For general laboratory use.

Print Protocol

Print Changes to Previous Version

mRNA Isolation Kit

Version 08

Content version: January 2009

Cat. No. 11 741 985 001

For isolation of at least 70 µg of poly (A^+) RNA

Store at +2 to +8°C

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Table of Contents

1. What this Product Does

Number of Tests

The kit is designed for isolation of at least 70 µg of poly (A^+) RNA.

Kit Contents

Vial	Label	Content
1 translucent vial	Lysis Buffer	1 × 100 ml, ready-to-use solution containing 0.1 M Tris buffer, 0.3 M LiCl, 10 mM EDTA, 1% lithium dodecylsulfate, 5 mM DTT (dithiothreitol), pH 7.5.
2 translucent vial, colorless cap	Streptavidin-coated Magnetic Particles	1 × 1.7 ml, ready-to-use suspension (10 mg/ml) in 50 mM Hepes, 0.1% bovine serum albumin, 0.1% chloracetamide, 0.01% methylisothiazolone, pH 7.4.
3 translucent vial, red cap	Oligo$(dT)_{20}$ probe,biotin-labeled	1 × 66 µl, ready-to-use solution containing 100 pmol biotin-labeled oligo$(dT)_{20}$ per µl of redist. water.
4 translucent vial	Washing Buffer	1 × 50 ml, ready-to-use solution containing 10 mM Tris buffer, 0.2 M LiCl, 1 mM EDTA, pH 7.5.
5 brown glass vial, white cap	Double-distilled Water, PCR Grade	1 × 4 ml
6 brown glass vial, white cap	Storage Buffer	1 × 7 ml ready-to-use solution containing 10 mM Tris buffer, 0.1% chloracetamide, 0.01% methylisothiazolone, pH 7.5.

⚠ If a precipitate is visible in vial 1, Lysis Buffer, dissolve it at +37°C in a shaking waterbath.

Storage and Stability

Store the kit protected from light at +2 to +8°C. When properly stored, the kit is stable until the expiration date printed on the label.

⚠ Do not store the kit at +15 to +25°C.

Additional Equipment and Reagents Required

- Magnetic Particle Separator
- Sterile tubes and cups
- Pipettes with sterile, disposable tips
- Syringe fitted with a 21-gauge needle (for tissue and cultured cells only)
- PBS (for preparing cultured cells only)
- Mortar, pestle, liquid nitrogen (for preparing tissue samples only)
- Spectrophotometer

Application

This kit is used for the isolation of mature mRNA from RNA preparations, cultured cells or tissues without intermediate isolation of total RNA. The isolated mRNA can be used for various downstream applications such as northern blotting, cDNA synthesis, RT-PCR or *in vitro* translation.

Assay Time

Total time : Approx. 30 min (starting from total RNA)
Hands-on time: Approx. 15 min (starting from total RNA)

2. How To Use this Product

2.1 Before You Begin

General considerations

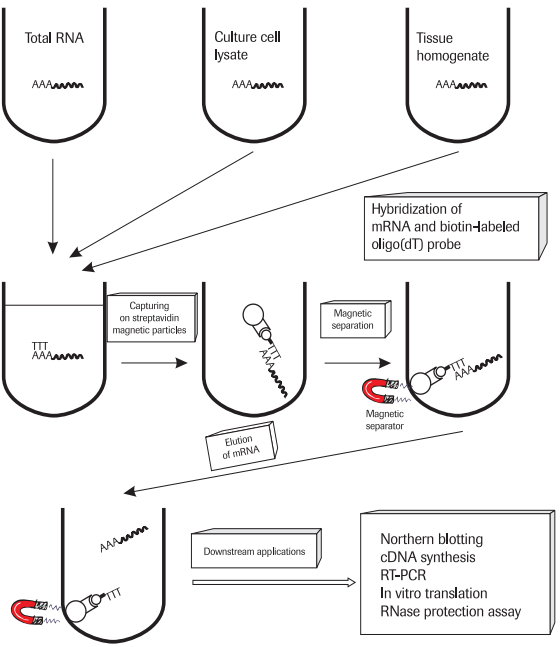
⚠ Make sure that the material which contacts the RNA is free of contaminating RNases. Protocols for decontamination of equipment are described in reference (1).

