Absolutely mRNA Purification Kit

INSTRUCTION MANUAL

Catalog #400806
Revision B.0

For Research Use Only. Not for use in diagnostic procedures.
400806-12

LIMITED PRODUCT WARRANTY

This warranty limits our liability to replacement of this product. No other warranties of any kind, express or implied, including without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by Agilent. Agilent shall have no liability for any direct, indirect, consequential, or incidental damages arising out of the use, the results of use, or the inability to use this product.

ORDERING INFORMATION AND TECHNICAL SERVICES

United States and Canada

Agilent Technologies
Stratagene Products Division
11011 North Torrey Pines Road
La Jolla, CA 92037
Telephone (858) 373-6300
Order Toll Free (800) 424-5444
Technical Services (800) 894-1304
Internet techservices@agilent.com
World Wide Web www.stratagene.com

Europe

Location	Telephone	Fax	Technical Services
Austria	0800 292 499	0800 292 496	0800 292 498
Belgium	00800 7000 7000	00800 7001 7001	00800 7400 7400
	0800 15775	0800 15740	0800 15720
France	00800 7000 7000	00800 7001 7001	00800 7400 7400
	0800 919 288	0800 919 287	0800 919 289
Germany	00800 7000 7000	00800 7001 7001	00800 7400 7400
	0800 182 8232	0800 182 8231	0800 182 8234
Netherlands	00800 7000 7000	00800 7001 7001	00800 7400 7400
	0800 023 0446	+31 (0)20 312 5700	0800 023 0448
Switzerland	00800 7000 7000	00800 7001 7001	00800 7400 7400
	0800 563 080	0800 563 082	0800 563 081
United Kingdom	00800 7000 7000	00800 7001 7001	00800 7400 7400
	0800 917 3282	0800 917 3283	0800 917 3281

All Other Countries

Please contact your local distributor. A complete list of distributors is available at www.stratagene.com.

ABSOLUTELY MRNA PURIFICATION KIT

CONTENTS

Absolutely mRNA Purification Kit

MATERIALS PROVIDED

Materials provided	Quantity[a]
Absolutely mRNA oligo (dT) Magnetic Particles[b]	500 µl
Absolutely mRNA Hybridization Buffer	4 ml
Absolutely mRNA Wash Buffer[b]	4 ml
Absolutely mRNA Elution Buffer	4 ml

[a] Sufficient reagents are provided to isolate mRNA from 10 samples of 100 µg total RNA each.

[b] Buffer contains 0.05% sodium azide.

STORAGE CONDITIONS

Store all components at room temperature. **Do not freeze the Absolutely mRNA oligo (dT) magnetic particles**.

ADDITIONAL MATERIALS REQUIRED

Magnetic bead separation stand (we recommend the Magnetight Separation Stand, available from VWR, Catalog #80031-368.)

Note *The separation stand should be configured to collect magnetic particles on the side, rather than the bottom, of a microcentrifuge tube.*

Revision B.0

INTRODUCTION

Highly pure mRNA is critical for downstream analyses such as RT-PCR, QRT-PCR and microarray target labeling. The Absolutely mRNA purification kit allows rapid isolation of high-quality and exceptionally pure polyA$^+$ RNA from total RNA samples of up to 1 mg. The kit technology is based on specially engineered oligo (dT) magnetic beads which have a larger surface area compared to standard magnetic beads and deliver higher yields of pure RNA. The microparticles have fast magnetic response times, allowing for efficient and effective purification, delivering pure mRNA in less than 20 minutes. The magnetic bead format also accommodates both small (50 µg) and large (up to 1 mg) total RNA samples, offering scalability and flexibility for a variety of downstream applications.

The purification protocol is summarized in Figure 1. In step 1, the Absolutely mRNA oligo (dT) magnetic particles are combined with the total RNA solution. PolyA$^+$ RNA hybridizes to the microparticles under optimized buffer conditions. In step 2, a magnetic field is applied to pull the microparticle-mRNA complexes out of solution. Contaminants are removed by aspiration, and then the microparticles are thoroughly washed. In step 3, purified mRNA is eluted from the microparticles in an aqueous solution, providing maximum flexibility for downstream applications.

