April 2022

RNeasy® Pure mRNA Bead Handbook

For purification of poly A+ RNA from total RNA

Sample to Insight

QIAGEN

Contents

Kit Contents

RNeasy Pure mRNA Bead Kit **Catalog no.** **No. of reactions**	**(48)** **180244** **48**
Pure mRNA Beads	8 x 600 µl
Buffer mRBB (binding buffer)	2 x 8 ml
Buffer OW2 (wash buffer)	3 x 19 ml
RNase-Free Water	2 x 10 ml
Buffer OEB (elution buffer)	3 x 1.5 ml
Small Spin Columns	48
Collection Tubes (1.5 ml)	144
Quick-Start Protocol	1

Storage

Store kit components, except Buffer mRBB and Pure mRNA Beads, at room temperature (15–25°C). Buffer mRBB and Pure mRNA Beads should be stored at 2–8°C. Do not freeze.

Some settling may occur during storage. The beads can be easily resuspended by vortexing before use. Under the storage conditions specified above, Pure mRNA Beads and the rest of the kit components are stable for 9 months upon delivery.

Intended Use

The RNeasy Pure mRNA Bead Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN® products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments or to other applicable guidelines.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at **www.qiagen.com/safety**, where you can find, view, and print the SDS for each QIAGEN kit and kit component.

Pure mRNA Beads contain sodium azide (NaN3) as a preservative. Sodium azide is highly toxic and may react explosively with lead or copper drainpipes. Take appropriate safety measures and wear gloves during handling. Dispose of azide-containing solutions according to your institution's waste-disposal guidelines.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of the RNeasy Pure mRNA Bead Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

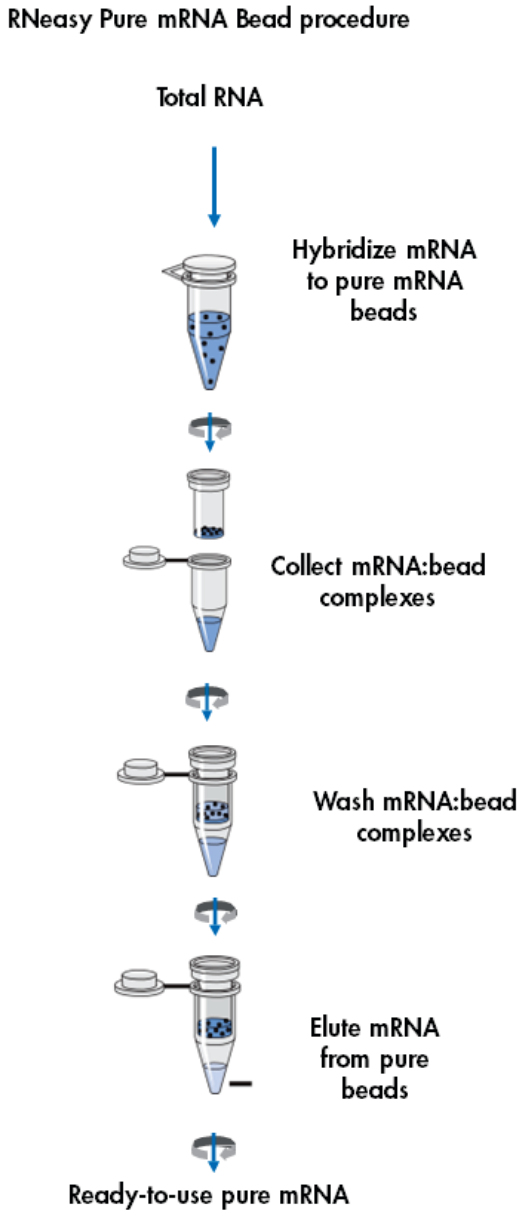
A typical mammalian cell contains 10–30 pg total RNA. The majority of RNA molecules, however, are tRNAs and rRNAs. mRNA accounts for only 1–5% of the total cellular RNA; however, the actual amount depends on the cell type and physiological state. Approximately 360,000 mRNA molecules are present in a single mammalian cell, with approximately 12,000 different mRNA species per cell. Some mRNAs comprise as much as 3% of the mRNA pool, whereas others account for less than 0.01%. These "rare" or "low abundance" mRNAs may have a copy number of only 5–15 molecules per cell. However, these rare species may account for as much as 11,000 different mRNA species, comprising 45% of the mRNA population. For more information, see Alberts, B. et al. (1).