The lysis buffer contained in the kit has been used for the preparation of mRNA from a number of different tissues (*e.g.* liver, spleen). Equivalent to the detergent buffer, guanidine thiocyanate (GTC) buffer can be used for mRNA extraction. GTC is a strong denaturant of RNases and may be especially useful, if mRNA has to be prepared from tissues that are particularly rich in RNases. The sample preparation procedure using GTC is described under 2.5 Related Procedures.

Sample Material

- Total RNA, 250 µg – 500 µg
- Culture Cells, 2×20^5- 1×10^8
- Tissue, 50 mg-200 mg

Experimental Overview



⚠ The procedures below apply only to the isolation of mRNA from the specified amounts of starting material (500 µg total RNA, 2×10^7 cultured cells, or 200 mg tissue).

To isolate mRNA from different amounts or volumes of sample, you must alter the amounts of reagents used in the procedures below.

For volumes of buffers, SMPs, and biotin-labeled oligo$(dT)_{20}$ probe required for different types and amounts of starting sample material see the table below:

Number of cells	1×10^8	2×10^7	1×10^7	2×10^6	2×10^5
Amount of total RNA		500 µg	250 µg		
Amount of tissue		200 mg	50–100 mg		
Volume of lysis buffer (bottle 1): cells/ tissue	15 ml	3 ml	1.5 ml	0.5 ml	0.1 ml
Volume of lysis buffer (bottle 1): total RNA (final volume)#		400 µl	200 µl		
Volume SMPs (vial 2)	1.5 ml (15 mg)	300 µl (3 mg)	150 µl (1.5 mg)	50 µl (0.5 mg)	50 µl (0.5 mg)
Volume lysis buffer (bottle 1) for preparation of SMPs	2.5 ml	500 µl	250 µl	70 µl	70 µl
Volume oligo$(dT)_{20}$ probe, biotin-labeled (vial 3)	15 µl (1.5 nmol)	3 µl (300 pmol)	1.5 µl (150 pmol)	50 pmol	50 pmol
Volume washing buffer (bottle 4)	3 × 2.5 ml	3 × 500 µl	3 × 250 µl	3 × 200 µl	3 × 200 µl
Volume redist. water (bottle 5)	250 µl	50 µl	25 µl	10 µl	5 µl

⚠ **#**Final volume mentioned refers to sum of sample volume and volume of Lysis Buffer to be added. Example (column 2): 500 µg of total RNA are dissolved in e.g. 50 µl (sample volume). In this case 350 µl lysis buffer (vial 1) have to be added to get the required final volume of 400 µl.

❶ **Prepare the sample:**

Total RNA

Dilute 500 µg total RNA (up to 200 µl) with Lysis Buffer (bottle 1) to a final volume of 400 µl. Incubate for 2 min at +65°C.

⚠ Do not dilute the Lysis Buffer more than twofold.

Cultured cells:

- Wash cells (2×10^7) twice with ice cold PBS.
- Add 3 ml Lysis Buffer (bottle 1) to the cell pellet.
- Shear DNA mechanically by passing 6 times through a 21-gauge needle.

Tissue:

- Snap freeze 200 mg of tissue. Grind frozen tissue to a homogeneous powder in a pre-cooled mortar.
- Chill 3 ml Lysis Buffer to ≤0°C in a sodium chloride-ice water bath, then add the frozen powder to the chilled Lysis Buffer.
- Homogenize the powder suspension by passing 4 times through a 21-gauge needle.
- Centrifuge the suspension at 11,000 × *g* for 30 s.
- Use only the supernatant for the capture procedure.

⚠ Perform all steps in the capture procedure below at a temperature between 0°C to –4°C.

