September 2014

GeneRead™ rRNA Depletion Handbook

For highly selective and efficient removal of ribosomal RNA (rRNA) or rRNA and globin mRNA for NGS applications

GeneRead rRNA Depletion Kit (6)

GeneRead rRNA Depletion Nano Kit (48)

GeneRead Globin mRNA Depletion Probes

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Sample & Assay Technologies

QIAGEN Sample and Assay Technologies

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- Nucleic acid and protein assays
- microRNA research and RNAi
- Automation of sample and assay technologies

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Contents

Kit Contents

	GeneRead rRNA Depletion Kit (6)	GeneRead rRNA Depletion Nano Kit (48)
Catalog no.	**180211**	**180224**
Number of reactions	**6**	**48**
HMR rRNA Depletion Probes	75 µl	4 x 75 µl
Antibody Solution	135 µl	2 x 135 µl
Hybridization Buffer	500 µl	500 µl
BioMag Protein G Beads	800 µl	3 x 800 µl
RNase-Free Water	1.9 ml	1.9 ml
RNase Inhibitor	25 µl	2 x 25 µl
Small Spin Columns	6	48
Reaction Tubes	12 (2 ml)	100 (1.5 ml)
Buffer RLT*	–	45 ml
Buffer RPE (concentrate)*	–	11 ml
RNase-Free Water*	–	10 ml
Collection Tubes (1.5 ml)*	–	50
Collection Tubes (2 ml)*	–	50
RNeasy® MinElute® Spin Columns (each in a 2 ml Collection Tube)*	–	50
Quick-Start Protocol	1	1

* These components are shipped in a separate box labeled 'RNeasy MinElute Cleanup Kit (50)'.

GeneRead Globin mRNA Depletion Probes	
Catalog no.	**180950**
Number of reactions	**25–50**
GeneRead Globin mRNA Depletion Probes	1 tube
Quick-Start Protocol	1

Storage

The GeneRead rRNA Depletion Kit (cat. no. 180211) and GeneRead rRNA Depletion Nano Kit (cat. no.180224) are shipped on ice and can be stored for up to 12 months. Upon receipt, HMR rRNA Depletion Probes, Antibody Solution, Hybridization Buffer, and BioMag Protein G Beads should be stored at 2–8°C. RNase Inhibitor should be stored at –15°C to –30°C. All other components can be stored at room temperature (15–25°C). When stored under these conditions and handled correctly, these products can be kept for 12 months without showing any reduction in performance.

GeneRead Globin mRNA Depletion Probes (cat. no. 180950) are shipped on ice. Upon receipt, store at 2–8°C. When stored under these conditions and handled correctly, these products can be kept for 12 months without showing any reduction in performance.

The RNeasy MinElute Cleanup Kit is supplied with the GeneRead rRNA Depletion Nano Kit. Store RNeasy MinElute spin columns immediately upon receipt at 2–8°C.The remaining components of the RNeasy MinElute Cleanup Kit should be stored at room temperature (15–25°C) under dry conditions. All components of the RNeasy MinElute Cleanup Kit are stable for at least 9 months under these conditions.

Intended Use

GeneRead rRNA Depletion Kits and GeneRead Globin mRNA Depletion Probes are intended for molecular biology applications. These products are 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.

CAUTION: DO NOT add bleach or acidic solutions directly to the sample preparation waste.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of the GeneRead rRNA Depletion Kits and GeneRead Globin mRNA Depletion Probes are tested against predetermined specifications to ensure consistent product quality.

Introduction

Ribosomal RNA is the most abundant molecule in total RNA, making up over 80% of the total amount. 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 GeneRead rRNA Depletion Kit effectively removes ribosomal RNA (rRNA), while ensuring complete recovery of mRNA and noncoding RNA from a wide variety of species, including human, mouse, and rat. In contrast to poly(A) enrichment, rRNA depletion preserves information on non-adenylated, non-coding, and regulatory RNAs, enabling investigation of RNA regulation, nascent transcription, RNA editing, and other phenomena that increase our understanding of the transcriptome's complexity. By improving the ratio of useful data, decreasing bias, and preserving non-coding RNA types, the kit provides high-quality RNA that is especially suited for NGS applications. The GeneRead rRNA Depletion Nano Kit efficiently removes >99.9% of all types of rRNA from 100 ng to 1 µg of human, mouse, or rat total RNA, while ensuring complete recovery of mRNA and non-coding RNA. In addition to rRNA, globin mRNA can be effectively depleted from total RNA isolated from human whole blood with GeneRead Globin mRNA Depletion Probes (globin mRNA can account for around 20% of total mRNA from whole blood).