Due to the low proportion of mRNA in the total cellular RNA pool, reducing the amount of rRNA and tRNA in a total RNA preparation increases the relative amount of mRNA. In applications such as RNA sequencing, it is of great interest to maximize the amount of information received from a sequencing run. Ribosomal RNA provides little information about the transcriptome and wastes valuable sequencing resources. The RNeasy Pure mRNA Bead Kit effectively enriches mRNA, while depleting rRNA and nonadenylated, noncoding, as well as regulatory RNA from a wide variety of eukaryotic species.

mRNA isolation directly from cells or tissue samples provides a reduced level of enrichment and poses a greater risk of interference by other cellular components. This leads to inaccurate results in sensitive applications such as next-generation sequencing (NGS). Purifying mRNA from total RNA samples is therefore more effective. The RNeasy Pure mRNA Bead Kit includes spin columns and all necessary reagents and buffers for isolation of pure poly A^+ mRNA from total RNA preparations in <45 minutes.

Principle and procedure

RNeasy Pure mRNA Bead technology combines the convenient handling of magnetic particles with the specificity of oligo-dT hybridization. Protocols require short pretreatment steps. Oligo-dT probes are covalently attached to the surface of pure mRNA beads. mRNA binds rapidly and efficiently to the oligo-dT probes on the Pure mRNA Beads in the presence of Buffer mRBB (see the flowchart on the next page). mRNA bound to the magnetic particles is then efficiently washed. Two wash steps are used to considerably improve the purity of the mRNA. Highly pure mRNA is eluted in the elution buffer (Buffer OEB). mRNA yields depend on sample input and sample storage.


RNeasy Pure mRNA Bead procedure
Total RNA
Hybridize mRNA to pure mRNA beads
Collect mRNA:bead complexes
Wash mRNA:bead complexes
Elute mRNA from pure beads
Ready-to-use pure mRNA

Automated purification of nucleic acids on QIAcube® instruments

Purification of nucleic acids can be fully automated on QIAcube Connect or the classic QIAcube. The innovative QIAcube instruments use advanced technology to process QIAGEN spin columns, enabling seamless integration of automated, low-throughput sample prep into your laboratory workflow. Sample preparation using QIAcube instruments follows the same steps as the manual procedure (i.e., lyse, bind, wash and elute), enabling you to continue using the AllPrep DNA/RNA Micro Kit for purification of high-quality nucleic acids.

QIAcube instruments are preinstalled with protocols for purification of plasmid DNA, genomic DNA, RNA, viral nucleic acids and proteins, plus DNA and RNA cleanup. The range of protocols available is continually expanding, and additional QIAGEN protocols can be downloaded free of charge at **www.qiagen.com/qiacubeprotocols**.

The QIAcube Connect.

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

- Water bath or heating block
- Sterile, RNase-free pipet tips
- Microcentrifuge
- Magnetic rack
- Disposable gloves
- Optional: RNase inhibitor

Important Notes

Description of protocol

Purification of mRNA with the RNeasy Pure mRNA Bead protocol (page 13) is an enrichment of poly A+ RNA from total RNA. This protocol can be used with total RNA purified by various methods, but better results are generally obtained with purer starting material. For best results, we recommend starting with total RNA purified using silica gel–membrane technology. For example, RNeasy kits can be used to isolate total RNA from a variety of starting materials and provide high-quality RNA ideal for use with RNeasy Pure mRNA Bead technology. See Ordering Information on page 25.

Poly A+ mRNA purified with RNeasy Pure mRNA Bead technology does not require further purification and is ready to use.

