January 2020

RNeasy® PowerMicrobiome® Kit Handbook

For the isolation of total RNA from stool and gut material

Sample to Insight

QIAGEN

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Kit Contents

RNeasy PowerMicrobiome Kit **Catalog no.** **Number of preps**	**(50)** **26000-50** **50**
PowerBead Tube, Glass 0.1 mm	50
Solution PM1	55 ml
Solution IRS	15 ml
Solution PM3	36 ml
Solution PM4	3 x 24 ml
Solution PM5	3 x 30 ml
DNase Digestion Solution	2 x 1.5 ml
Solution PM7	23 ml
RNase-Free Water	10 ml
DNase I (RNase-free)	1 vial (1500 units)
MB RNA Spin Columns	50
Collection Tubes (2 ml)	4 x 50
Quick-Start Protocol	1

Storage

The RNeasy PowerMicrobiome Kit contains lyophilized DNase I. Remove and store at 2–8°C. All other reagents and components should be stored at room temperature (15–25°C) until the expiration date printed on the label. After resuspension, DNase I must be stored at –30°C to –15°C. DNase is sensitive to physical denaturation. Do not vortex the resuspended DNase.

Intended Use

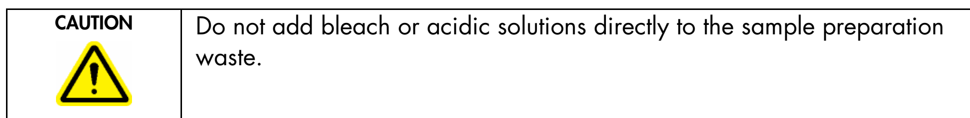
All RNeasy products are intended for molecular biology applications. These products are not intended for the diagnosis, prevention or treatment of a disease.

QIAcube® Connect is designed to perform fully automated purification of nucleic acids and proteins in molecular biology applications. The system is intended for use by professional users trained in molecular biological techniques and the operation of QIAcube Connect.

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 RNA 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.

The sample preparation waste contains guanidine hydrochloride from Solutions PM1 and PM7 (guanidine thiocyanate) and Solution PM3 (guanidine hydrochloride), which can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water and then with 1% (v/v) sodium hypochlorite.

Quality Control

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

Introduction

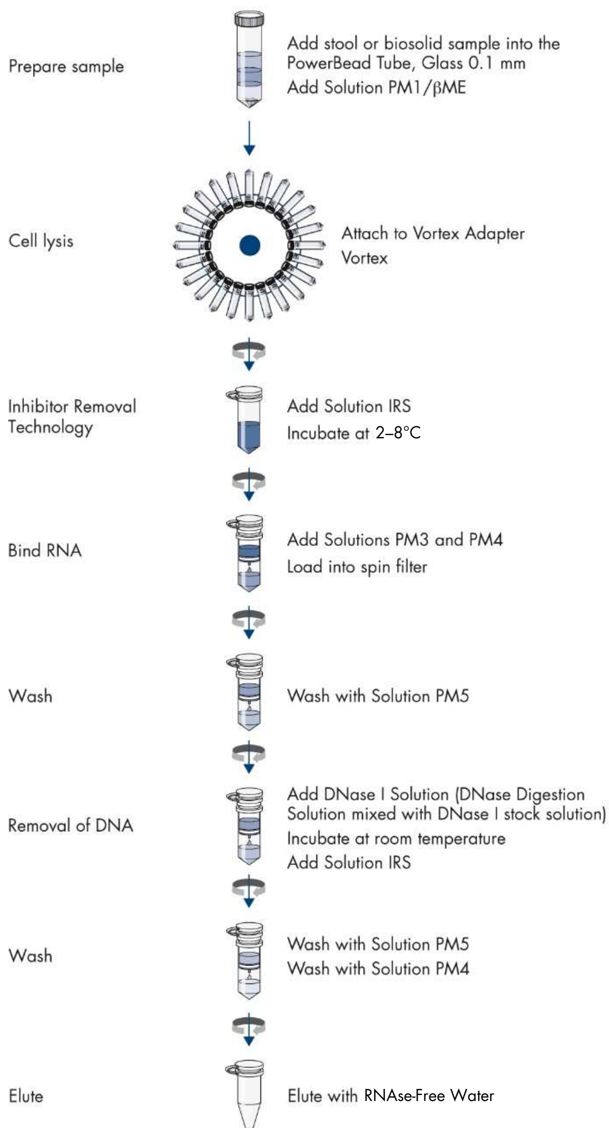
The RNeasy PowerMicrobiome Kit is designed for fast and easy purification of total RNA from samples high in PCR inhibitors, including stool, gut material, dried feces, contaminated buccal swabs and secretions. Inhibitor Removal Technology® (IRT) ensures complete removal of the inhibitory substances often contained in these materials (e.g., undigested plant material in the gut or heme compounds from lysed red blood cells, abundant in stool). The result is RNA that is ready to use in the most demanding downstream applications. Genomic DNA is removed on column by using the provided DNase I and reaction buffer. RNA is eluted in RNase-free water and is ready for downstream applications.