The protocol may be adapted for use without a magnetic separation stand by substituting centrifugation for magnetic force to achieve separation of mRNA from contaminants. See *Appendix 2* for a complete protocol.

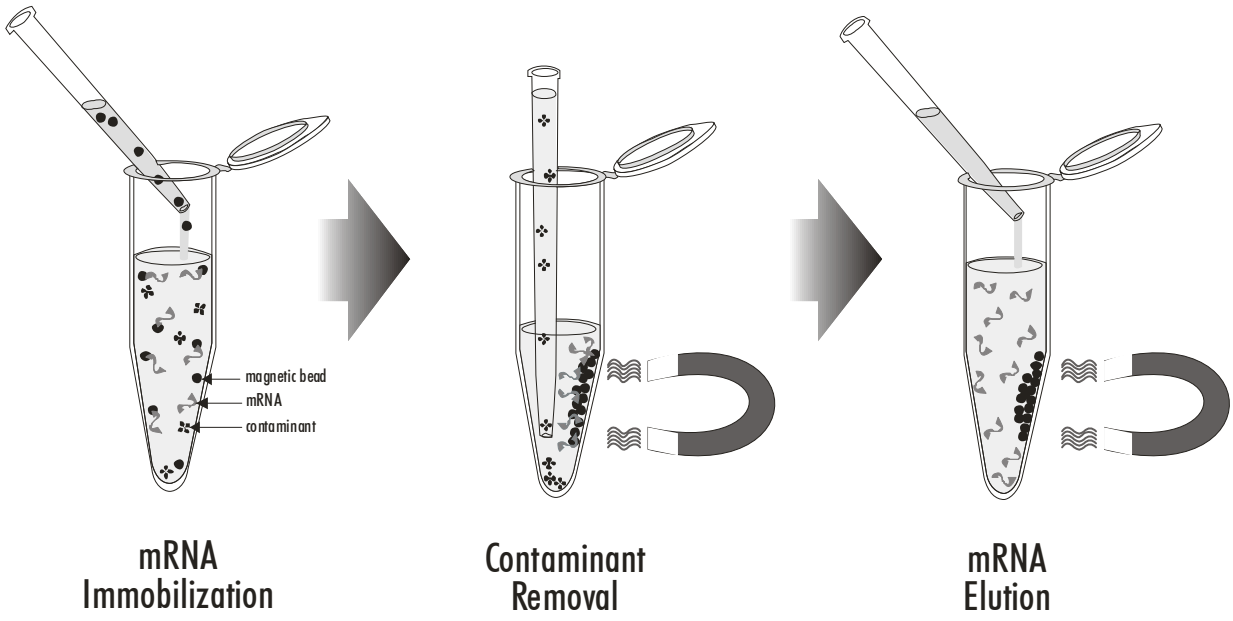

FIGURE 1 Absolutely mRNA purification method.

PROTOCOL

The Absolutely mRNA purification protocol given below is for purification of mRNA from 100 µg total RNA. Reactions may be scaled down to 0.5× (for 50 µg total RNA) or up to 10× (for up to 1 mg total RNA) in a single reaction tube. When scaling up or down, simply increase or decrease the volumes of all components, including the Absolutely mRNA oligo (dT) magnetic particles.

1. Prepare the total RNA (100 µg) in 100 µl of Absolutely mRNA elution buffer or 100 µl of RNase-free H_2O.

   Note *If the total RNA is more dilute than 1 µg/µl, the 100 µg of RNA may be added in a larger sample volume. In this case, increase the volume of Absolutely mRNA hybridization buffer used in step 4 to equal the initial volume of the total RNA sample.*

2. Heat the total RNA solution to 65°C for 3 minutes, and then transfer the sample to ice.

3. Swirl or roll the vial of Absolutely mRNA oligo (dT) magnetic particles to ensure that the particles are in a homogeneous suspension. Transfer 50 µl of the particle suspension to an RNase-free microcentrifuge tube. Wash the particles twice with 100 µl of Absolutely mRNA hybridization buffer. During each wash, mix by pipetting and then collect the beads using a magnetic separator stand, according to the manufacturer's instructions.