Quantification of starting RNA

For highly concentrated samples, the concentration of RNA should be determined prior to the RNeasy Pure mRNA Bead purification procedure by measuring the absorbance at 260 nm (A_{260}) using, for example, the QIAxpert® System. Nucleic acids have an absorption peak at 260 nm (A_{260}). The QIAxpert instrument reads this absorption with high accuracy and reproducibility. The nucleic acid concentration is calculated from the optical density (OD) at 260 nm using the Beer–Lambert Law, which relates absorption and concentration. The proportionality factor depends on the type of molecule. For example, an A_{260} reading of 1.0 at a 10 mm path length is equivalent to approximately 50 ng/µl dsDNA, 33 ng/µl for ssDNA, or 40 ng/µl RNA. As discussed hereafter, the ratio between the absorbance values at 260 and 280 nm gives an estimate of RNA purity.

Purity of starting RNA

DNA or RNA purity analysis is done by calculating the A_{260}/A_{230} and A_{260}/A_{280} ratios. As a general rule, high-quality RNA will have an A_{260}/A_{280} ratio in the range 1.8–2.0 and an A_{260}/A_{230} of 2.0 or greater.

The solution properties, pH and ionic strength, can affect the $A_{260}A_{230}$ and the A_{260}/A_{280} ratios. Therefore, we recommend using a buffered solution such as Buffer TE (pH 8.0) as both the solution for diluting the nucleic acid and the solution used to calibrate the instrument to normalize readings during the measurement. Pure water often has an acidic pH and can lower the A_{260}/A_{280} ratio, while TE buffer has an intrinsic UV absorption below 240 nm.

With the RNeasy Pure mRNA Bead protocol, superior results are generally obtained with purer starting material. Our experiments suggest that RNA purified in the absence of phenol is typically purer for most downstream applications, including subsequent mRNA purification. For best results, we recommend starting with total RNA purified using silica gel–membrane technology.

Protocol: Purification of Poly A$^+$ RNA from Total RNA

This protocol is for mRNA enrichment of total RNA. Store columns and Buffers OW2 and OEB at room temperature (15–25°C) and Pure mRNA Beads and Buffer mRBB at 2–8°C.

Things to do before starting

- Vortex the bottle containing Pure mRNA Beads for 3 min (before first use) or 1 min (before subsequent uses) to ensure that the magnetic particles are fully resuspended.
- Heat a water bath or heating block to 70°C, and heat Buffer OEB.
- Unless otherwise indicated, all protocol steps, including centrifugation, should be performed at 20–30°C. Steps 5–10 can be processed using a centrifuge or a magnetic rack.
- Symbols: ■ indicates processing by centrifugation using a small spin column; ▲ indicates processing using a microcentrifuge tube and a magnetic rack.

Procedure

1. Determine the amount of starting RNA (max. 1 µg). Pipet total RNA into an RNase-free, 2 ml microcentrifuge tube, and adjust the volume with RNase-free water (if necessary) to 250, 500, or 650 µl (see Table 1).

   Optional: Add 1µl RNase Inhibitor (4 U/µl) to the sample.

2. Add appropriate volumes of Buffer mRBB and Pure mRNA Beads (see Table 1), and vortex.

Table 1. Volumes of Buffer mRBB and Pure mRNA Beads required for mRNA binding

Amount of starting RNA (µg)	Volume RNA solution (µl)	Volume of Buffer mRBB (µl)	Volume of Pure mRNA Beads (µl)
<5	250	250	25
≤50	250	250	50
≤100	250	250	75
≤250	500	500	150
≤500	500	500	200
≤1000	650	650	400

3. Incubate the sample for 3 min at 70°C in a water bath or heating block.

   This step disrupts the secondary structure of RNA.

4. Remove the sample from the water bath/heating block and place at room temperature for 10 min.

   This step allows hybridization between the oligo-dT of the pure mRNA Beads and the poly A tail of the mRNA.

5. ▪ Pellet the mRNA:bead complex by centrifugation for 2 min at maximum speed, and carefully remove the supernatant by pipetting.