❷ **Prepare the Streptavidin-coated Magnetic Particles:**

- Re-suspend SMPs (vial 2) thoroughly.
- Pipette 300 µl from vial 2 into a fresh cup or tube.
- Immobilize the SMPs on the side of the container with a Magnetic Particle Separator.
- Remove the Storage Buffer from the SMPs.
- Re-suspend the SMPs in 500 µl Lysis Buffer (vial 1).
- Again immobilize the SMPs with the Magnetic Particle Separator.
- Remove all the Lysis Buffer.

⚠ Never let the SMPs dry out.

❸ **Hybridize the mRNA to the Biotin-labeled Oligo(dT)$_{20}$ probe:**

- Add 3 µl Biotin-labeled Oligo(dT)$_{20}$ probe (cup 3) to the sample (from step 1).
- Mix sample and Oligo(dT)$_{20}$ to form the hybridization mix.

❹ **Immobilize the biotinylated dT-A hybrids with Streptavidin-coated Magnetic Particles:**

- Add hybridization mix to the tube containing the prepared SMPs (from Step 2).
- Re-suspend SMPs in the hybridization mix.
- Incubate for: EITHER 5 min at +37°C (if you started with total RNA or cultured cells) OR at 0° (if you started with tissue).

❺ • Separate the SMPs from the fluid with a Magnetic Particle Separator.

This step takes about 3 min.

❻ **Wash the Streptavidin-coated Magnetic Particles 3 times. For each wash:**

- Add 500 µl Wash Buffer (vial 4) to the tube.
- Re-suspend the SMPs in the Wash Buffer.
- Separate the SMPs from the buffer with a Magnetic Particle Separator.
- Remove all Wash Buffer and discard.
- Make sure that the Washing Buffer is quantitatively removed after the last washing step.

❼ **Elute the mRNA:**

- Add 50 µl redist. water (vial 5) to the SMPs.
- Re-suspend SMPs in the double-distilled water.
- Incubate for 2 min at +65°C.
- Separate the SMPs from the eluate with a Magnetic Particle Separator.

❽ Transfer the supernatant (containing the mRNA) to a fresh RNase-free tube.

❾ Determine amount of mRNA spectrophotometrically; for conversion of A_{260} units into concentration see table 5.

An eluate containing pure RNA has a A_{260}/A_{280} ratio of 2.0 ± 0.1. Ensure that the sample is free of SMPs.

⚠ Avoid contact of mRNA with RNase contaminated material. Guidelines for the decontamination of equipment are described in reference (1).

Interruption of Protocol

If you apply the standard protocol described above, it is best to perform all steps successively without any interruption. Nevertheless, some minutes interruption before the step 3 (hybridization) are tolerable. If you need to interrupt the RNA isolation protocol, follow the GTC protocol (please refer to 2.5). This allows to store the lysate at +4°C for some hours without the risk of RNA degradation.

Reuse of Streptavidin-coated Magnetic Particles

If cross-contamination of mRNA preparations does not matter (*e.g.* isolation of mRNA from several samples of the same cell line or tissue), the SMPs can be used up to four times in total. In that case, the used SMPs have to be heated for 2 min at +65°C in 100 µl of redist. water prior to reusing them. Besides, follow exactly the mRNA isolation protocol described above. In particular, the sample material (total RNA, cell lysate, tissue homogenate) has to be supplemented with biotin-labeled oligo$(dT)_{20}$ probe despite the fact that the already used magnetic particles have bound the probe.

The binding capacity decreases when using SMPs repeatedly: the yield will be about 70% for the second and third use, and about 35% for the fourth use.

⚠ Do not re-generate SMPs by alkali treatment.

Storage of used SMPs

Used SMPs can be stored as a 10 mg/ml suspension in storage buffer (vial 6) at +2 to +8°C. Do not dry up or freeze SMPs.

⚠ Keep SMPs away from light. Do not store SMPs in water.

Controls

⚠ It is the user's own responsibility to apply an appropriate control concept.