Principle and procedure

GeneRead rRNA Depletion Kits use a highly efficient and specific hybridization method to selectively deplete rRNA (see flowchart, page 9). The method is based on hybridization of specific oligonucleotide probes that are designed to hybridize to the large (18s, 28s), small (5s, 5.8s), and mitochondrial (12s, 16s) rRNAs. The RNA:DNA hybrid is recognized by an antibody and the resulting antibody-hybrid complex is efficiently captured on a BioMag Protein G Bead. These beads can then be separated from the sample, removing the rRNA in the process. The rRNA-depleted sample retains the diversity of RNA types, including poly(A) mRNA, non-adenylated mRNA, non-coding RNA, and regulatory RNAs. Multiple short oligonucleotides are used per rRNA to ensure that, even in the presence of degraded target RNA or mutations, the rRNA will be completely removed from the sample. The probes are carefully designed to ensure that cross-reactivity to non-rRNA molecules is minimized. The probes in these kits are designed for human, mouse, and rat, and will remove >99.9% (determined by qPCR) of the rRNA molecules from a sample from these species. The probes will also effectively remove rRNA from other eukaryotic species, though the efficiency may not be as high due to variations in rRNA sequence. For example,

the probes in these kits have been shown to be effective for rabbit, pig, and hamster, in addition to human, mouse, and rat.

Unlike affinity-tag approaches, only hybridized probes will be recognized by the antibody and bead capture system. This leads to very high efficiencies and fast reaction times. Residual probes are removed during RNA cleanup using the RNeasy MinElute Cleanup Kit (included with the GeneRead rRNA Depletion Nano Kit).

Description of protocols

For depletion of rRNA from up to 5 µg of human, mouse, or rat total RNA , see page 12.

For depletion of rRNA from 100 ng to 1 µg of human, mouse, or rat total RNA, see page 15.

For RNA cleanup, see page 18.

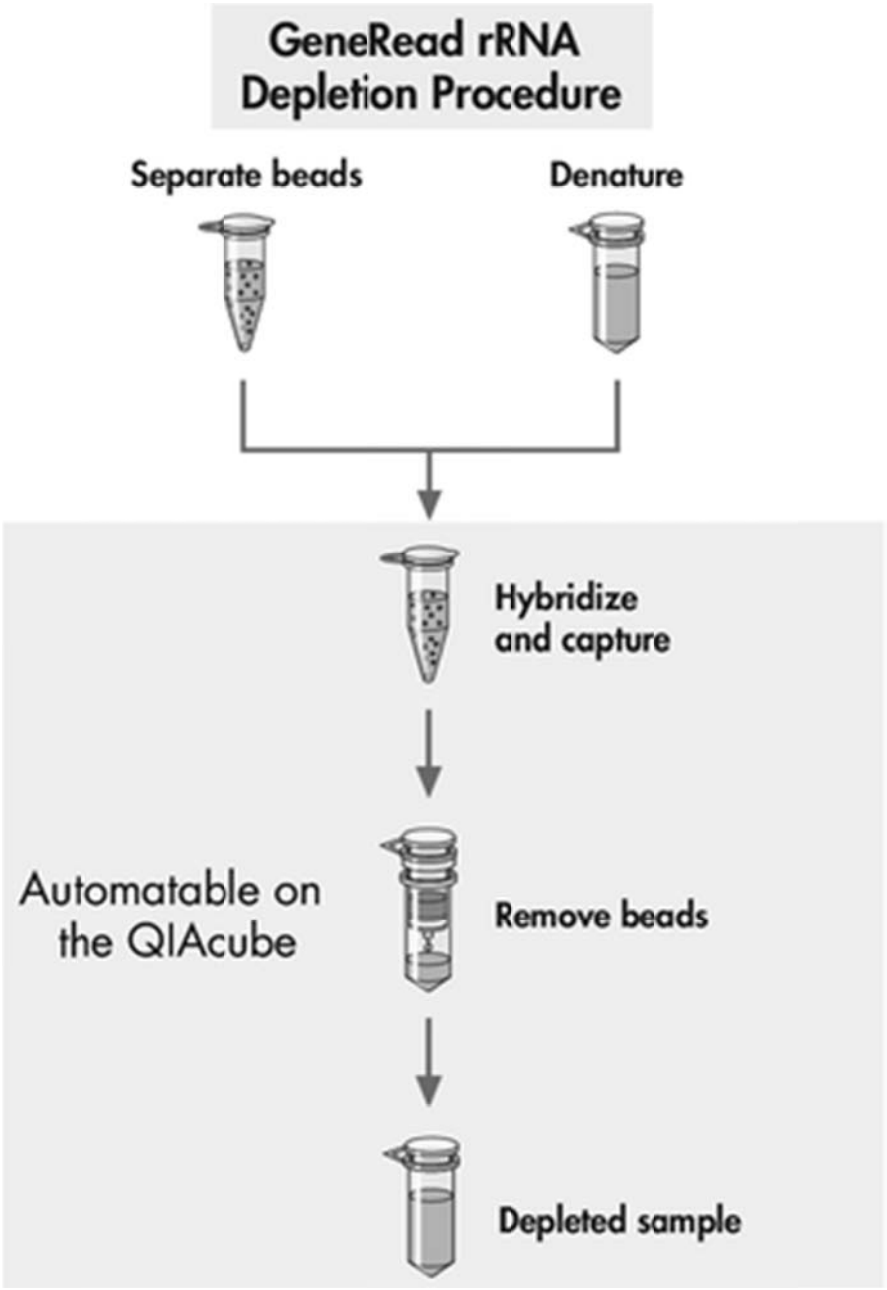
For depletion of globin mRNA together with rRNA from 100 ng to up to 5 µg of total RNA from human whole blood, see page 20.

