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1. PRODUCT DESCRIPTION

1.1 Kit Content

Dynabeads mRNA DIRECT Kit		
Product number	**610.11**	**610.12**
Dynabeads Oligo $(dT)_{25}$*	5 ml	10 ml
Lysis/Binding Buffer	30 ml	60 ml
Washing Buffer A	60 ml	120 ml
Washing Buffer B	30 ml	60 ml
10 mM Tris-HCl (Elution Buffer)	15 ml	15 ml

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

Product # 610.11 provides enough reagents for 20 standard isolations.
Product # 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.

1.2. Intended Use

This product has been designed for a 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.

1.3 Principle

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 (ref. 1). The high capture efficiency facilitates detection of mRNA by

reverse transcriptase (RT)-PCR from highly specialized cells (e.g. isolated from a heterogenous 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 section 2.5).

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.

1.4 Binding Capacity

1 mg of beads (200 µl) will bind up to 2 µg of mRNA.
A typical mammalian cell contains about 10-30 pg of total RNA, from which 1-5 % is mRNA.

1.5 Description of Materials

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

Dynabeads are uniform, superparamagnetic beads. They are stable in the pH range of 4-13. Do not freeze the 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:	120 ± 25 × 10-6 m^3/kg

Buffers

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

Please note that precipitate may form in the buffers. Dissolve precipitate before use by warming to room temperature and mixing thoroughly.

Additional Material Required

- Magnets: See www.invitrogen.com/magnets for magnet recommendations.
- RNase free pipette tips and pipettors.
- RNase free microtubes.
- Mixer allowing both tilting and rotation.
- Heat block and/or incubator at 65-80°C for elution step (if required).

For isolation of mRNA from tissue samples:

- Liquid nitrogen.
- Mechanical or manual tissue grinder.
- Syringe and 21 gauge needle.
- Benchtop microcentrifuge.

2. INSTRUCTIONS FOR USE

2.1 Technical Advice

- Keep Dynabeads Oligo $(dT)_{25}$ in liquid suspension during storage and all handling steps. Resuspend well before use.
- Work RNase free and wear gloves.
- Bring all buffers, except the 10mM Tris-HCl (Elution Buffer), to room temperature prior to use. The 10mM Tris-HCl buffer should be stored on ice or at 2-8°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 carry over 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.

2.2 Schematic Diagram of mRNA Isolation

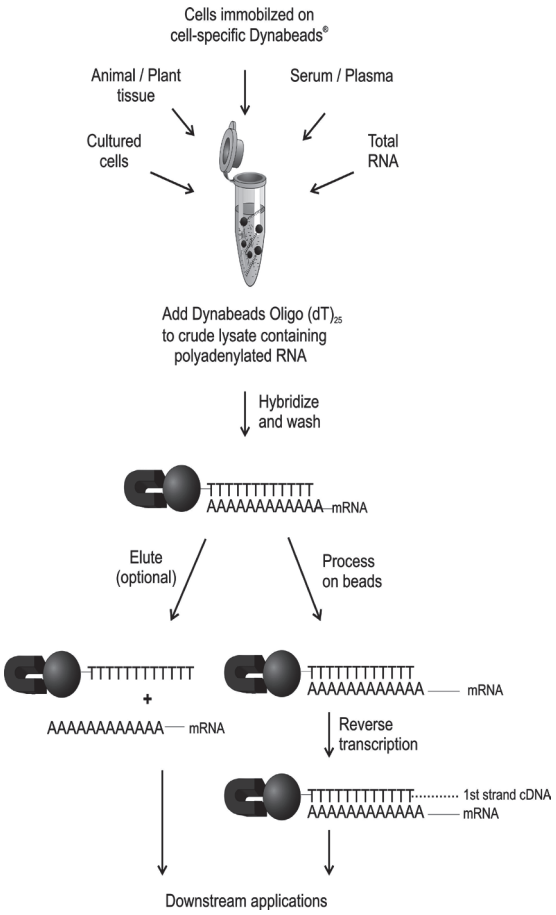

Fig 1: Outline of the protocol for isolating mRNA from a crude starting sample using Dynabeads Oligo$(dT)_{25}$. The isolated mRNA is suitable for use in all downstream molecular biology applications.