   Notes *The hybridization buffer may precipitate when stored at cooler temperatures. Store the buffer at room temperature and do not place the buffer on ice. If a precipitate is present, warm the buffer to 50°C and swirl the solution to redissolve the components.*

   Whenever the particles are collected using the magnetic separator, leave the tube in the magnetic stand while removing the supernatant.

4. Resuspend the washed particles in 100 µl of Absolutely mRNA hybridization buffer by repeated pipetting.

5. Add 100 µl of the total RNA solution to the resuspended magnetic particles. Mix by repeated pipetting.

6. Incubate the mixture at room temperature with gentle agitation for 5 minutes to allow hybridization of the polyA-RNA to the particles.

7. Collect the magnetic particles complexed with mRNA using a magnetic separator stand.

8. Remove the supernatant with a pipet tip.

   Note *The supernatant, containing the unbound fraction of the total RNA may be retained, if desired, to facilitate troubleshooting or may be discarded at this step.*

9. Wash the mRNA-bound magnetic particles four times using 100 µl of Absolutely mRNA wash buffer for each wash. Gently resuspend the particles during each wash, and then re-collect the particles using the magnetic separator stand. Be sure to remove all of the wash buffer when completing the final wash.

10. Remove the tube from the magnetic stand and then add 100 µl of Absolutely mRNA elution buffer to the particles. Incubate the tube at room temperature with gentle agitation for 5 minutes to release the mRNA from the particles.

11. Collect the magnetic particles using a magnetic separator stand.

12. Draw off the 100-µl eluate, containing the purified mRNA, and transfer the solution to a fresh, RNase-free tube. The RNA can be stored at –20°C for up to one month or at –80°C for long-term storage.

Expected RNA Yields and Quantification

To quantify mRNA isolated from 50 µg–1 mg total RNA, a highly sensitive fluorescence-based system (e.g., RiboGreen® RNA quantitation kit, Molecular Probes, Inc.) may be used. The expected yield of mRNA is 1–5% of the amount of total RNA starting material.

TROUBLESHOOTING

Observation	Suggestion
RNA is degraded	Wear gloves throughout the procedure and when handling equipment and solutions used for RNA work. See *Appendix: Preventing Sample Contamination* for additional recommendations.
	RNase may be present within the total RNA sample. To assess the contribution of sample contamination to RNA degradation during the procedure, incubate an aliquot of the total RNA sample at 65°C for 3 minutes, then at room temperature for 15 minutes. Analyze the sample by agarose gel electrophoresis. RNase contamination is indicated by loss or smearing of the 18S and 28S rRNA bands.
Final mRNA solution is too dilute for downstream applications	Purified mRNA may be concentrated by vacuum centrifugation (e.g. using a SpeedVac® concentrator).
mRNA is contaminated with excess rRNA	Ribosomal RNA may co-purify as the result of mRNA-rRNA interactions. Ensure that the total RNA is heated to 65°C prior to addition to the oligo (dT) beads and that the hybridization and wash steps are carried out at room temperature. If rRNA levels are unacceptably high for downstream applications, the mRNA preparation can be subjected to a second round of purification using fresh beads. Note that performing an additional round of purification is expected to reduce the mRNA yield.
	Using the alternative protocol for particle separation by centrifugation (*Appendix 2*) instead of separation by magnetic force may increase the extent of rRNA contamination.
Magnetic particles are trapped in lid or in drops on the sides of microcentrifuge tubes or stock vial	Briefly spin the tube in a microcentrifuge. Resuspend the pelleted particles by swirling or rolling the tube, or by repeated pitpetting, prior to continuing the protocol.
Purified mRNA concentration measurement by optical density (OD_{260}) or the OD_{260}/OD_{280} ratio is unexpectedly low	The presence of residual magnetic particles in the purified mRNA solution interferes with measurement of optical density. Return the sample to the magnetic stand to collect the residual particles and then remeasure OD_{260} and OD_{280}.
The purified mRNA solution has a brownish coloration	Brown coloration is indicative of incomplete magnetic particle removal. Return the sample to the magnetic stand to collect the residual particles and then transfer the mRNA to a fresh tube, taking care to aspirate the supernatant with a pipet tip without disturbing the magnetic particle pellet.
	Use a magnetic stand that contains a stronger magnet. See *Additional Materials Required* for stand recommendations.
Hybridization buffer contains a precipitate	Components of the hybridization buffer may precipitate when stored at cooler temperatures. If a precipitate is present, warm the buffer to 50°C and swirl the solution to redissolve the components.