Isolation of mRNA by using GTC for cell lysis

For preparation of buffers see table below:

Solution	Preparation	Stability/ Temp.	Notes
GTC lysis buffer	4.0 M GTC (Guanidine thiocyanate) 0.1 M Tris-HCl, pH 8.0 1.0 % DTT (w/v) 0.5 % lauroylsarcosine (w/v)	Store aliquots at −15 to −25°C	Recommended if mRNA has to be prepared from RNase rich tissues
GTC dilution buffer	Volumes for preparation of 250 ml: 25 ml 1 M Tris-HCl, pH 7.5 (at 15-25°C) 20 ml 5 M LiCl 10 ml 0.5 M, EDTA Adjust pH to 8.0–8.5 Adjust volume to 250 ml with redist. water	Stable if sterile	Only required if GTC buffer is used for cell lysis

For volumes of buffers, SMPs, and biotin-labeled oligo(dT)20 probe required for different amounts of starting sample material see the table below:

:

Amount of tissue	200 mg	50–100 mg
Volume of GTC lysis buffer (not contained in the kit)	0.8 ml	0.4 ml
Volume of GTC dilution buffer (not contained in the kit)	2.2 ml	1.1 ml
Volume SMPs (cup 2)	300 µl (3 mg)	150 µl (1.5 mg)
Volume lysis buffer (bottle 1) for preparation of SMPs	500 µl	250 µl
Volume oligo(dT)$_{20}$, biotin-labeled (cup 3)	3 µl (300 pmol)	1.5 µl (150 pmol)
Volume washing buffer (bottle 4)	3 × 500 µl	3 × 250 µl
Volume redist. water (bottle 5)	50 µl	25 µl

❶	• Grind frozen tissue in a mortar on liquid nitrogen to a homogeneous powder (prevent thawing of the material). • Add the frozen powder to the Lysis Buffer (0°C to -4°C, sodium chloride ice water bath).
❷	• Homogenize by passing four times through a 21-gauge needle and spin 30 s at 11,000 × *g*. • Separate the supernatant for the next steps, the homogenate has to be diluted with GTC dilution buffer afterwards.
❸	• Perform the following hybridization, immobilization, separation and washing steps at 0°C to 4°C (according to protocol 2.2). ⚠ Complete lysis of the cells is essential for the yield of mRNA. For some types of tissues it might be necessary to use other tools for homogenization than described here. Fast lysis of cells and RNA preparation is of prime importance for the isolation of intact mRNA from RNase rich tissues. Keep reagents and samples on ice during the procedure.

- Yield: Variable, depending upon the type and amount of starting sample material:

Starting material	Total RNA (μg)	mRNA (μg)
Cultured cells (10^7 cells)	30 - 500	0.3 - 25
Tissue (100 mg)		
Mouse brain	200	7
Mouse liver	700	14
Mouse lung	130	10

- Purity: The isolated mRNA is free of DNA and other RNAs according to current quality control procedures.

Possible contamination of mRNA with rRNA

The degree of contamination of isolated mRNA with rRNA depends on the type of tissue or cells used for the preparation. Minute amounts of rRNA usually do not affect most downstream applications (*e.g.,* northern analysis, RT-PCR).

If the rRNA content has to be further reduced we recommend to repeat the isolation procedure once or twice.