2.3 Protocols

Protocols included

2.3.1 Preparation of Sample Lysate
2.3.2 Preparation of Dynabeads Oligo $(dT)_{25}$
2.3.3 Direct mRNA Isolation Protocol
2.3.4 Re-use of Dynabeads Oligo $(dT)_{25}$ for Large Scale Isolations
2.3.5 Elimination of rRNA contamination

2.3.1 Preparation of Sample Lysate

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. Dynabeads mRNA DIRECT™ Kit protocols can be scaled up or down to suit specific sample source and quantity.
Please see section 2.4 before preparing the sample, and for recommended bead and buffer volumes (Table 1 and 2).

A) Preparation of Lysate from 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, 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 preventing mRNA degradation.
4. A DNA-shear step is advised for samples containing over 500,000 cells. Force the lysate 3-5 times through a 21 gauge needle using a 1-2 ml syringe to shear the DNA. The reduction in viscosity should be noticeable. 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 –80°C for later use.
5. Prepare Dynabeads Oligo $(dT)_{25}$ as described below in section 2.3.2, and subsequently proceed with the mRNA isolation in section 2.3.3.

B) Preparation of Lysate from Cultured Cells or Cell Suspension

1. Pellet cells by centrifugation (e.g. at 400 g for 8 minutes at 4°C) and wash the pellet by resuspending in phosphate-buffered saline (PBS). Pellet cells by centrifugation again. 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. A DNA-shear step is advised for samples containing over 500,000 cells. Force the lysate 3-5 times through a 21 gauge needle using a 1-2 ml syringe to shear the DNA. The reduction in viscosity should be noticeable. 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 –80°C for later use.
4. Prepare Dynabeads Oligo $(dT)_{25}$ as described below in section 2.3.2, and subsequently proceed with the mRNA isolation in section 2.3.3.

2.3.2 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. DynaMag™-2).
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 section 2.3.3. below.

2.3.3 Direct mRNA Isolation Protocol

1. Remove the Lysis/Binding Buffer from the pre-washed Dynabeads Oligo$(dT)_{25}$ (section 2.3.2.4) by placing on the magnet for 30 seconds, or until the suspension is clear.
2. Remove the microtube from the magnet and add the sample lysate.
3. Pipette to resuspend the beads completely in the sample lysate. 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. 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.
7. 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.
8. If elution of mRNA from the beads is desired, add an appropriate volume of 10 mM Tris-HCl (Elution Buffer) and incubate at 65-80°C for 2 min. 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.

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

Please note that the buffers supplied with the kit (product no. 610.11 and 610.12) may not be sufficient for large scale mRNA isolations.

Multiple isolations from the same sample can be performed by re-using Dynabeads Oligo$(dT)_{25}$ after mRNA elution.
Simply follow the protocol described in section 2.3.3. After elution of the mRNA, wash the beads once in Lysis/Binding Buffer (section 2.3.2 above). 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.

2.3.5 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. 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 Sodiumpyrophosphate before the isolation of mRNA.

1. Follow the isolation protocol (section 2.3.3 above). Elute the mRNA in 10 mM Tris-HCl (Elution Buffer). Transfer the eluted mRNA to a new tube and place on ice. Do not discard the beads.
2. Wash the beads two times in Washing Buffer B.
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. Remove the Washing Buffer B from the beads, by placing the tube on the magnet, and add the diluted mRNA. Incubate with mixing at room temperature for 3-5 min.
5. Continue with the isolation protocol (section 2.3.3, starting at step 4.)