APPENDIX 1: ALTERNATE PURIFICATION PROTOCOL USING PARTICLE CENTRIFUGATION

The purification protocol given below is for purification of mRNA from 100 µg total RNA. Reactions may be scaled down to 0.5× (for 50 µg total RNA) or up to 10× (for up to 1 mg total RNA) in a single reaction tube. When scaling up or down, simply increase or decrease the volumes of all components, including the Absolutely mRNA oligo (dT) magnetic particles.

1. Prepare the total RNA (100 µg) in 100 µl of Absolutely mRNA elution buffer or 100 µl of RNase-free H_2O.

   Note *If the total RNA is more dilute than 1 µg/µl, the 100 µg of RNA may be added in a larger sample volume. In this case, increase the volume of Absolutely mRNA hybridization buffer used in step 4 to equal the initial volume of the total RNA sample.*

2. Heat the total RNA solution to 65°C for 3 minutes, and then transfer the sample to ice.

3. Swirl or roll the vial of Absolutely mRNA oligo (dT) magnetic particles to ensure that the particles are in a homogeneous suspension. Transfer 50 µl of the particle suspension to an RNase-free microcentrifuge tube. Wash the particles twice with 100 µl of Absolutely mRNA hybridization buffer. During each wash, mix by pipetting and then collect the beads by centrifugation at 10,000 × *g* for 2 minutes.

   Notes *The hybridization buffer may precipitate when stored at cooler temperatures. Store the buffer at room temperature and do not place the buffer on ice. If a precipitate is present, warm the buffer to 50°C and swirl the solution to redissolve the components.*

   Whenever the particles are collected by centrifugation, remove supernatant carefully to avoid disturbing the pelleted particles.

4. Resuspend the washed particles in 100 µl of Absolutely mRNA hybridization buffer by repeated pipetting.

5. Add 100 µl of the total RNA solution to the resuspended magnetic particles. Mix by repeated pipetting.

6. Incubate the mixture at room temperature with gentle agitation for 5 minutes to allow hybridization of the polyA-RNA to the particles.

7. Collect the magnetic particles complexed with mRNA by centrifugation at 10,000 × *g* for 2 minutes.

8. Remove the supernatant with a pipet tip.

   Note *The supernatant, containing the unbound fraction of the total RNA may be retained, if desired, to facilitate troubleshooting or may be discarded at this step.*

9. Wash the mRNA-bound magnetic particles four times using 100 µl of Absolutely mRNA wash buffer for each wash. Gently resuspend the particles during each wash, and then re-collect the particles by centrifugation at 10,000 × *g* for 2 minutes. Be sure to remove all of the wash buffer when completing the final wash.

10. Add 100 µl of Absolutely mRNA elution buffer to the particles. Incubate the tube at room temperature with gentle agitation for 5 minutes to release the mRNA from the particles.

11. Collect the magnetic particles by centrifugation at 10,000 × *g* for 2 minutes.

12. Draw off the 100-µl eluate, containing the purified mRNA, and transfer the solution to a fresh, RNase-free tube. The RNA can be stored at –20°C for up to one month or at –80°C for long-term storage.