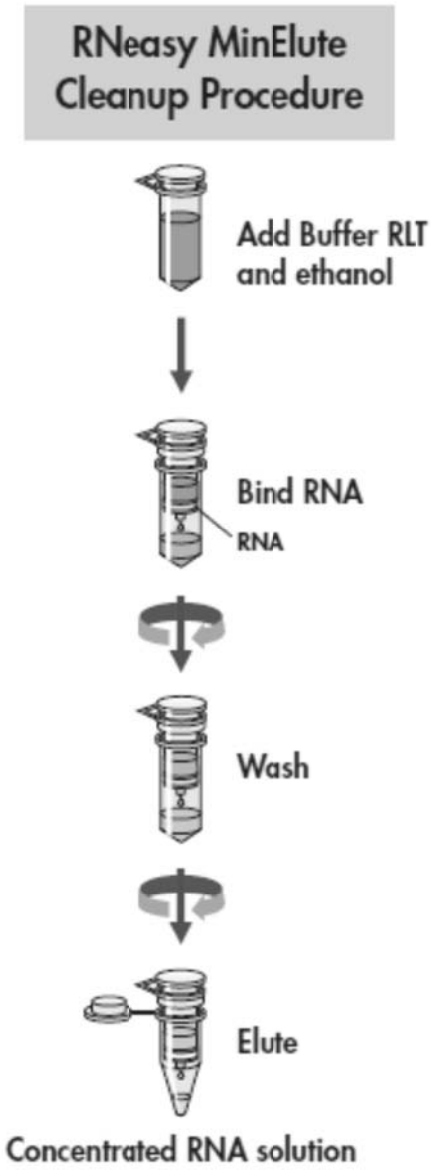
Automated purification

The majority of the procedure, from hybridization and capture through to RNA cleanup, can be fully automated on the QIAcube®. The innovative QIAcube uses advanced technology to process QIAGEN spin columns, enabling seamless integration of automated, low-throughput sample prep into your laboratory workflow. rRNA depletion using the QIAcube follows the same steps at the manual procedure. For more information about the automated procedure, see the relevant protocol sheet available at www.qiagen.com/MyQIAcube.

The QIAcube is 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/MyQIAcube.

The QIAcube.


GeneRead rRNA
Depletion Procedure
Separate beads
Denature
Hybridize
and capture
Automatable on
the QIAcube
Remove beads
Depleted sample


RNeasy MinElute
Cleanup Procedure
Add Buffer RLT
and ethanol
Bind RNA
RNA
Wash
Elute
Concentrated RNA solution

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.

- Sterile, RNase-free pipet tips
- Magnetic stand
- Vortexer
- Microcentrifuge
- Shaker-incubator such as the Thermomixer comfort (cat. no. 5355 000.011) and a thermoblock for 24 x 2 ml tubes (cat. no. 5362 000.019) from Eppendorf (www.eppendorf.com)*
- QIAGEN's RNeasy MinElute Cleanup Kit (cat. no. 74204). **Note**: This kit is already included with the GeneRead rRNA Depletion Nano Kit (cat. no.180224).

* This is not a complete list of suppliers and does not include many important vendors of biological supplies.