2.4 Sample Guidelines and Scaling

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

Table 1. Estimated total RNA yield from mammalian cells and tissues

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
107 cultured fibroblasts	50-80 µg
107 cultured epithelial cells	100-120 µg

Table 2. Recommended volumes of Dynabeads Oligo$(dT)_{25}$ and buffers for use with different amounts of starting material

Components	**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 × 10^6	1-4 × 10^6	0.15-1 × 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

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

Table 3. mRNA DIRECT from animal tissues

Tissue	Species	References
Adrenals	Rat	4
Brain	Mouse, Trout	12, 4
Brain (cereberal cortex, preoptic area, dentate gyrus)	Rat	13, 14, 15
Cartilage	Human	16
Organ of Corti and spiral ganglion	Guineapig, 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

Table 4. mRNA DIRECT 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

Table 5. 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 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 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

Table 6. Direct isolation of viral polyA 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.

2.6 Troubleshooting

Problem	Clumping of beads during incubation step with sample lysate.
Possible Cause	DNA in the sample lysate has not been completely sheared.
Suggested Solution	i) Pipette the solution several times through a 1 ml pipette. ii) Increase force/number of passages through the needle in future shearing steps.

Problem	mRNA is contaminated with DNA.
Possible Cause	i) Incomplete DNA shearing. ii) Incomplete removal of sample lysate after hybridization step, and subsequent carry over to wash and elution steps. iii) Inefficient washing. iv) Incomplete removal of wash buffers. v) Sample-to-beads ratio too high.
Suggested Solution	i) Increase the force and /or the number of passages through the needle in the DNA shearing step. ii) Completely remove the sample lysate after hybridization. iii) Make sure the beads/mRNA complex is fully resuspended in washing buffer. iv) Completely remove the sample/washing buffers. v) Dilute sample lysate or increase the amount of beads. vi) Re-extract the mRNA from the eluate.

Problem	mRNA yield is lower than expected.
Possible Cause	i) Inefficient elution of mRNA from the beads. ii) Beads-to-sample ratio is too low. iii) Cells/tissue incompletely lysed.
Suggested Solution	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 homogenization step.

Problem	The beads/cDNA complex is clumped and sticking to the tubes after reverse transcription.
Possible Cause	Non-specific electrostatic interactions between the cDNA molecules and the plastic materials of the tubes/pipette tips.

Suggested Solution	i) 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. ii) Where appropriate, add 0.05% Tween-20 to the reaction buffers. iii) Alternatively, dilute the beads/cDNA solution (after reverse transcription) with an equal volume of the 1 × reverse transcription reaction buffer containing 0.05% Tween-20. Mix by pipetting and transfer the suspension to a new tube. If there are any remaining beads stuck to the tube walls, remove by washing with a fresh aliquot of buffer containing Tween-20. Pool these beads with the original bead suspension. Place the pooled beads on a magnet and remove the supernatant, then wash 2-3 times with the buffer containing Tween-20. Store the solid-phase cDNA library in an appropriate buffer containing 0.05% Tween-20.

Problem	Unable to detect specific mRNA molecules.
Possible Cause	i) The beads-to-sample ratio is too low. ii) Inappropriate sample volume. iii) Hybridization time too short.
Suggested Solution	i) Increase the amount of beads. ii) Reduce sample volume/increase sample concentration. iii) Increase hybridization incubation time to 10-15 min.
Comment	Using the appropriate bead-to-sample ratio (volume and concentration) 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.

For references, see section 4.

3.GENERAL INFORMATION

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

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

3.2 Technical Support

Please contact Invitrogen Dynal for further technical support (see contact details). Certificate of Analysis/Compliance is available upon request.

3.3 Warning and Limitations

This product is for research use only. The product is not for use in human diagnostics or therapeutic procedures. Follow appropriate laboratory guidelines.

This product contains 0.02% sodium azide (NaN_3) 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.

Material Safety Data Sheet (MSDS) is available at
http: //www.invitrogen.com.

