Buffer A
- Washing Buffer B
- Elution Buffer (10 mM Tris-HCl, pH 7.5)

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Solid Plant or Animal Tissue

1. Aliquot (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 (see the following table), as using too much tissue reduces the mRNA yield and purity.

Component	Std (mg)	Micro (mg)	Mini (mg)	Maxi (mg)
Plant Tissue	20–100	≤4	4–20	100–400
Animal Tissue	10–50	≤2	2–10	50–200

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 (≈1–2 min). Rapid lysis in the Lysis/Binding Buffer is critical for preventing mRNA degradation.

Std	Micro	Mini	Maxi
1250 µL	300 µL	300 µL	5 mL

4. A DNA-shear step is recommended for samples containing over 5×10^5 cells.

   Force the lysate through a 21 gauge needle 3–5 times using a 1–2 mL syringe to shear the DNA. The reduction in viscosity should be noticeable.

   Note: Repeated shearing causes foaming of the lysate due to detergent in the buffer, however, this should not affect the mRNA yield. The foam can be reduced by a 30 second centrifugation. The lysate can be frozen and stored at –80°C for later use.

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Cultured Cells or Cell Suspension

1. Pellet the appropriate number of cells (indicated in the following table), by centrifugation (e.g., 400 × *g* for 8 minutes at 4°C).

Std	Micro	Mini	Maxi
1–4 × 10^6	≤150,000	0.15–1 × 10^6	4–20 × 10^6

2. Wash the pellet by resuspending in phosphate-buffered saline (PBS). Pellet cells by centrifugation again. Use the cell pellet immediately, or freeze in liquid nitrogen or at –80°C for later use.
3. Add the appropriate volume of Lysis/Binding Buffer to either a frozen cell pellet or to a fresh cell pellet.

Std	Micro	Mini	Maxi
1250 µL	300 µL	300 µL	5 mL

4. 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.
5. A DNA-shear step is recommended for samples containing over 5 × 10^5 cells.

   Force the lysate through a 21 gauge needle 3–5 times using a 1–2 mL syringe to shear the DNA. The reduction in viscosity should be noticeable.

   Note: Repeated shearing causes foaming of the lysate due to detergent in the buffer, however, this should not affect the mRNA yield. The foam can be reduced by a 30 second centrifugation. The lysate can be frozen and stored at –80°C for later use.

Prepare Dynabeads® Oligo (dT)$_{25}$

Prepare 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 an RNase-free 1.5-mL microcentrifuge tube and place the tube on a magnet (e.g., DynaMag™-2).

Std	Micro	Mini	Maxi
250 µL	10 µL	50 µL	1 mL

3. After 30 seconds (or when the suspension is clear), remove the supernatant.
4. Remove the tube from the magnet and wash the beads by resuspending in an equivalent volume of fresh Lysis/Binding Buffer.
   Optional: For very small bead volumes (mini- and micro- isolations) use 50–100 µL wash volume to ease handling.
5. Proceed to **Direct mRNA Isolation Protocol**, page **9**.

Direct mRNA Isolation Protocol

Direct mRNA Isolation Protocol

1. Remove the Lysis/Binding Buffer from the pre-washed Dynabeads® Oligo(dT)$_{25}$ (**Prepare Dynabeads® Oligo (dT)$_{25}$**, step 4) by placing on the magnet for 30 seconds, or until the suspension is clear and discard the supernatant.
2. Remove the microcentrifuge tube from the magnet and add the appropriate volume of sample lysate indicted in the following table.

Std	Micro	Mini	Maxi
1250 µL	300 µL	300 µL	5 mL

3. Pipet to resuspend the beads completely in the sample lysate.
4. Incubate with continuous mixing (rotating or roller mixer) for 3–5 min at room temperature to allow the polyA tail of the mRNA to hybridize to the oligo (dT)$_{25}$ on the beads. **Note:** Increase the incubation time if the solution is viscous.
5. Place the vial on the magnet for 2 minutes and remove the supernatant. If the solution is noticeably viscous, increase the time to approximately 10 minutes.
6. Wash the beads/mRNA complex two times with the appropriate volume of Washing Buffer A (see the following table) at room temperature. Use the magnet to separate the beads from the solution between each washing step.

Std	Micro	Mini	Maxi
1–2 mL	600 µL	600 µL	10 mL

Continued on next page

Direct mRNA Isolation Protocol, continued

7. Wash the beads/mRNA complex once with the appropriate volume of Washing Buffer B (see the following table) at room temperature. Use the magnet to separate the beads from the solution.

Std	Micro	Mini	Maxi
1–1.5 mL	300 µL	300 µL	5 mL

8. If the isolated mRNA is to be used in enzymatic downstream applications (e.g., RT-PCR), one extra wash in Washing Buffer B is recommended. This should be followed by a final wash in the enzymatic buffer to be used (e.g., RT-PCR buffer without the enzyme or primers).

   Note: Perform cDNA synthesis as recommended by the manufacturer of the reverse transcriptase. When using a thermostable reverse transcriptase and the bead-bound oligo (dT) as primer for first strand cDNA synthesis, an initial incubation at 50°C for 5 minutes is necessary before proceeding at the recommended temperature.