Protocol: Depletion of rRNA from 1–5 µg Total RNA Using the GeneRead rRNA Depletion Kit

This protocol is optimized for depletion of 18s, 28s, 5s, 5.8s, 12s, and 16s ribosomal RNA from 1–5 µg of total RNA from human, mouse, or rat.

Important points before starting

- If preparing RNA for the first time, read Appendix A, page 25.
- Use of total RNA isolated using RNeasy Kits is recommended.
- Use of RNA of the highest quality is recommended.
- See Appendix B, page 27, for analysis of yield and ribosomal RNA removal.

Things to do before starting

- Remove all components from refrigerator or freezer.
- Preheat incubator to 70°C and incubator-shaker to 50°C.

Procedure

1. **Mix the stock solution of BioMag Protein G Beads by vortexing for 15 s. For each sample, aliquot 125 µl of BioMag Protein G Beads into a separate 2 ml reaction tube (supplied). Place the reaction tube on a magnet rack or magnetic stand for a minimum of 2 min. Remove and discard the supernatant. Retain the bead pellet in the reaction tube.**
2. **For each sample, prepare a depletion mix according to Table 1 in a separate 2 ml reaction tube (supplied).**

Table 1. Composition of the GeneRead rRNA depletion mix

Component	Volume per reaction
Hybridization Buffer	10 µl
Purified total RNA	1–5 µg in 1–59 µl
HMR rRNA Depletion Probes	10 µl
Antibody Solution	20 µl
RNase Inhibitor	1 µl
RNase-Free Water	Variable
Total volume per reaction	**100 µl**

3. **Mix the depletion reaction thoroughly and incubate at 70°C for 5 min. After incubation, centrifuge the reaction mix briefly in a microcentrifuge to ensure all liquid is in the base of the tube.**
4. **Transfer the depletion mix to the bead pellet from step 1. Vortex briefly to resuspend beads, and incubate at 50°C with continuous shaking at 900 rpm for 30 min.**

   Optional: If a heated shaker is not available, then the depletion mix can be incubated at 50°C in a standard heat block. In this case, intermittently vortex the depletion mix and return to heat, no less than once every 5 min.
5. **Transfer the depletion mix to the small spin column in a 1.5 ml collection tube (supplied). Close the lid gently, and centrifuge for 30 s at ≥8000 x *g* (≥10,000 rpm). Remove the small spin column from the collection tube and discard. The flow-through contains the depleted RNA sample.**

   Optional: The beads used for depletion are magnetic. If magnetic separation is desired, remove the small spin column from the included collection tube. Place the depletion mix on a magnet rack or magnetic stand for a minimum of 2 min. Transfer the supernatant, being careful not to disturb the bead pellet, to the collection tube. The supernatant contains the depleted RNA sample.
6. **Clean up the depleted RNA sample using the RNeasy MinElute Kit (cat. no. 74204) as described on page 18.**

   IMPORTANT: Cleanup is an essential step. For reliable cleanup of the depleted RNA sample, the RNeasy MinElute Cleanup Kit is recommended. Without cleanup, or with cleanup methods such as ethanol precipitation,

excess capture probes will not be removed efficiently, and will result in problems during library preparation and sequencing.

Note: The RNeasy MinElute Cleanup Kit needs to be purchased separately. Visit www.qiagen.com or contact your local sales representative for more information.

Note: Efficiency of ribosomal removal and yield of RNA can be evaluated as described in Appendix B, page 27.

Protocol: Depletion of rRNA from 100 ng to 1 µg Total RNA Using the GeneRead rRNA Depletion Nano Kit

This protocol is optimized for depletion of 18s, 28s, 5s, 5.8s, 12s, and 16s ribosomal RNA from 100 ng to 1 µg of human, mouse, or rat total RNA.

Important points before starting

- If preparing RNA for the first time, read Appendix A, page 25.
- Use of total RNA isolated using RNeasy Kits is recommended.
- Use of RNA of the highest quality is recommended.
- See Appendix B, page 27, for analysis of yield and ribosomal RNA removal.

Things to do before starting

- Remove all components from refrigerator or freezer.
- Preheat incubator to 70°C and incubator-shaker to 50°C.