	Possible Cause	Recommendation
Low Yield or no Yield	Insufficient homogenization of sample material	Eliminate all visible clumps in lysate (step 1) by homogenization, etc. 🔍 mRNA trapped inside sample clumps cannot be isolated by this procedure.
	Too much sample material per volume of lysis buffer (lysate viscosity too high)	Use not more than the recommended amount of starting material. ⚠ The viscosity of the lysate is critical to successful mRNA isolation.
	Elution temperature too low	Incubate suspension in double-distilled water for 2 min at +65°C (step 7).
	Elution volume too low	Repeat the elution step.
	Improper preparation and reuse of magnetic particles	Follow the instructions in the Instructions for Use if one intends to reuse the SA-MPs. ⚠ Do not regenerate SA-MPs with alkali.
	Wrong lysis buffer or lysis buffer with too much guanidine thiocyanate (GTC)	• Use only the Lysis Buffer provided in the kit. • If you use a lysis buffer containing GTC, follow the guidelines in the Instructions for Use for GTC concentration and hybridization temperature.
	RNase contamination in buffers	• Check all buffers for RNase contamination. • Follow published procedures for RNase contamination [*e.g.*, Farrel, R.A. (1983) RNA Methodologies: A Laboratory Guide for Isolation and Characterization. Academic Press, NY].
Degraded mRNA	RNase contamination in buffers 🔍 Degradation of mRNA would lead to a typical smear pattern: You would see distinct bands and additionally at each band a short smear would appear.	Check all buffers for RNase contamination. Follow published procedures (a.m.) for RNase decontamination.
	Sample material processed too slowly or at too high a temperature	Follow the sample preparation guidelines in step 1

	Possible Cause	Recommendation
Overall smear –no distinct bands	Hybridization was not working correctly	To differentiate whether it is a hybridization or isolation problem, Check a sample isolated with a previous lot of the kit or with another isolation method with the currently used hybridization method. Possibilites to overcome low specificity in hybridization are: • Reduce probe concentration in hybridization mix. • Increase hybridization amount. • Reduce salt concentration in the hybridization mix.
rRNA or DNA contamination	Too much sample material per volume of lysis buffer (lysate viscosity too high)	• Use not more starting material than the recommended amount. • Use the isolated mRNA as starting material and repeat the isolation protocol.
mRNA not detectable	If only a (rather insensitive) gel staining with Et Br is performed, the mRNA is smeared all over the lane and cannot be detected if the gel is not totally overloaded, whereas the rRNA are concentrated as distinct bands corresponding to 18S and 28S.	mRNA can sensitively only be detected after blotting.
A_{260}/A_{280} Ratio < 2.0	Protein present in the sample OR mRNA digested by RNases	Run a spectrum of the results and check, if distinct peaks are visible or if the absorbance is mainly the baseline. If there is a peak with a 280 nm shoulder, there should be protein present in the sample. To check this we recommend to perform a phenol extraction as your customer has already done, but this time with enough material and again run a spectrum with OD_{260} and $OD_{280} > 0.1$ Absorbance units. If no distinct peaks are observed, the mRNA is probably digested by RNases

	Possible Cause	Recommendation
Viscous Liquid remains at Magnetic Particles after magnetic Separation	This problem is probably caused by DNA contamination (if *e.g.* cultured cells have been used as starting material). It is observed if shearing of the DNA has not been done completely. The viscosity is not due to buffer components but due to the DNA itself.	Shear rigorously, e.g. using a 21-gauge needle at least 6 times. Foam formation is normal. ⚠ Shearing should be done in a vessel that is large enough. Later the sample can be transferred in a smaller vial taking care not to carry over the foam.

5. Additional Information on this Product

How this Product Works

Posttranscriptional polyadenylation is a common feature of the biogenesis of most eucaryotic mRNAs. Initial sizes of poly(A) tails are approx. 200 nucleotides in length. These long tails shorten during aging of mRNA to lengths of 40 – 65 adenylate residues. The poly(A) tails allow to purify polyadenylated RNA species from non-polyadenylated RNA (rRNA and tRNA). The method relies on base-pairing between the poly(A) residues at the 3'-ends of the mRNAs and biotin-labeled oligo(dT)$_{20}$ probe, which itself can be used for immobilization on avidin- or streptavidin-coated solid supports. Non-adenylated RNA species are not bound and easily washed off. The bound mRNA is eluted from the solid support by lowering the salt concentration. Immobilization of mRNA on streptavidin magnetic particles opens a convenient way for efficient isolation and purification of poly(A)-tailed RNA.