9. If elution of mRNA from the beads is desired, add an appropriate volume (see the following table) of 10 mM Tris-HCl pH 7.5 (Elution Buffer) and incubate at 65°C to 80°C for 2 minutes. Immediately place the tube on the magnet, transfer the supernatant containing the mRNA to a new RNase-free tube and place this tube on ice.

Std	Micro	Mini	Maxi
10–25 µL	10 µL	10 µL	50–100 µL

Large Scale Isolations

Reuse of Dynabeads® Oligo (dT)$_{25}$

Note: The buffers supplied with the kit may not be sufficient for large scale mRNA isolations.

Multiple isolations from the same sample can be performed by reusing Dynabeads® Oligo(dT)$_{25}$ after mRNA elution. Simply follow the protocol described in **Direct mRNA Isolation Protocol** (page **9**).

- After elution of the mRNA, wash the beads once in Lysis/Binding Buffer (**Prepare Dynabeads® Oligo (dT)$_{25}$**, page **8**).
- Add a new lysate sample to the beads and continue the isolation as usual. Alternatively, washed beads can be re-applied to the same sample lysate until all the mRNA has been captured.

Elimination of rRNA Contamination

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 such as cDNA library construction and microarray analysis, rRNA contamination should be avoided.

Ribosomal RNA is effectively eliminated by re-extracting the mRNA from the eluate. Reuse of the same Dynabeads® Oligo $(dT)_{25}$ used for the original isolation is recommended. If new beads are used, wash the beads in 50 mM sodium pyrophosphate before the isolation of mRNA.

1. Follow the **Direct mRNA Isolation Protocol** (page **9**). Elute the mRNA in Elution Buffer.
2. Transfer the eluted mRNA to a new RNase-free tube and place on ice. Do not discard the beads.
3. Wash the beads two times in Washing Buffer B (**Direct mRNA Isolation Protocol,** step 6 on page **10**).
4. Dilute the eluted mRNA with 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).
5. Remove the Washing Buffer B from the beads, by placing the tube on the magnet, and add the diluted mRNA.
6. Incubate with mixing at room temperature for 3–5 minutes.
7. Continue with the **Direct mRNA Isolation Protocol** (page **9**) starting at step 4.

Troubleshooting

Observation	Cause	Solution
Clumping of beads during incubation with sample lysate.	DNA in the sample lysate has not been completely sheared.	Pipet the solution several times through a 1-mL pipette.
		Increase force/number of passages through the needle in future shearing steps.
mRNA is contaminated with DNA.	Incomplete DNA shearing.	Increase the force and/or the number of passages through the needle in the DNA shearing step.
	Incomplete removal of sample lysate after hybridization step, and subsequent carry over to wash and elution steps.	Completely remove the sample lysate after hybridization.
	Inefficient washing.	Make sure the beads/mRNA complex is fully resuspended in washing buffer.
	Incomplete removal of wash buffers.	Completely remove the sample/washing buffers.
	Sample-to-beads ratio too high.	Dilute sample lysate or increase the amount of beads.
		Re-extract the mRNA from the eluate.
mRNA yield lower than expected.	Inefficient elution of mRNA from the beads.	Increase the elution volume/time/temperature or perform the elution step two times, pooling the eluate.
	Beads-to-sample ratio is too low.	Increase the amount of beads.
	Cells/tissue incompletely lysed.	Repeat the homogenization step.

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Observation	Cause	Solution
The beads-cDNA complex is clumped and sticking to the tubes after reverse transcription.	Non-specific electrostatic interactions between the cDNA molecules and the plastic materials of the tubes/pipette tips.	• Add BSA (0.2–1.0% final concentration) to the reverse transcription mix before performing the cDNA synthesis. This is to reduce clumping of the beads for a more efficient cDNA synthesis. **Note:** Use best possible BSA quality. • Where appropriate, add 0.05% Tween-20 to the reaction buffers. • Alternatively, 1. Dilute the beads/cDNA solution (after reverse transcription) with an equal volume of the 1X reverse transcription reaction buffer containing 0.05% Tween-20. 2. Mix by pipetting and transfer the suspension to a new tube. 3. If there are any remaining beads stuck to the tube walls, remove by washing with a fresh aliquot of buffer containing Tween-20. 4. Pool these beads with the original bead suspension. Place the pooled beads on a magnet and remove the supernatant. 5. Wash 2–3 times with the buffer containing Tween-20. 6. Store the solid-phase cDNA library in an appropriate buffer containing 0.05% Tween-20.

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Troubleshooting, continued

Observation	Cause	Solution
Unable to detect specific mRNA molecules.	The beads-to-sample ratio is too low.	Increase the amount of beads.
	Inappropriate sample volume.	Reduce sample volume/increase sample concentration.
	Hybridization time too short.	Increase hybridization incubation time to 10–15 min.

Appendix

Accessory Products

Introduction

The following products may be used with the Dynabeads® mRNA DIRECT™ Kit. For details, visit **www.lifetechnologies.com** or contact **Technical Support** (see page **21**).