Principle and procedure

Stool or biosolid samples are processed through a lysis step that uses bead beating and a strong chemical lysis buffer for efficient extraction of tough microorganisms. We recommend starting with 0.25 grams of material.

In the next step, the binding solution (PM3) holds the total nucleic acids in the lysate. DNA is removed using on-column DNase followed by a wash solution. This removes the enzyme and any digested nucleic acids. The RNA eluted in RNase-free water is ready to use in any downstream application.

Note: Stool samples must be stored at –90°C to –65°C as soon as possible after collection to protect the integrity of the RNA.


RNeasy PowerMicrobiome Kit
Prepare sample
Add stool or biosolid sample into the PowerBead Tube, Glass 0.1 mm
Add Solution PM1/βME
Cell lysis
Attach to Vortex Adapter
Vortex
Inhibitor Removal Technology
Add Solution IRS
Incubate at 2–8°C
Bind RNA
Add Solutions PM3 and PM4
Load into spin filter
Wash
Wash with Solution PM5
Removal of DNA
Add DNase I Solution (DNase Digestion Solution mixed with DNase I stock solution)
Incubate at room temperature
Add Solution IRS
Wash
Wash with Solution PM5
Wash with Solution PM4
Elute
Elute with RNAse-Free Water

Automated purification of RNA on QIAcube Instruments

Purification of RNA 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 RNeasy PowerMicrobiome Kit for purification of high-quality RNA.

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**.

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.

- Microcentrifuge (13,000 x *g*)
- Pipettor (1.5–1000 µl)
- Vortex-Genie® 2 Vortex
- Vortex Adapter for 24 (1.5–2.0 ml) tubes (cat. no. 13000-V1-24)
- β–mercaptoethanol (β-ME)
- Phenol–chloroform–isoamyl alcohol (25:24:1) pH 6.7–8.0 (optional)

Protocol: Experienced User

Notes before starting

- Solution PM1 must be warmed at 55°C for 5–10 min prior to use.
- Shake to mix Solution PM5 before use.
- Prepare Solution PM1 by adding 10 µl β-mercaptoethanol (β-ME) for every 990 µl Solution PM1 (a total of 1 ml for each prep).
- Prepare DNase I stock solution by adding 550 µl RNase-Free Water to the DNase I (RNase-free) lyophilized powder and mixing gently. Aliquot the DNase I stock enzyme in 50 µl portions and store at –30°C to –15°C for long-term storage (but do not freeze–thaw more than 3 times). To prepare DNase I solution, thaw and combine 5 µl DNase I stock enzyme with 45 µl DNase Digestion Solution per prep.

Procedure

1. Place 0.25 g stool or biosolid sample into a PowerBead Bead Tube, Glass 0.1 mm.
   Note: If phenol-based lysis is desired, add 100 µl phenol–chloroform–isoamyl alcohol (pH 6.5–8.0) to the PowerBead Tube before adding the sample.
2. Add 650 µl Solution PM1–β-ME to the PowerBead Tube. Alternatively, you may add 650 µl PM1 and 6.5 µl β-ME to the PowerBead Tube.
3. Secure the PowerBead Tube horizontally to a Vortex Adapter (cat. no. 13000-V1-24). Orient tube caps to point toward the center of the Vortex Adapter.
4. Vortex at maximum speed for 10 min. Centrifuge at 13,000 x *g* for 1 min at room temperature (15–25°C). Transfer the supernatant to a clean 2 ml Collection Tube (provided).