   ▲ Briefly centrifuge the 2 ml sample tube to remove drops of liquid from the inside of the lid and place the tube on a magnetic rack, wait (approx. 2 min) until bead separation has been completed, and remove the supernatant.

   Note: Save the supernatant until certain that satisfactory binding and elution of poly A^+ mRNA has occurred.

6. Resuspend the mRNA:bead pellet in 400 µl Buffer OW2 by vortexing or pipetting.

7. ▪ Pipet onto a small spin column (provided). Centrifuge for 1 min at maximum speed.

p Pipet the solution into a 1.5 ml microcentrifuge tube. Place the tube on a magnetic rack, wait (~1 min) until bead separation has been completed, and remove the supernatant.

8. ▪ Transfer the spin column to a new RNase-free 1.5 ml microcentrifuge tube (provided), and apply 400 µl Buffer OW2 to the column. Centrifuge 1 min at maximum speed and discard the flow-through.

   ▲ Apply 400 µl Buffer OW2 to the pellet. Pipet the solution into a 1.5 ml microcentrifuge tube. Place the tube on a magnetic rack, wait (~1 min) until bead separation has been completed, and remove the supernatant.

9. ▪ Transfer the spin column to a new RNase-free 1.5 ml microcentrifuge tube (provided). Pipet 20 µl hot (70°C) Buffer OEB onto the column, pipet up and down 3–4 times to resuspend the beads, and centrifuge for 1 min at maximum speed.

   ▲ Pipet 20 µl hot (70°C) Buffer OEB to a 1.5 ml microcentrifuge tube and pipet up and down 3–4 times to resuspend the beads. Place the tube on a magnetic rack, wait (approx. 1 min) until bead separation has been completed, and remove the supernatant and collect it in a new 1.5 ml microcentrifuge.

   Note: The volume of Buffer OEB used depends on the expected or desired concentration of poly A^+ mRNA.

10. For maximal yield, pipet another 20 µl hot (70°C) Buffer OEB onto the beads. Pipet up and down 3–4 times to resuspend the beads ▪ and centrifuge for 1 min at maximum speed. Alternatively, ▲ place the tube on a magnetic rack, wait (approx.1 min) until bead separation has been completed, and remove the supernatant and collect it in a new 1.5 ml microcentrifuge.

11. To keep the elution volume low, the first eluate may be used for a second elution. Reheat the eluate to 70°C, and elute in the same microcentrifuge tube. However, for maximal yield, additional volume of Buffer OEB is recommended.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page in our Technical Support Center: **www.qiagen.com/FAQ/FAQList.aspx**. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information or protocols in this handbook (for contact information, visit **support.qiagen.com**).

		Comments and suggestions
Clogged spin column		
a)	Partially undissolved total RNA	If starting with precipitated RNA, dissolve the RNA pellet in the appropriate amount of RNase-free water by heating the tube for 3 min at 60°C followed by vortexing for 5 s and sharply flicking the tube. Repeat at least twice.
b)	Impure starting RNA	Better results are generally obtained with purer starting material. We recommend using total RNA isolated or cleaned up using RNeasy Kits (see page 25 for Ordering Information).
c)	Centrifugal force too low	Ensure that points a and b above are not a factor. Increase centrifugal force or centrifugation time.
Low or no yield of RNA		
a)	Elution volume too low	Repeat elution steps with another aliquot of hot (70°C) Buffer OEB.
b)	Temperature of elution Buffer OEB too low	Make sure that Buffer OEB has been heated to 70°C. Remember that small volumes cool down quickly. Buffer OEB should therefore be added immediately to the sample. With multiple samples, it may be necessary to place the entire microcentrifuge tube (with spin column, Pure mRNA Beads, and sample) into a 70°C heating block to maintain the temperature while preparing the next samples.
c)	Insufficient elution	Be sure to follow the full elution procedure: add hot (70°C) Buffer OEB or eluate to the spin column, pipet up and down to resuspend Pure mRNA Beads, and spin to elute.
d)	RNase contamination	Although all buffers have been tested and are guaranteed RNase-free, RNases can be introduced during use. Make sure RNases are not introduced during the procedure or during handling. See Appendix A, page 19.