Background Information

Spectrophotometric conversion

1 A_{260} unit	Concentration (mg/ml)
Single-stranded RNA	40

Product Characteristics

Specifications of Streptavidin-coated Magnetic Particles

Binding capacity: 1 mg streptavidin magnetic particles binds:

> 1,800 pmol of free biotin,

> 150 pmol of biotin-labeled oligonucleotide.

Particle: Polydisperse core-shell polystyrene particles.

Particle size: 1 μm mean diameter.

Specific gravity: 1.1 – 1.4 g/cm^3

Reference

1 Sambrook, J., Fritsch, E. F & Maniatis, 1 (1989): Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Scientific Publications

Wickens, M. (1990) How the messenger got its tail: addition of poly(A) in the nucleus. *Trends Biochem. Sci.* **15,** 277-281.

Rotola A et al. (1998) U94 of human herpesvirus 6 is expressed in latently infected peripheral blood mononuclear cells and blocks viral gene expression in transformed lymphocytes in culture. *Proc. Natl. Acad. Sci* **95**, 13911-13916.

Keppler, OT et al (1999) 'UDP-GlcNAc 2-epimerase: A regulator of cell surface sialylation *Science* **284,** 1372-1376.

Quality Control

Binding capacity of streptavidin magnetic particles:

Each lot of magnetic particles has been tested for the guaranteed minimum binding capacity with free biotin.

Absence of ribonuclease activity:

All reagents included in the kit are checked for the absence of ribonuclease activity.

6. Supplementary Information

6.1 Conventions

Text Conventions

To make information consistent and memorable, the following text conventions are used in this document:

Text Convention	Use
Numbered stages labeled ①, ②, *etc.*	Stages in a process that usually occur in the order listed
Numbered instructions labeled ❶, ❷, *etc.*	Steps in a procedure that must be performed in the order listed
Asterisk *	Denotes a product available from Roche Applied Science.

Symbols

In this document, the following symbols are used to highlight important information:

Symbol	Description
	Information Note: Additional information about the current topic or procedure.
	Important Note: Information critical to the success of the procedure or use of the product.

Abbreviations

In this document the following abbreviations are used:

Abbreviation	Meaning
SMPs	Streptavidin-coated Magnetic Particles
GTC	Guanidine thiocyanate

6.2 Changes to Previous Version

- Editorial Changes

6.3 Trademarks

All product names and trademarks are the property of their respective owners.

6.4 Regulatory Disclaimer

For general laboratory use.

6.5 Ordering Information

Roche Applied Science offers a large selection of reagents and systems for life science research. For a complete overview of related products and manuals, please visit and bookmark our home page www.roche-applied-science.com and our Special Interest Sites including:

- Nucleic Acid Isolation and Purification: http://www.roche-applied-science.com/napure
- PCR - Innovative Tools for Amplification: http://www.roche-applied-science.com/pcr

Product	Pack size	Cat. No.
Streptavidin Magnetic Particles	20 mg (2 ml) 100 mg (10 ml)	11 641 778 001 11 641 786 001
Reverse Transcriptase, AMV	500 U	11 495 062 001
Reverse Transcriptase, M-MuLV	500 U	11 062 603 001
Tth DNA Polymerase	2 × 250 U	11 480 022 001
Transcriptor Reverse Transcriptase	250 U (25 reactions) 500 U (50 reactions) 4 × 500 U (200 reactions)	03 531 317 001 03 531 295 001 03 531 287 001
PCR Nucleotide Mix	200 µl	11 581 295 001
PCR Core Kit	100 reactions	11 578 553 001
cDNA Synthesis System	Kit for 25 µg of RNA	11 117 831 001
mRNA Isolation Kit for Blood/Bone Marrow	1 kit	11 934 333 001
Transcriptor First Strand cDNA Synthesis Kit	1 kit (50 reactions)	04 379 012 001
Protector RNase Inhibitor	2,000 U 10,000 U	03 335 399 001 03 335 402 001

Contact and Support

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We also want you to contact us if you have suggestions for enhancing RAS product performance or using our products in new or specialized ways. Such customer information has repeatedly proven invaluable to RAS and the worldwide research community.

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