3.4 Trademarks

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3.6 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 are 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 that have been altered outside Invitrogen Dynal, nor shall it apply to any products that 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. REFERENCES

1. Karrer EE, et al. (1995) *In situ* isolation of mRNA from individual plant cells: Creation of cell-specific cDNA libraries. *Proc Natl Acad Sci USA* 92:3814-3818.
2. Raineri I, et al. (1991) Improved efficiency for single-sided PCR by creating a reusable pool of first strand cDNA coupled to a solid phase. *Nucleic Acids Research* 19(14):4010.
3. Lambert KN and Williamson VM. (1993) cDNA library construction from small amounts of RNA using paramagnetic beads and PCR. *Nucleic Acids Research* 21(3):775-776.
4. Jakobsen KS, et al. (1994) Direct mRNA isolation using Magnetic Oligo(dT) Beads: A protocol for all types of cell cultures, animal and plant tissues. *Advances in Biomagnetic Separation*, Ed. Uhlèn M, Hornes E and Olsvik Ø, Eaton Publishing 61-71.
5. Rodriguez IR and Chader GJ. (1992) A novel method for the isolation of tissue-specific genes. *Nucleic Acids Research* 20(13):3528.
6. Sharma P, et al. (1993) PCR-based construction of subtractive cDNA library using magnetic beads. *BioTechniques* 15(4):610-611.
7. Schraml P, et al. (1993) cDNA subtraction library construction using a magnet-assisted subtraction technique (MAST). *Trends in Genetics* 9(3):70-71.
8. Aasheim HC, et al. (1994) A simple subtraction method for the isolation of cell-specific genes using magnetic monodisperse polymer particles. *Biotechniques* 16(4):716-721.
9. Coche T, et al. (1994) Generation of an unlimited supply of a subtracted probe using magnetic beads and PCR. *Nucleic Acids Research* 22(7):1322-1323.

10. Borgnes A, et al. Detection of isolated colon carcinoma cells in peripherial blood- or bone marrow mononuclear cell suspensions. Micrometastasis Meeting Munich, June 1996 (Abstract).
11. Dazert SB, et al. (2007) Hearing development and spiral ganglion neurite growth in VASP deficient mice. *Brain Research* 1178:73-82.
12. Moyal M, et al. (1992) Mutations in the UL53 gene of SV-1 abolish virus neurovirulence to mice by the intracerebral route of infection. *Virus Research* 26:99-112.
13. Pines G, et al. (1992) Cloning and expression of a rat brain L- glutamate transporter. *Nature* 360:464-467.
14. Alme MN, et al. (2007) Chronic Fluoxetine Treatment Induces Brain Region-Specific Upregulation of Genes Associated with BDNF-Induced Long-Term Potentiation. *Neuronal Plasticity*:1-9.
15. Kim K, et al. (1994) Competitive PCR for quantification of gonadotropinreleasing hormone mRNA level in a single micropunch of the rat preoptic area. *Mol Cel Endocrinol* 97:153-158.
16. Winterpacht A, et al. (1994) Alter-native splicing as the result of a type II procollagen gene (COL2A1) mutation in a patient with Kneist Dysplasia. *Hum Mol Genet* 3(10):1891-1893.
17. Safieddine S and Wenthold RJ. (1997) The glutamate receptor subunit d1 is highly expressed in hair cells of the auditory and vestibular systems. *J Neurosci* 17:7523-7531.
18. de Andrès B, et al. (1995) Improved method for mRNA extraction from paraffin-embedded tissues. *BioTechniques* 18(1):42-44.
19. Hengerer B. (1993) A rapid procedure for mRNA extraction from a large number of samples. *Biotechniques* 14(4):522-524.
20. Bicknell G, et al. (1996) Separate extraction of mRNA from glomerular epithelial and mesangial cells. Pathological Society of Great Britain and Ireland, 172nd Meeting.
21. Shamsher MK, et al. (1995) Novel mutations in keratin 16 gene underly focal non-epidermolytic palmoplantar keratoderma (NEPPK) in two families. *Hum Mol Genet* 4(10):1875-1881.
22. Schauder B, et al. (1994) Cloning of a cDNA encoding an ectoenzyme that degrades thyrotropin-releasing hormone. *Proc Natl Acad Sci USA* 91:9534-9538.
23. Schüssler P, et al. (1995) Combined isolation of nucleic acids and protein from small amounts of tissue. *Trends in Genetics* 11(10):378-379.
24. Rubin D. Magnetic Bead Isolation of mRNA from Plant Tissue. In: The Red Book Bulletin, Current Protocols in Molecular Biology, Subscriber's Notebook,Anonymous 1992, p. 1,Suppl.20.
25. Ivanov R. et al. (2008) EFFECTOR OF TRANSCRIPTION2 is involved in xylem differentiation and includes a functional DNA single strand cutting domain. *Developmental Biology* (313):93-106.
26. Magrelli A, et al. (1994) Splicing of the rolA transcript of Agrobacterium rhizogenes in Arabidopsis. *Science* 266:1986-1988.
27. Zhu QH, et al. (2007) compact shoot and leafy head 1, a mutation affects leaf initiation and developmental transition in rice (*Oryza sativa L.). Plant Cell Reports* (26):421-427.
28. Hewelt A, et al. (1994) Promoter tagging with a promoterless ipt gene leads to cytokinin-induced phenotypic variability in transgenic tobacco plants: implications of gene dosage effects. *Plant Journal* 6(6):879-891.
29. Breton C, et al. (1995) PCR-generated cDNA library of transition-stage maize embryos: cloning and expression of calmodulin genes during early embryogenesis. *Plant Mol.Biol.* 27:105-113.
30. Allen RL and Lonsdale DM. (1993) Molecular characterization of one of the maize poly-galacturonase gene family members which are expressed during late pollen development. *Plant Journal* 3(2):261-271.