Procedure

1. **Mix the stock solution of BioMag Protein G Beads by vortexing for 15 s. For each sample, aliquot either 30 µl (for a starting amount of <500 ng total RNA) or 45 µl (for a starting amount of 0.5–1 µg total RNA) of BioMag Protein G Beads into a separate 1.5 ml reaction tube (supplied). Place the reaction tube on a magnet rack or magnetic stand for a minimum of 2 min. Remove and discard the supernatant. Retain the bead pellet in the reaction tube.**
2. **For each sample, prepare a depletion mix according to Table 2 in a separate 1.5 ml reaction tube (supplied).**

Table 2. Composition of the GeneRead rRNA depletion mix

Component	Volume per reaction (<500 ng RNA)	Volume per reaction (0.5–1 µg RNA)
Hybridization Buffer	5 µl	5 µl
Purified total RNA	100 ng to <500 ng in 1–36 µl	0.5–1 µg in 1–34 µl
HMR rRNA Depletion Probes	5 µl	5 µl
Antibody Solution	3 µl	5 µl
RNase Inhibitor	1 µl	1 µl
RNase-Free Water	Variable	Variable
Total volume per reaction	**50 µl**	**50 µl**

3. **Mix the depletion reaction thoroughly and incubate at 70°C for 5 min. After incubation, centrifuge the reaction mix briefly in a microcentrifuge to ensure all liquid is in the base of the tube.**
4. **Transfer the depletion mix to the bead pellet from step 1. Vortex briefly to resuspend beads, and incubate at 50°C with continuous shaking at 900 rpm for 30 min.**

   Optional: If a heated shaker is not available, then the depletion mix can be incubated at 50°C in a standard heat block. In this case, intermittently vortex the depletion mix and return to heat, no less than once every 5 min.
5. **Transfer the depletion mix to the small spin column in a 1.5 ml collection tube (supplied). Close the lid gently, and centrifuge for 30 s at ≥8000 x *g* (≥10,000 rpm). Remove the small spin column from the collection tube and discard. The flow-through contains the depleted RNA sample.**

   Optional: The beads used for depletion are magnetic. If magnetic separation is desired, remove the small spin column from the included collection tube. Place the depletion mix on a magnet rack or magnetic stand for a minimum of 2 min. Transfer the supernatant, being careful not to disturb the bead pellet, to the collection tube. The supernatant contains the depleted RNA sample.
6. **Clean up the depleted RNA sample using the RNeasy MinElute Kit (cat. no. 74204) as described on page 18.**

IMPORTANT: Cleanup is an essential step. For reliable cleanup of the depleted RNA sample, the RNeasy MinElute Cleanup Kit is recommended. Without cleanup, or with cleanup methods such as ethanol precipitation, excess capture probes will not be removed efficiently, and will result in problems during library preparation and sequencing.

Note: The RNeasy MinElute Cleanup Kit is included with the GeneRead rRNA Depletion Nano Kit.

Note: Efficiency of ribosomal removal and yield of RNA can be evaluated as described in Appendix B, page 27.

Protocol: RNA Cleanup Using the RNeasy MinElute Cleanup Kit

This protocol is designed for use with the RNeasy MinElute Cleanup Kit (cat. no. 74204) for RNA cleanup following after ribosomal RNA depletion, using the. GeneRead rRNA Depletion Kits.

Important points before starting

- If using the GeneRead rRNA Depletion Kit (cat. no. 180211), the RNeasy MinElute Cleanup Kit (cat. no. 74204) needs to be purchased separately. The RNeasy MinElute Cleanup Kit is included in the GeneRead rRNA Depletion Nano Kit (cat. no.180224)
- If preparing RNA for the first time, read Appendix A, page 25.
- Buffer RLT contains a guanidine salt* and is therefore not compatible with disinfecting reagents containing bleach. See page 6 for safety information.
- Perform all steps of the procedure at room temperature (15–25°C). During the procedure, work quickly.
- Perform all centrifugation steps at 20–25°C in a standard microcentrifuge. Ensure that the centrifuge does not cool below 20°C.

Things to do before starting

- Buffer RPE is supplied as a concentrate. Before using for the first time, add 4 volumes of ethanol (96–100%) as indicated on the bottle to obtain a working solution.
- Preheat Buffer RLT as it may form a precipitate during storage. If necessary, redissolve by warming, and then place at room temperature (15–25°C).

Procedure

1. **Add 350 µl Buffer RLT to the RNA sample and mix well by pipetting. Do not centrifuge. Proceed immediately to step 2.**
2. **Add 250 µl of 96–100% ethanol to the diluted RNA, and mix well by pipetting. Do not centrifuge. Proceed immediately to step 3.**
3. **Transfer the sample to an RNeasy MinElute spin column placed in a 2 ml collection tube (supplied). Close the lid gently, and centrifuge for 15 s at 8000 x *g* (10,000 rpm). Discard the flow-through.***

* Flow-through contains Buffer RLT and is therefore not compatible with bleach. See page 6 for safety information.