   Note: If you added phenol–chloroform–isoamyl alcohol, remove the upper aqueous layer and transfer to a clean 2 ml Collection Tube (provided).
5. Add 150 µl Solution IRS, and vortex briefly to mix. Incubate at 2–8C for 5 min.

6. Centrifuge at 13,000 x *g* for 1 min.
7. Avoiding the pellet, transfer the supernatant to a clean 2 ml Collection Tube (provided).
   Note: Do not transfer more than 650 µl at this step.
8. Add 650 µl each of Solution PM3 and Solution PM4. Vortex briefly to mix.
   Note: To prevent small RNAs (5s RNAs, tRNAs and degraded RNAs) from co-purifying with mRNA and rRNA, use 650 µl 70% ethanol instead of Solution PM4. To purify small RNAs, such as microRNAs and siRNAs, transfer the lysate to a larger tube to accommodate a higher volume (2.6 ml) and add an additional 650 µl 100% ethanol (user supplied) to the lysate.
9. Load 650 µl supernatant into an MB RNA Spin Column and centrifuge the tubes at 13,000 x *g* for 1 min. Discard the flow-through and repeat until all the supernatant has been processed through the spin column.
10. Shake to mix Solution PM5. Add 650 µl Solution PM 5 to the MB RNA Spin Column and centrifuge at 13,000 x *g* for 1 min.
    Note: Skip steps 11–13 if you want to isolate both RNA and DNA.
11. Discard flow-through and centrifuge at 13,000 x *g* for 1 min to remove residual wash.
12. Place the MB RNA Spin Column into a clean 2 ml Collection Tube (provided). To the center of the Spin Column, add 50 µl DNase I Solution (prepared by mixing 45 µl DNase Digestion Solution and 5 µl DNase I stock enzyme; see "Notes before starting").
13. Incubate at room temperature for 15 min. Add 400 µl Solution PM7 and centrifuge at 13,000 x *g* for 1 min.
14. Discard flow-through. Add 650 µl Solution PM5. Centrifuge at 13,000 x *g* for 1 min.
15. Discard flow-through. Add 650 µl Solution PM4. Centrifuge at 13,000 x *g* for 1 min.
16. Discard flow-through. Centrifuge at 13,000 x *g* for 2 min.
17. Place the MB RNA Spin Column into a clean 2 ml Collection Tube (provided).

18. Add 100 µl RNase-Free Water (provided) to the center of the white filter membrane. Incubate at room temperature for at least 1 min.
    Note: Eluting with 100 µl RNase-Free Water will maximize RNA yield. For more concentrated RNA, a **minimum** of 50 µl RNase-Free Water can be used.
19. Centrifuge at 13,000 x *g* for 1 min. Discard the MB Spin Column. The RNA is now ready for downstream applications.

Protocol: Detailed

Notes before starting

- Solution PM1 must be warmed at 55°C for 5–10 min prior to use.
- Shake to mix Solution PM5 before use.
- Prepare Solution PM1 by adding 10 µl β-mercaptoethanol (β-ME) for every 990 µl Solution PM1 (a total of 1 ml for each prep).
- Prepare DNase I stock solution by adding 550 µl RNase-Free Water to the DNase I (RNase-free) lyophilized powder and mixing gently. Aliquot the DNase I stock enzyme in 50 µl portions and store at–30°C to –15°C for long term storage (but do not freeze–thaw more than 3 times). To prepare DNase I solution, thaw and combine 5 µl DNase I stock enzyme with 45 µl DNase Digestion solution per prep.

Procedure

1. Place 0.25 g stool or biosolid sample into a PowerBead Tube, Glass 0.1 mm.
   Note: If phenol-based lysis is desired, add 100 µl phenol–chloroform–isoamyl alcohol (pH 6.5–8.0) to the PowerBead Tube before adding the sample.
2. Add 650 µl Solution PM1–β-ME to the PowerBead Tube. Alternatively, you may add 650 µl PM1 and 6.5 µl β-ME to the PowerBead Tube.
3. Secure the PowerBead Tube horizontally to a Vortex Adapter (cat. no. 13000-V1-24). Orient tube caps to point toward the center of the Vortex Adapter.