31. Jakobsen KS, et al. (1990) Purification of mRNA directly from crude plant tissues in 15 minutes using oligo dT microspheres. *Nucleic Acids Research* 18(12):3669.
32. Stacy RAP, et al. (1995) Evolution of the group 1 late embryogenesis abundant (Lea) genes: analysis of the Lea B19 gene family in Barley. *Plant Mol Biol* 28:1039-1054.
33. Kumar V and Trick M. (1994) Expression of the S-locus receptor kinase multigene familiy in Brassica oleracea. *Plant Journal* 6(6):807-813.
34. Bachem CWB, et al. (1994) Antisense expression of polyphenol oxidase genes inhibits enzymatic browning in potato tubers. *Biotechnology* 12:1101-1105.
35. Espelund M, et al. (1992) Late embryogenesis-abundant genes encoding proteins with different numbers of hydrophilic repeats are regulated differentially by abscisic acid and osmotic stress. *Plant Journal* 2(2):241-252.
36. Aalen R, et al. (1994) Transcripts encoding oleosin and a dormancy-related protein are present in both the aleurone layer and the embryo of developing barley (Hordeum vulgare L.) seeds. *Plant Journal* 5(3):385-396.
37. Giranton JL, et al. (1995) The S locus receptor kinase gene encodes a soluble glycoprotein corresponding to the SRK extracellular domain in Brassica oleracea. *Plant Journal* 8(6):827-834.
38. Larsen F, et al. (1993) A tight cluster of five unrelated human genes on chromosome 16q22.1. *Hum Mol Genet* 2(10):1589-1595.
39. Luna L, et al. (1994) Molecular cloning of a putative novel human bZIP transcription factor on chromosome 17q22. *Genomics* 22:553-562.
40. Maas RA, et al. (1995) Immuno-magnetic purification of human breast carcinoma cells allows tumor-specific detection of multidrug resistance gene-1 mRNA by reverse transcriptase polymerase chain reaction in fine needle aspirates. *Lab Invest* 72(6):760-764.
41. Slupphaug G, et al. (1991) Cell cycle regulation and in vitro hybrid arrest analysis of the major human uracil-DNA glycosylase. *Nucleic Acids Research* 19:5131-5137.
42. Sprecher E and Becker Y. (1992) Detection of IL-1, TNF- and IL-6 gene transcription by the polymerase chain reaction in keratinocytes: Langerhans cells and peritoneal exudate cells during infection with herpes simplex virus-1. *Arch Virology* 126:253-269.
43. Day S and Ross J. (1995) mRNA based method for typing the HLA-C locus gene by automated sequencing based typing. *Eur J Immunogenet* 23(1):92.
44. Osnes LTN, et al. (1995) Lipopolysaccharide activation of human monocytes mediated by CD14, results in a coordinated synthesis of tissue factor TNF-alpha and IL-6. *J Endotoxin Res* 2:27-35.
45. Bristulf J, et al. (1994) Interleukin-1 stimulates the expression of type I and type II interleukin-1 receptors in the rat insulinoma cell line Rinm5f; sequencing of a rat type II interleukin-1 receptor cDNA. *Eur Cytokine Netw* 5(3):319-330.
46. Thanhäuser A, et al. (1993) Pentoxifylline: a potent inhibitor of IL-1 and IFNgamma biosynthesis and BCG-induced cytotoxicity. *Immunology* 80:151-156.
47. Raineri I and Senn HP. (1992) HIV-1 promotor insertion revealed by selective detection of chimeric provirus-host gene transcripts. *Nucleic Acids Research* 20:6261-6266.
48. Obata F, et al. (1993) A single universal primer for the T-cell receptor (TCR) variable genes enables enzymatic amplification and direct sequencing of TCR cDNA of various T-cell clones. *Human Immunology* 36:163-167.
49. Spurkland A. (1992) Magnetic isolation of mRNA for in vitro amplification. *Trends in Genetics* 8(7):225-226.