Product	Quantity	Cat. no.
Dynabeads® Oligo(dT)$_{25}$	5 mL	61005
DynaMag™-2 Magnet	Each	12321D
SampleRack (for DynaMag™-2)	Each	12322D
DynaMag™-15 Magnet	Each	12301D
DynaMag™-96 Side Skirted	Each	12027
HulaMixer® Sample Mixer	Each	15920D
SuperScript® III Reverse Transcriptase	2000 units	18080-093
SuperScript® III First-Strand Synthesis System	50 reactions	18080-051
SuperScript® III One-Step RT-PCR System with Platinum® Taq	100 reactions	12574-035
SuperScript® VILO™ cDNA Synthesis Kit	50 reactions	11754-050
UltraPure™ DNase/RNase-free Distilled Water	500 mL	10977-015

Sample Types from which mRNA has been Isolated Using Dynabeads® Oligo (dT)$_{25}$

Animal Tissues

Isolated mRNA from animal tissues

Tissue	Species	References
Adrenals	Rat	4
Brain	Mouse, Trout	12, 4
Brain (cerebral cortex, preoptic area, dentate gyrus)	Rat	13, 14, 15
Cartilage	Human	16
Organ of Corti and spiral ganglion	Guinea Pig, Rat	17
Ear (cochleae)	Mouse	11
Eggs	Trout	4
Gut (paraffin 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
Nematode (frozen rehydrated cysts)	*Globodera rostochiensis*	58
Ovaries	Trout, Xenopus	4
Pancreas	Rat	19*
Paraffin embedded lung, liver, gut	Human	18
Paraffin embedded keratinocytes	Human	21
Pituitary	Rat	22
Plasma	Human	59
Pronephos	Trout	4
Skin (dried)	Frog	57
Spleen	Rat	4, 13
Trematode	*Schistosoma mansoni*	23
Whole insect	Drosophila	4

*Lysis buffer with 4 M urea and 1 % SDS

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Sample Types from which mRNA has been Isolated Using Dynabeads® Oligo (dT)$_{25}$, continued

Plant Tissues

Isolated mRNA from plant tissues

Tissue	Species	References
Whole plants	*Arabidopsis thaliana*, Rice, Oryza sativa	23, 24, 25, 26, 27
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, *Brassica oleracea*, Maize, Potato, Tobacco, Tomato	31, 4, 32, 33, 29, 27, 34, 28, 1
Ovules	Maize	29
Roots	Barley, *Brassica oleracea*, Spruce, Maize	31, 33, 6, 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 leaf cells	Tomato	1
Stem	Tobacco	28
Stigma	*Brassica oleracea*	33, 37
Stolon tips	Potato	34

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Sample Types from which mRNA has been Isolated Using Dynabeads® Oligo (dT)$_{25}$, continued

Cells

Isolated mRNA from different types of cells

Cell type/cell line	Species	References
Chondrocytes	Human	16
Cervical cancer cells (HeLa)	Human	38, 39
Colon carcinoma cell line (COLO320)	Human	40
Fibroblast cells line (ST-1 and SKB-1)	Human	41, 9
Fibroblast (D551)	Human	8, 9
Fibroblast (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 (T47D)	Human	40
Monocytes	Human	44
Pancreas, insulinoma Rinm5F cells	Rat	45
Peripheral blood mononuclear cells (PBMC)	Human	46
Peritoneal exuduate 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

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Sample Types from which mRNA has been Isolated Using Dynabeads® Oligo (dT)$_{25}$, continued

Viruses

Direct isolation of viral polyA RNA with Dynabeads® Oligo (dT)$_{25}$

Starting Material	Virus	References
Cells in bronchoalveolar washes	HIV-1	47*
Cerebrospinal fluid	HIV-1	53*
Cell line	HTLV-I/II	54**
Peripheral blood mononuclear cells (PBMC)	HIV-1	47*
Plasma	HIV-1/HIV-2	54*, **
Serum	HIV-1	55*, 56*, 54*, **
T-lymphocytes cell line (CD4+)	HIV-1	47*

*Lysis/binding buffer: 1 M LiCl, 2% SDS, 2XTE, 50 µg tRNA, Vanadyl ribonucleosyl complexes.

**Lysis/binding buffer: 4 M GTC, 0.5% sarkosyl, 1% DTT, 0.5 M LiCl, 0.1 M Tris pH8.

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Technical Support

Obtaining Support

For the latest services and support information for all locations, go to **www.lifetechnologies.com**.

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support (**techsupport@lifetechnologies.com**)
- Search for user documents, Safety Data Sheets (SDSs), vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

Safety Data Sheets (SDS)

Safety Data Sheets (SDSs) are available at **www.lifetechnologies.com/support**.

Certificate of Analysis

The Certificate of Analysis is available at **www.lifetechnologies.com/support**. Search for the Certificate of Analysis by product lot number, which is printed on the box.

Limited Product Warranty

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Purchaser Notification

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General Information

Life Technologies AS complies with the Quality System Standards ISO 9001:2008 and ISO 13485:2003.

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