4. **Place the RNeasy MinElute spin column in a new 2 ml collection tube (supplied). Add 500 µl Buffer RPE to the spin column. Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash the spin column membrane. Discard the flow-through.**

   Reuse the collection tube in step 5.

   Note: Buffer RPE is supplied as a concentrate. Ensure that ethanol is added to Buffer RPE before use (see "Things to do before starting").

5. **Add 500 µl of 80% ethanol to the RNeasy MinElute spin column. Close the lid gently, and centrifuge for 2 min at ≥8000 x *g* (≥10,000 rpm) to wash the spin column membrane. Discard the flow-through and collection tube.**

   Note: After centrifugation, carefully remove the RNeasy MinElute spin column from the collection tube so that the column is not in contact with the flow-through. Otherwise, carryover of ethanol will occur.

6. **Place the RNeasy MinElute spin column in a new 2 ml collection tube (supplied). Open the lid of the spin column, and centrifuge at full speed for 5 min to dry the membrane. Discard the flow-through and collection tube.**

   To avoid damage to their lids, place the spin columns into the centrifuge with at least one empty position between columns. Orient the lids so that they point in a direction opposite to the rotation of the rotor (e.g., if the rotor rotates clockwise, orient the lids counterclockwise). It is important to dry the spin column membrane since residual ethanol may interfere with downstream reactions. Centrifugation with the lids open ensures that no ethanol is carried over during RNA elution.

7. **Place the RNeasy MinElute spin column in a new 1.5 ml collection tube (supplied). Add 14 µl RNase-free water directly to the center of the spin column membrane. Close the lid gently, and centrifuge for 1 min at full speed to elute the RNA.**

   As little as 10 µl RNase-free water can be used for elution if a higher RNA concentration is required, but the yield will be reduced by approximately 20%. Do not elute with <10 µl RNase-free water as the spin column membrane will not be sufficiently hydrated.

   The dead volume of the RNeasy MinElute spin column is 2 µl; elution with 14 µl RNase-free water results in a 12 µl eluate.

Protocol: Depletion of Globin mRNA Together with rRNA Using the GeneRead rRNA Depletion Kit or GeneRead rRNA Depletion Nano Kit

GeneRead Globin mRNA Depletion Probes (cat. no. 180950) are designed for use with either the GeneRead rRNA Depletion Kit (cat. no. 180211) or the GeneRead rRNA Depletion Nano Kit (cat. no. 180224). This protocol is for use with GeneRead Globin mRNA Depletion Probes for the depletion of globin mRNA in addition to rRNA from total RNA isolated from human whole blood.

Important points before starting

- If preparing RNA for the first time, read Appendix A, page 25.
- Use of total RNA isolated using RNeasy Kits is recommended.
- Use of RNA of the highest quality is recommended.
- See Appendix B, page 27, for analysis of yield and ribosomal RNA removal.

Things to do before starting

- Remove all components from refrigerator or freezer.
- Preheat incubator to 70°C and incubator-shaker to 50°C.

Procedure

1. **In step 2 of the rRNA depletion protocol, adjust the composition of the GeneRead rRNA depletion mix according to Table 3 (if using the GeneRead rRNA Depletion Kit) or Table 4 (if using the GeneRead rRNA Depletion Nano Kit) on the next page.**
2. **Perform the rest of the protocol as described in pages 11–14 or 15–17.**

Table 3. Composition of the GeneRead rRNA depletion mix (if using the GeneRead rRNA Depletion Kit)

Component	Volume/reaction (1–5 µg RNA)
Hybridization Buffer	10 µl
Purified total RNA	1–5 µg in 1–50 µl
HMR rRNA Depletion Probes	10 µl
GeneRead Globin mRNA Depletion Probes	2 µl
Antibody Solution	20 µl
RNase Inhibitor	1 µl
RNase-Free Water	Variable
Total volume per reaction	**100 µl**

Table 4. Composition of the GeneRead rRNA depletion mix (if using the GeneRead rRNA Depletion Nano Kit)

Component	Volume per reaction (<500 ng RNA)	Volume per reaction (0.5–1 µg RNA)
Hybridization Buffer	5 µl	5 µl
Purified total RNA	100 ng to <500 ng in 1–35 µl	0.5–1 µg in 1–33 µl
HMR rRNA Depletion Probes	5 µl	5 µl
GeneRead Globin mRNA Depletion Probes	1 µl	1 µl
Antibody Solution	3 µl	5 µl
RNase Inhibitor	1 µl	1 µl
RNase-Free Water	Variable	Variable
Total volume per reaction	**50 µl**	**50 µl**

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 at 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 and protocols in this handbook or sample and assay technologies (for contact information, see back cover or visit www.qiagen.com).