50. Murtagh JJ, et al. (1994) Alternative splicing of the guanine nucleotide-binding regulatory protein Goa generates four distinct mRNAs. *Nucleic Acids Res.* 22(5):842-849.
51. Tebbe CC, et al. (1995) Direct detection of recombinant gene expression by two genetically engineered Yeasts in soil on the transcriptional and translational levels. *Appl Environ Microbiology* 61(12):4296-4303.
52. Faulkner JDB and Minton NP. (1993) Rapid small-scale isolation of mRNA from whole yeast cells. *Biotechniques* 14(5):718-720.
53. Chiodi F, et al. (1992) Human immuno-deficiency virus type 1 is present in the cerebrospinal fluid of a majority of infected individuals. *J Clin Microbiol* 30(7):1768-1771.
54. Meijer A, et al. (1995) Development of molecular methods for detection and epidemiological investigation of HIV-1, HIV-2 and HTLVI/II infections. Report 118504 001 National Inst. Publ. Health and Env. Protection Bilthoven, The Netherlands.
55. Albert JA, et al. (1992) Persistence of azidothymidine-resistant human immunodeficiency virus type 1 RNA genotypes in posttreatment sera. *J Virol* 66(9):5627-5630.
56. Scarlatti G, et al. (1993) Comparison of variable region 3 sequences of human immunodeficiency virus type 1 from infected children with the RNA and DNA sequences of the virus population of their mothers. *Proc Natl Acad Sci USA* 90:1721-1725.
57. Sin Y, et al. (2008) Skin bradykinin-related peptides (BRPs) and their biosynthetic precursors (kininogens): Comparisons between various taxa of Chinese and North American ranid frogs. *Peptides* 29:393-403.
58. Lu X, et al. (2007) A comparison of CFU-GM, BFU-E and endothelial progenitor cells using *ex vivo* expansion of selected cord blood $CD133^+$ and $CD34^+$ cells. *Cytotherapy* 9:292 - 300.
59. Silva J, et al. (2007) Circulating Bmi-1 mRNA as a possible prognostic factor for advanced breast cancer patients. *Breast Cancer Research* 9:1-9.
60. Jost R, et al. (2007) Magnetic quantitative reverse transcription PCR: A high-throughput method for mRNA extraction and quantitative reverse transcription PCR. *BioTechniques* 43:206-211.

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