		Comments and suggestions
e)	Insufficient hybridization time	Repeat the procedure with the supernatant saved during the procedure. Add fresh Pure mRNA Beads, and repeat the protocol beginning with the 70°C incubation or room-temperature hybridization step. Extend hybridization time if necessary.
f)	Lysate or starting RNA solution too viscous or concentrated	Make sure not to exceed the recommended amount of total RNA. Dilute the supernatant saved during the procedure. Add fresh Pure mRNA Beads, and repeat the protocol beginning with the 70°C incubation.
g)	Temperature too high during hybridization	Hybridization should be carried out at 20–30°C as indicated in the protocol. If the ambient temperature is higher, a cooled water bath may be necessary. Repeat the procedure with the supernatant saved during the procedure. Add fresh Pure mRNA Beads, and repeat the protocol beginning with the 70°C incubation.
mRNA degradation		
RNase contamination		Although all buffers have been tested and are guaranteed RNase-free, RNases can be introduced during use. Make sure RNases are not introduced during the procedure or during handling. Add RNase inhibitor to your total RNA. See Appendix A, page 19.
Poor downstream performance of mRNA		
Carryover of salts or cellular components		Be sure to thoroughly resuspend the Pure RNA Bead pellet as described in the protocol following centrifugation steps or collection using the magnetic rack. Incomplete resuspension can leave salts or cellular components trapped in the Pure RNA Bead pellet. If necessary, also carefully resuspend the Pure RNA Bead in the spin column during the Buffer OW2 wash steps.
rRNA contamination		
Standard protocols		The standard procedures provide significant enrichment of poly A^+ RNA. However, for somewhat higher enrichment of mRNA, the following enrichment steps can be added: following step 5, add equal amounts of RNase-free water and Buffer mRBB. Mix the contents thoroughly by pipetting or flicking the tube. Repeat steps 3–5, and then continue the relevant protocol with step 6.

References

1. Alberts, B., Bray D., Lewis, J., Raff, M., Roberts, K., and Watson, J.D. (1994) *Molecular Biology of the Cell*. 3rd ed. New York: Garland Publishing, Inc.

Appendix A: General Remarks on Handling RNA

Handling RNA

Ribonucleases (RNases) are very stable and active enzymes that generally do not require cofactors to function. Because RNases are difficult to inactivate and even minute amounts are sufficient to destroy RNA, do not use any plasticware or glassware without first eliminating possible RNase contamination. Great care should be taken to avoid inadvertently introducing RNases into the RNA sample during or after the purification procedure. To create and maintain an RNase-free environment, the following precautions must be taken during pretreatment and use of disposable and nondisposable vessels and solutions while working with RNA.

General handling

Proper microbiological, aseptic technique should always be used when working with RNA. Hands and dust particles may carry bacteria and molds and are the most common sources of RNase contamination. Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contamination from the surface of the skin or from dusty laboratory equipment. Change gloves frequently and keep tubes closed whenever possible. Keep purified RNA on ice when aliquots are pipetted for downstream applications. To remove RNase contamination from bench surfaces, nondisposable plasticware, and laboratory equipment (e.g., pipets and electrophoresis tanks), use general laboratory reagents. To decontaminate plasticware, rinse with 0.1 M NaOH, 1 mM EDTA* followed by RNase-free water (see "Solutions", page 20), or rinse with chloroform* if the plasticware is chloroform resistant. To decontaminate electrophoresis tanks, clean with detergent (e.g., 0.5% SDS),* rinse with RNase-free water, rinse with ethanol (if the tanks are ethanol-resistant), and allow to dry.

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

Disposable plasticware

The use of sterile, disposable polypropylene tubes is recommended throughout the procedure. These tubes are generally RNase-free and do not require pretreatment to inactivate RNases.* Glassware should be treated before use to ensure that it is RNase-free. Glassware used for RNA work should be cleaned with a detergent,* thoroughly rinsed and oven baked at 240°C for at least 4 hours (overnight, if more convenient) before use. Autoclaving alone will not fully inactivate many RNases. Alternatively, glassware can be treated with diethyl pyrocarbonate (DEPC)*, as described in "Solutions" below.