	Comments and suggestions
***rRNA depletion*:**	
Poor ribosomal RNA removal	
a) Insufficient shaking	At the very least, intermittent vortexing of the depletion mix (every 5 min or less) is required to keep the beads in suspension and properly capture the hybridized ribosomal RNA.
b) Insufficient probe, antibody, or bead concentration	For efficient removal of rRNA, the depletion mix must have a minimum concentration, as shown in Table 1. Using less reagent or diluting the reaction to greater than 100 µl will decrease efficiency of rRNA depletion.
c) Insufficient hybridization time	Decreasing hybridization time below 30 min will negatively impact rRNA depletion. Increased time of over 30 min has minimal effect.
d) Severely degraded starting RNA	GeneRead rRNA Depletion Kits will remove rRNA even from samples that are significantly degraded (RIN values <3). However, the likelihood that a fragment of rRNA will have no probe hybridization site increases with increasing fragmentation of the sample.
Depletion of non-ribosomal RNA	
Low temperature during hybridization	Be sure to preheat incubator. If the RNA depletion mix is incubated significantly below 50°C, the probes may non-specifically hybridize to undesired targets.

	Comments and suggestions
High percentage of ribosomal reads despite depletion/RNA does not perform well in downstream experiments	
Cleanup not performed after rRNA depletion	Cleanup is an essential step. Excess capture probes will not be removed efficiently without cleanup, resulting in problems during library preparation and sequencing. Cleanup methods such as ethanol precipitation are not sufficient. For reliable cleanup of the depleted RNA sample, the RNeasy MinElute Cleanup Kit is recommended.
RNA cleanup:	
Low or no recovery of RNA	
a) RNase-free water incorrectly dispensed	Pipet RNase-free water to the center of the RNeasy MinElute spin column membrane to ensure that the membrane is completely covered.
b) Ethanol carryover	After the wash with 80% ethanol, be sure to centrifuge at full speed for 5 min to dry the RNeasy MinElute spin column membrane. After centrifugation, carefully remove the RNeasy MinElute spin column from the collection tube so that the column does not contact the flow-through. Otherwise, carryover of ethanol will occur.
Clogged RNeasy MinElute spin column	
Centrifugation temperature too low	The centrifugation temperature should be 20–25°C. Some centrifuges may cool to below 20°C even when set at 20°C. This can cause formation of precipitates that can clog the spin column. If this happens, set the centrifugation temperature to 25°C.

Comments and suggestions	
Low A_{260}/A_{280} value	
Water used to dilute RNA A_{260}/A_{280} measurement	Use 10 mM Tris·Cl, pH 7.5, not RNase-free water, to dilute the sample before measuring purity (see Appendix B, page 27).
RNA does not perform well in downstream experiments	
Ethanol carryover	After the wash with 80% ethanol, be sure to centrifuge at full speed for 5 min to dry the RNeasy MinElute spin column membrane. After centrifugation, carefully remove the RNeasy MinElute spin column from the collection tube so that the column does not contact the flow-through. Otherwise, carryover of ethanol will occur.

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. Since 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 of RNaseKiller (cat. no 2500080) from 5 PRIME (www.5prime.com) is recommended. RNase contamination can alternatively be removed using general laboratory reagents. To decontaminate plasticware, rinse with 0.1 M NaOH, 1 mM EDTA* followed by RNase-free water (see "Solutions", page 26), 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.

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.

*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.

Glassware

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 DEPC* (diethyl pyrocarbonate), 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: GeneRead and 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 of RNA

Purified RNA may be stored at –20°C or –70°C in RNase-free water. Under these conditions, no degradation of RNA is detectable after 1 year.

Quantification of RNA

The concentration of RNA can be determined by measuring the absorbance at 260 nm (A_{260}) in a spectrophotometer, for example, the QIAxpert (QIAGEN, cat. no. 9002340) (see "Spectrophotometric quantification of RNA" below). For small amounts of RNA, however, it may not be possible to accurately determine amounts photometrically. Small amounts of RNA can be quantified by qRT-PCR or fluorogenic assays.

Spectrophotometric quantification of RNA

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 → 44 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 28), 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 26). 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)

* 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.