Expected RNA Yields and Quantification

To quantify mRNA isolated from 50 µg–1 mg total RNA, a highly sensitive fluorescence-based system (e.g., RiboGreen® RNA quantitation kit, Molecular Probes, Inc.) may be used. The expected yield of mRNA is 1–5% of the amount of total RNA starting material.

APPENDIX 2: PREVENTING SAMPLE CONTAMINATION

Preventing RNase Contamination

Ribonucleases are very stable enzymes that hydrolyze RNA. RNase A can be temporarily denatured under extreme conditions, but it readily renatures. RNase A can therefore survive autoclaving and other standard methods of protein inactivation. The following precautions can help prevent RNase contamination:

- **Wear gloves at all times** during the procedures and while handling materials and equipment, as RNases are present in the oils of the skin.
- Exercise care to ensure that all equipment and supplies (e.g., centrifuge tubes, etc.) are free from contaminating RNases. Avoid using equipment or workspaces that have been exposed to RNases. Use only sterile tubes and micropipet tips.
- Micropipettor bores can be a source of RNase contamination, since material accidentally drawn into the pipet or produced by gasket abrasion can fall into RNA solutions during pipetting. Clean micropipettors according to the manufacturer's recommendations. We recommend rinsing both the interior and exterior of the micropipet shaft with 70% ethanol or 70% methanol.

Sterilizing Labware

Disposable Plasticware

Disposable sterile plasticware is generally free of RNases. If disposable sterile plasticware is unavailable, components such as microcentrifuge tubes can be sterilized and treated with diethylpyrocarbonate (DEPC), which chemically modifies and inactivates enzymes, according to the following protocol:

Caution *DEPC is toxic and extremely reactive. Always use DEPC in a fume hood.* ***Read and follow the manufacturer's safety instructions.***

1. Add DEPC to deionized water to a final concentration of 0.1% (v/v) and mix thoroughly.
2. Place the plasticware to be treated into a separate autoclavable container. Carefully pour the DEPC-treated water into the container until the plasticware is submerged.
3. Leave the container and the beaker used to prepare DEPC-treated water in a fume hood overnight.

4. For disposal, pour the DEPC-treated water from the plasticware into another container with a lid. Autoclave the bottle of waste DEPC-treated water and the container with the plasticware for at least 30 minutes. Aluminum foil may be used to cover the container, but it should be handled with gloves and cut from an area untouched by ungloved hands.

Nondisposable Plasticware

Remove RNases from nondisposable plasticware with a chloroform rinse. Before using the plasticware, allow the chloroform to evaporate in a hood or rinse the plasticware with DEPC-treated water.

Electrophoresis Gel Boxes

To inactivate RNases on electrophoresis gel boxes, treat the gel boxes with 3% (v/v) hydrogen peroxide for 10–15 minutes and then rinse them with RNase-free water.

Glassware or Metal

To inactivate RNases on glassware or metal, bake the glassware or metal for a minimum of 8 hours at 180°C.

Treating Solutions with DEPC

Treat water and solutions (except those containing Tris base) with DEPC at 0.1% (v/v). During preparation, mix the 0.1% solution thoroughly, incubate the solution overnight at room temperature, and then autoclave it prior to use. If a solution contains Tris base, prepare the Tris solution with autoclaved DEPC-treated water.

Preventing Nucleic Acid Contamination

If the isolated RNA will be used for cDNA synthesis for cDNA library construction or PCR amplification, it is important to remove any residual nucleic acid from equipment that was used for previous nucleic acid isolations.

ENDNOTES

RiboGreen® is a registered trademark of Molecular Probes, Inc.
SpeedVac® is a registered trademark of Savant Instruments, Inc.

MSDS INFORMATION

The Material Safety Data Sheet (MSDS) information for Stratagene products is provided on the web at *http://www.stratagene.com/MSDS/*. Simply enter the catalog number to retrieve any associated MSDS's in a print-ready format. MSDS documents are not included with product shipments.