Solutions

Solutions (water and other solutions) should be treated with 0.1% DEPC. DEPC is a strong, but not absolute, inhibitor of RNases. It is commonly used at a concentration of 0.1% to inactivate RNases on glass or plasticware or to create RNase-free solutions and water. DEPC inactivates RNases by covalent modification. Add 0.1 ml DEPC to 100 ml of the solution to be treated and shake vigorously to bring the DEPC into solution. Let the solution incubate for 12 hours at 37°C. Autoclave for 15 minutes to remove any trace of DEPC. DEPC will react with primary amines and cannot be used directly to treat Tris* buffers. DEPC is highly unstable in the presence of Tris buffers and decomposes rapidly into ethanol and CO_2. When preparing Tris buffers, treat water with DEPC first, and then dissolve Tris to make the appropriate buffer. Trace amounts of DEPC will modify purine residues in RNA by carbethoxylation. Carbethoxylated RNA is translated with very low efficiency in cell-free systems. However, its ability to form DNA:RNA or RNA:RNA hybrids is not seriously affected unless a large fraction of the purine residues have been modified. Residual DEPC must always be eliminated from solutions or vessels by autoclaving or heating to 100°C for 15 minutes.

Note: RNeasy buffers are guaranteed RNase-free without using DEPC treatment and are therefore free of any DEPC contamination.

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

Appendix B: Storage, Quantification, and Determination of Quality of RNA

Storage

Purified RNA may be stored at –70°C to –15°C in RNase-free water. Under these conditions, no degradation of RNA is detectable after 1 year.

Quantification of RNA

The concentration of RNA should be determined by measuring the absorbance at 260 nm (A_{260}) in a spectrophotometer (see "Spectrophotometric quantification of RNA" below). For small amounts of RNA, however, it may be difficult to determine amounts photometrically. Small amounts of RNA can be quantified using quantitative RT-PCR or fluorometric quantification.

Spectrophotometric quantification of RNA

Using the QIAxpert UV/VIS Spectrophotometer for microvolume analysis

To determine the concentration of your RNA sample purified with RNeasy QIAGEN kit, use the corresponding RNeasy App on the QIAxpert. For more information, see the QIAxpert product page (**www.qiagen.com/qiaxpert-system**).

Using a standard spectrophotometer

To ensure significance, A_{260} readings should be greater than 0.15. An absorbance of 1 unit at 260 nm corresponds to 44 µg of RNA per ml (A_{260} = 1 → 4 µg/ml). This relation is valid only for measurements at a neutral pH. Therefore, if it is necessary to dilute the RNA sample, this should be done in a buffer with neutral pH.* As discussed below (see "Purity of RNA", page 23), the ratio between the absorbance values at 260 and 280 nm gives an estimate of RNA purity. When measuring RNA samples, be certain that cuvettes are RNase-free, especially if the RNA is to be recovered after spectrophotometry. This can be accomplished by washing cuvettes with 0.1 M NaOH, 1 mM EDTA,* followed by washing with RNase-free water (see "Solutions", page 20). Use the buffer in which the RNA is diluted to zero the spectrophotometer. An example of the calculation involved in RNA quantification is shown below:

Volume of RNA sample	= 100 µl
Dilution	= 10 µl of RNA sample + 490 µl of 10 mM Tris·Cl,* pH 7.0 (1/50 dilution)

Measure absorbance of diluted sample in a 1 ml cuvette (RNase-free)

A_{260}	= 0.2
Concentration of RNA sample	= 44 µg/ml x A_{260} x dilution factor = 44 µg/ml x 0.2 x 50 = 440 µg/ml
Total amount	= concentration x volume in milliliters = 440 µg/ml x 0.1 ml = 44 µg of RNA

* When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

Purity of RNA

The assessment of RNA purity will be performed routinely, when using the QIAxpert with the corresponding RNeasy App. See the QIAxpert user manual for more information (**www.qiagen.com/qiaxpert-system/user-manual**).