† 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.

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

Purity of RNA

The ratio of the readings at 260 nm and 280 nm (A_{260}/A_{280}) provides an estimate of the purity of RNA with respect to contaminants that absorb in the UV spectrum, such as protein. However, the A_{260}/A_{280} ratio is influenced considerably by pH. Since water is not buffered, the pH and the resulting A_{260}/A_{280} ratio can vary greatly. 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 27).

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. While the RNeasy MinElute Cleanup procedure will remove the vast majority of cellular DNA from the RNA fraction, trace amounts may still remain, depending on the amount and nature of the sample. The starting RNA used for rRNA depletion should be reasonably free of DNA in order to avoid complications

*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.

Ordering Information

Product	Contents	Cat. no.
GeneRead rRNA Depletion Kit (6)*	For 6 x 100 µl reactions: HMR rRNA Depletion Probes, Antibody Solution, Hybridization Buffer, BioMag Protein G Beads, RNase-free Water, RNase Inhibitor, Small Spin Columns, Reaction Tubes	180211
GeneRead rRNA Depletion Nano Kit (48)*	For 6 x 100 µl reactions: HMR rRNA Depletion Probes, Antibody Solution, Hybridization Buffer, BioMag Protein G Beads, RNase-free Water, RNase Inhibitor, Small Spin Columns, Reaction Tubes	180224
GeneRead Globin mRNA Depletion Probes	For 25–50 reactions; to be used with the GeneRead rRNA Depletion Kit (cat. no. 180211) or the GeneRead rRNA Depletion Nano Kit (cat. no. 180224)	180950
Accessories		
RNeasy MinElute Cleanup Kit — for RNA cleanup and concentration with small elution volumes		
RNeasy MinElute Cleanup Kit (50)	50 RNeasy MinElute Spin Columns, Collection Tubes, RNase-free Reagents and Buffers.	74204
QIAGEN RNase Inhibitor — for potent inhibition of RNase A-type ribonucleases		
QIAGEN RNase Inhibitor (500 reactions)	500 µl (4 U/µl) RNase Inhibitor in 2 mM KH_2PO_4, 8.0 mM Na_2HPO_4, 3.0 mM KCl, 150 mM NaCl, pH 7.4, and 50% glycerol	129916

Product	Contents	Cat. no.
QIAcube — for fully automated sample preparation using QIAGEN spin-column kits		
QIAcube (110 V)* QIAcube (230 V)†	Robotic workstation for automated purification of nucleic acids or proteins using QIAGEN spin-column kits, 1-year warranty on parts and labor‡	9001292* 9001293†
Starter Pack, QIAcube	Pack includes: reagent bottle racks (3); rack labeling strips (8); 200 µl filter-tips (1024); 1000 µl filter-tips (1024); 1000 µl filter-tips, wide-bore (1024); 30 ml reagent bottles (18); rotor adapters (120); rotor adapter holder	990395
Related products		
GeneRead Pure mRNA Kit — for highly selective and efficient purification of mRNA for NGS applications		
GeneRead Pure mRNA Kit (48)§	For 48 preps: Pure mRNA Beads, Buffers, Small Spin Columns, Collection Tubes, and RNase-Free Water	180244
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

* US, Canada, and Japan

†. Rest of the world.

‡ Agreements for comprehensive service coverage are available; please inquire.

§ Fully automatable on the QIAcube. See www.qiagen.com/MyQIAcube for protocols

Product	Contents	Cat. no.
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
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 Micro Kit — for purification of up to 45 µg total RNA from cell and tissue samples		
RNeasy Micro Kit (50)*	50 RNeasy MinElute Spin Columns, Collection Tubes (1.5 ml and 2 ml), RNase-free DNase I, Carrier RNA, RNase-Free Reagents and Buffers	74004

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

Product	Contents	Cat. no.
RNeasy Mini Kit — for purification of total RNA from cells, tissues, and yeast, and for RNA cleanup		
RNeasy Mini Kit (50)*	50 RNeasy Mini Spin Columns, Collection Tubes, RNase-Free Reagents and Buffers	74104
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	75142
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
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

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

Limited License Agreement for GeneRead rRNA Depletion Kits and GeneRead Globin mRNA Depletion Probes

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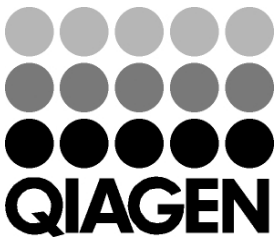

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Sample & Assay Technologies