4. Vortex at maximum speed for 10 min. Centrifuge at 13,000 x *g* for 1 min at room temperature (15–30°C). Transfer the supernatant to a clean 2 ml Collection Tube (provided).
   Note: If you added phenol–chloroform–isoamyl alcohol, remove the upper aqueous layer and transfer to a clean 2 ml Collection Tube (provided).
   Note: The sample is homogenized using mechanical bead beating and a lysis buffer that protects the RNA released into the supernatant. As the sample spins, proteins and cellular debris are pelleted with the beads and the supernatant contains RNA and DNA from both human and bacterial cells.
5. Add 150 µl Solution IRS and vortex briefly to mix. Incubate at 2–8°C for 5 min.
   Note: Solution IRS– is the Inhibitor Removal Solution which completes the IRT process and removes the contaminants from the sample that would cause problems with PCR and other downstream applications.
6. Centrifuge at 13,000 x *g* for 1 min.
7. Avoiding the pellet, transfer the supernatant to a clean 2 ml Collection Tube (provided).
   Note: Do not transfer more than 650 µl at this step.
8. Add 650 µl each of Solution PM3 and Solution PM4. Vortex briefly to mix.
   Note: To prevent small RNAs (5s RNAs, tRNAs and degraded RNAs) from co-purifying with mRNA and rRNA, use 650 µl 70% ethanol instead of Solution PM4. To purify small RNAs, such as microRNAs and siRNAs, transfer the lysate to a larger tube to accommodate a higher volume (2.6 ml) and add an additional 650 µl 100% ethanol (user supplied) to the lysate.
   Note: Solution PM3 contains the binding salts for total nucleic acid purification and Solution PM4 is 100% ethanol. These solutions set up the conditions for RNA and DNA binding to the Spin Filter.
9. Load 650 µl supernatant into an MB RNA Spin Column and centrifuge the tubes at 13,000 x *g* for 1 min. Discard the flow-through and repeat until all the supernatant has been processed through the Spin Column.
   Note: Total nucleic acids are bound to the Spin Column by passing through the membrane using centrifugation.

10. Shake to mix Solution PM5. Add 650 µl Solution PM 5 to the MB RNA Spin Column and centrifuge at 13,000 x *g* for 1 min.

    Note: Skip steps 11–13 if you want to isolate both RNA and DNA.

    Note: Solution PM5 is a wash buffer that contains isopropanol to remove salts from the membrane for optimal performance of the on-column DNase step.

11. Discard flow-through and centrifuge at 13,000 x *g* for 1 min to remove residual wash.
12. Place the MB RNA spin column into a clean 2 ml Collection Tube (provided). To the center of the Spin Column, add 50 µl DNase I Solution (prepared by mixing 45 µl DNase Digestion Solution and 5 µl DNase I stock enzyme; see "Notes before starting").
13. Incubate at room temperature for 15 min. Add 400 µl Solution PM7 and centrifuge at 13,000 x *g* for 1 min.

    Note: DNase Digestion Solution is a DNase digestion buffer. The DNase in DNase Digestion Solution soaks into the membrane and digests the genomic DNA in the column. Solution PM7 inactivates the DNase enzyme and removes it from the column membrane along with digested DNA.

14. Discard flow-through. Add 650 µl Solution PM5. Centrifuge at 13,000 x *g* for 1 min.
15. Discard flow-through. Add 650 µl Solution PM4. Centrifuge at 13,000 x *g* for 1 min.

    Note: Solution PM5 and PM4 are isopropanol- and ethanol-containing wash buffers, respectively, and are used to desalt the column before the elution step.

16. Discard flow-through. Centrifuge at 13,000 x *g* for 2 min.

    Note: The final dry spin ensures all ethanol is cleared from the membrane so that the elution will be efficient.

17. Place the MB RNA Spin Column into a clean 2 ml Collection Tube (provided).
18. Add 100 µl RNase-Free Water (provided) to the center of the white filter membrane. Incubate at room temperature for at least 1 min.

    Note: Eluting with 100 µl RNase-Free Water will maximize RNA yield. For more concentrated RNA, a **minimum** of 50 µl RNase-Free Water can be used.

19. Centrifuge at 13,000 x *g* for 1 min. Discard the MB Spin Column. The RNA is now ready for downstream applications.

    Note: RNA is solubilized from the Spin Filter membrane into RNase-Free Water and is ready for use.