For standard photometric measurements, the ratio of the readings at 260 and 280 nm (A_{260}/A_{280}) provides an estimate of the purity of RNA with respect to contaminants, such as protein, that absorb in the UV spectrum. However, the A_{260}/A_{280} ratio is influenced considerably by pH. Because water is not buffered, the pH and the resulting A_{260}/A_{280} ratio can vary greatly when using pure water. Lower pH results in a lower A_{260}/A_{280} ratio and reduced sensitivity to protein contamination.* For accurate values, we recommend measuring absorbance in 10 mM Tris·Cl, pH 7.5. Pure RNA has an A_{260}/A_{280} ratio of 1.9–2.1† in 10 mM Tris·Cl, pH 7.5. Always be sure to calibrate the spectrophotometer with the same solution used for dilution. For determination of RNA concentration, however, we recommend dilution of the sample in a buffer with neutral pH since the relationship between absorbance and concentration (A_{260} reading of 1 = 44 µg/ml RNA) is based on an extinction coefficient calculated for RNA at neutral pH (see "Spectrophotometric quantification of RNA", page 21).

DNA contamination

No currently available purification method can guarantee that RNA is completely free of DNA, even when it is not visible on an agarose gel. RNeasy kits will, however, remove the vast majority of cellular DNA. gDNA Eliminator solution helps to further reduce genomic DNA contamination; however, trace amounts of genomic DNA may still remain, depending on the amount and nature of the sample. For analysis of very low abundance targets, any interference by residual DNA contamination can be detected by performing real-time RT-PCR control experiments in which no reverse transcriptase is added prior to the PCR step.

* Wilfinger, W.W., Mackey, M., and Chomczynski, P. (1997) Effect of pH and ionic strength on the spectrophotometric assessment of nucleic acid purity. BioTechniques **22**, 474.

† Values up to 2.3 are routinely obtained for pure RNA (in 10 mM Tris·Cl, pH 7.5) with some spectrophotometers.

To prevent any interference by DNA in real-time RT-PCR applications, such as with Applied Biosystems® and Rotor-Gene® instruments, we recommend designing primers that anneal at intron splice junctions so that genomic DNA will not be amplified. QuantiTect Primer Assays from QIAGEN are designed for SYBR® Green-based real-time RT-PCR analysis of RNA sequences (without detection of genomic DNA) where possible (see **www.qiagen.com/GeneGlobe**). For real-time RT-PCR assays where amplification of genomic DNA cannot be avoided, we recommend using the QuantiTect Reverse Transcription Kit for reverse transcription. The kit integrates fast cDNA synthesis with rapid removal of genomic DNA contamination (see Ordering Information, page 25).

Integrity of RNA

The integrity and size distribution of total RNA purified with RNeasy Plus Universal Kits can be checked by denaturing agarose gel electrophoresis and ethidium bromide staining* or by using the QIAxcel® system or Agilent 2100 Bioanalyzer. Ribosomal RNAs should appear as sharp bands or peaks. The apparent ratio of 28S rRNA to 18S rRNA should be approximately 2:1. If the ribosomal bands or peaks of a specific sample are not sharp but appear as a smear towards smaller sized RNAs, it is likely that the sample suffered major degradation either before or during RNA purification. As a useful measure of RNA integrity, the QIAxcel Advanced system and the Agilent 2100 Bioanalyzer provide an RNA integrity score and an RNA integrity number, respectively. Ideally, the value should be close to 10, but in many cases (particularly with tissue samples), RNA quality is greatly influenced by how well the original sample was preserved.

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

Ordering Information

Product	Contents	Cat. no.
RNeasy Pure mRNA Bead (48)*	For 48 preps: Pure mRNA Beads, Buffers, Small Spin Columns, Collection Tubes, and RNase-Free Water	180244
QIAcube Connect — for fully automated nucleic acid extraction with QIAGEN spin-column kits		
QIAcube Connect	Instrument, connectivity package, 1-year warranty on parts and labor	9002864
Starter Pack, QIAcube	Reagent bottle racks (3); 200 µl filter-tips (1024); 1000 µl filter-tips (1024); 30 ml reagent bottles (12); rotor adapters (240); rotor adapter holder	990395
RNeasy Plus Universal Mini Kit — for purification of total RNA from all types of tissue using gDNA Eliminator Solution		
RNeasy Plus Universal Mini Kit (50)†	For 50 RNA minipreps: RNeasy Mini Spin Columns, gDNA Eliminator Solution, Collection Tubes, RNase-Free Water and Buffers	73404

* Fully automatable on the QIAcube. See **www.qiagen.com/MyQIAcube** for protocols.
† Other kit sizes/formats available; see **www.qiagen.com**.

Product	Contents	Cat. no.
RNeasy Plus Micro Kit — for purification of total RNA from small cell and tissue samples using gDNA Eliminator columns		
RNeasy Plus Micro Kit (50)*	50 RNeasy MinElute® Spin Columns,50 gDNA Eliminator Mini Spin Columns, Collection Tubes, Carrier RNA, RNase-Free Reagents and Buffers	74034
RNeasy Plus Mini Kit — for purification of up to 100 µg total RNA from cultured cells and tissues using gDNA Eliminator columns		
RNeasy Plus Mini Kit (50)*	For 50 minipreps: RNeasy Mini Spin Columns, gDNA Eliminator Spin Columns, Collection Tubes, RNase-Free Water and Buffers	74134
RNeasy Midi Kit — for purification of up to 1 mg total RNA from cells, tissues, and yeast		
RNeasy Midi Kit (10)*	10 RNeasy Midi Spin Columns, Collection Tubes (15 ml), RNase-Free Reagents and Buffers	75144
RNeasy Maxi Kit — for purification of up to 6 mg total RNA from cells, tissues, and yeast		
RNeasy Maxi Kit (12)	12 RNeasy Maxi Spin Columns, Collection Tubes (50 ml), RNase-Free Reagents and Buffers	75162

* Other kit sizes/formats available; see **www.qiagen.com**

Product	Contents	Cat. no.
RNeasy FFPE Kit — for purification of total RNA from formalin-fixed, paraffin-embedded tissue sections		
RNeasy FFPE Kit (50)	50 RNeasy MinElute Spin Columns, Collection Tubes, Proteinase K, RNase-Free DNase I, DNase Booster Buffer, RNase-Free Buffers, RNase-Free Water	73504
QIAxpert System — for accelerated DNA, RNA, and protein quantification and quality control		
QIAxpert Instrument	QIAxpert instrument with 1 year warranty coverage including parts, labor, and shipping; repair by sending to a regional repair center	9002340

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

Document Revision History

Date	Changes
05/2014	Initial release.
03/2021	Updated the product name. Updated the Safety Information section. Updated text and ordering information for the QIAcube Connect. Deleted the section “Important points before starting”. Inserted the Reference section. Updated Appendices A and B.
04/2022	Updated the Kit Contents table. Inserted Table 1.

Notes

Notes

Limited License Agreement for RNeasy Pure mRNA Bead Kit

Use of this product signifies the agreement of any purchaser or user of the product to the following terms:

1. The product may be used solely in accordance with the protocols provided with the product and this handbook and for use with components contained in the kit only. QIAGEN grants no license under any of its intellectual property to use or incorporate the enclosed components of this kit with any components not included within this kit except as described in the protocols provided with the product, this handbook, and additional protocols available at www.qiagen.com. Some of these additional protocols have been provided by QIAGEN users for QIAGEN users. These protocols have not been thoroughly tested or optimized by QIAGEN. QIAGEN neither guarantees them nor warrants that they do not infringe the rights of third-parties.
2. Other than expressly stated licenses, QIAGEN makes no warranty that this kit and/or its use(s) do not infringe the rights of third-parties.
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