Appendix I: Sample Storage Temperatures

The yield and integrity of the RNA isolated from microbes in stool is greatly influenced by the state of the digestive system, diet of the individual and the length of time between collection of the sample and preservation at –90°C to –65°C. The main components of stool are water (between 65–85%), undigested food, bile, bilirubin, which is derived from dead red blood cells, and dead bacterial cells (up to 50%). Because of the high content of dead and decaying bacterial and human cells, RNA isolated from stool will typically appear to have some level of degradation using standard analysis methods. Quantitative PCR assays developed for RNA species from stool or gut should be designed to detect small fragments to increase detection sensitivity.

To optimize the quality of RNA from stool, process the sample as quickly as possible after collection. It may be preferable to freeze the samples at –90°C to –65°C in small aliquots to avoid freeze–thaw cycles of the bulk sample, which can increase the lysis of cells and degradation of RNA. Samples should be processed rapidly by adding Solution PM1, which is a lysis buffer that contains β-ME, to the bead tube before the sample has fully thawed. Homogenize immediately to saturate the cellular RNA in the protective lysis buffer. For fresh (non-frozen) samples, rapid homogenization in lysis buffer is especially critical to isolate the highest quality RNA.

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/or protocols in this handbook or sample and assay technologies. For contact information, visit **www.qiagen.com**.

		Comments and suggestions
RNA		
a)	RNA degradation	The optimal method for storing stool samples prior to RNA isolation is to freeze at –90°C to –65°C and to avoid multiple freeze–thaw cycles. The primary solid component of stool is dead bacteria, which contains a high level of degraded RNA. Co-isolation of degraded RNA along with intact RNA is expected. Removal of small RNA is achieved by reducing the concentration of ethanol used at the binding step (step 8). Instead of Solution PM4, use 650 µl 70% ethanol. If using stool samples preserved in RNA*later*®, use only 0.1 g sample to avoid clogging the column. RNA*later*-preserved samples will require additional DNase treatment post-purification to remove contaminating DNA. The use of phenol–chloroform–isoamyl alcohol (25:24:1) buffered to pH 6.7–8.0 is optional and may assist in protecting the integrity of the RNA during homogenization of the sample. Add 100 µl phenol–chloroform–isoamyl alcohol to the PowerBead Tube, Glass 0.1 mm before adding the sample. For most solid samples, an organic phase and an aqueous phase may not be visible. However, for watery samples you may see a separation of phases. Always take the upper aqueous phase for the next step.
b)	Low purity	Expected purity readings for RNA are 1.8–2.1 for the A_{260}/A_{280} ratio and >1.5 for the A_{260}/A_{230} ratio. If your sample has low purity, it may be related to the sample composition. If the A_{260}/A_{230} readings are low, increase the amount of Solution PM2 to 200 µl to remove more inhibitors. Alternatively, use less sample if the purity is not improved with the addition of more Solution PM2.

		Comments and suggestions
c)	Genomic DNA contamination	Depending on the quality of bacterial cells in the starting sample, it is possible to still have residual genomic DNA in the RNA even after the on-column DNase digest. To completely remove the genomic DNA, the DNase treatment using the DNase Max Kit (cat. no. 15200-50) will ensure removal of DNA without the addition of EDTA or heat inactivation of the enzyme.

Ordering Information

Product	Contents	Cat. no.
RNeasy PowerMicrobiome Kit (50)	For the isolation of total RNA from stool and gut material	26000-50
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	Inquire
Starter Pack, QIAcube	Filter-tips, 200 µl (1024), 1000 µl filter-tips (1024), 30 ml reagent bottles (12), rotor adapters (240), elution tubes (240), rotor adapter holder	990395
Related products		
DNase Max Kit (50)	For the removal of genomic DNA contamination in RNA preparations	15200-50
PowerLyzer® 24 Homogenizer (110/220 V)	For the most efficient and complete lysis and homogenization of any biological sample.	13155
Vortex Adapter for 24 (1.5–2.0 ml) tubes	For maximizing homogenization with the Vortex-Genie® 2 Vortex	13000-V1-24

* All QIAcube Connect instruments are provided with a region-specific connectivity package, including tablet and equipment necessary to connect to the local network. Further, QIAGEN offers comprehensive instrument service products, including service agreements, installation, introductory training and preventive subscription. Contact your local sales representative to learn about your options.

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
August 2018	Updated volume of RNase-Free Water (550 µl) added to DNase I to make a stock solution.
January 2020	Updated text, ordering information and intended use for QIAcube Connect.

Limited License Agreement for RNeasy PowerMicrobiome 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.
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Notes