# RNeasy® PowerFecal® Pro Kit Handbook

For the isolation of microbial RNA from stool and gut samples, sludge, or wastewater



### Contents

Kit Contents	4
Storage	5
Intended Use	5
Safety Information	6
Quality Control	7
Introduction	8
Principle and procedure	8
Automated purification of RNA on QIAcube Instruments	10
Equipment and Reagents to Be Supplied by User	11
Important Notes	12
Sample storage and preservation	12
Disruption and homogenization of starting material	13
Protocol: Experienced User	14
Protocol: Detailed	17
Troubleshooting Guide	20
Appendix: General Remarks on Handling RNA	22
Handling RNA	22
General handling	22
Disposable plasticware	22
Non-disposable plasticware	23
Glassware	23
Electrophoresis tanks	

Solutions	24
Ordering Information	25
Document Revision History	27

## Kit Contents

RNeasy PowerFecal Pro Kit	(50)
Catalog no.	78404
Number of preps	50
PowerBead Pro Tubes	50
Solution CD1	40 ml
Solution CD2	15 ml
MB RNA Spin Columns	50
Collection Tubes 2 ml	100
Solution EA	175 ml
Solution C5	30 ml
RNase free water	<i>7</i> ml
DNase Digestion Solution	1,5 ml
DNase I (lyophilized)	1 tube
Microcentrifuge Tubes, 2 ml	100
Elution Tubes, 1.5 ml	50
Quick-Start Protocol	1

### Storage

Solution CD2 and Lyophilized DNase I should be stored at 2–8°C upon arrival. All other kit components and reagents can be stored at room temperature (15–25°C) until the expiry date printed on the box label.

When stored correctly, RNeasy PowerFecal Pro Kit is stable until the expiration date printed on kit box lid.

### Intended Use

The RNeasy PowerFecal Pro Kit is intended for the purification of microbial RNA from stool, gut samples, sludge or wastewater. The RNeasy PowerFecal Pro Kit is intended for research use only. This product is not intended for the diagnosis, prevention, or treatment of a disease.

QIAcube<sup>®</sup> 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 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.

WARNING



Solution FA and Solution C5 are flammable.

CAUTION



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

Solution CD1 and Solution EA contain chaotropic salts, 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 the RNeasy PowerFecal Pro Kit is tested against predetermined specifications to ensure consistent product quality.

### Introduction

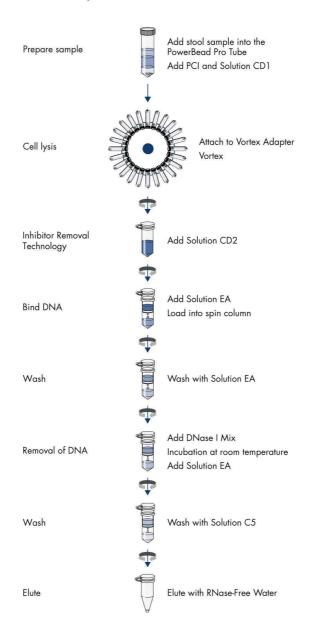
The RNeasy PowerFecal Pro Kit comprises a novel and proprietary method for isolating total RNA from samples high in PCR inhibitors, including stool, gut samples, sludge, or wastewater. The kit uses QIAGEN's second-generation Inhibitor Removal Technology® (IRT) and is intended for use with samples containing inhibitory substances commonly found in stool, such as polysaccharides, heme compounds, and bile salts. Improved IRT combined with more efficient bead beating and lysis chemistry yields high-quality RNA that can be used immediately in downstream applications, including RT-PCR, qPCR and next-generation sequencing (e.g., RNA-seq or metatranscriptome).

#### Principle and procedure

The recommended starting material is 50–200 mg of stool. Each sample is homogenized in a 2 ml bead beating tube containing a mixture of lysis beads. Lysis of host and microbial cells is facilitated by both mechanical collisions between beads and chemical disruption of cell membranes. IRT is then used to remove common substances in stool samples that interfere with PCR and other downstream applications. The lysate is then passed through a MB RNA Spin Column. The DNA is removed using on-column DNase digestion 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 applications.

**Note**: Stool samples should be preserved as soon as possible after collection to protect the integrity of the RNA. Storage in PowerProtect DNA/RNA or by freezing (at -80 or  $-20^{\circ}$ C) is the preferred method of preserving the samples.

#### RNeasy PowerFecal Pro Kit Procedure



# 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 PowerFecal Pro Kit for purification of high-quality RNA.

The 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.

- Phenol-chloroform-isoamyl alcohol (25:24:1) or QIAzol<sup>®</sup> Lysis Reagent (cat.no. 79306)
- Pipettes and disposable pipette tips with aerosol barriers (20–1000 μl)
- Disposable gloves
- Microcentrifuge (with rotor for 1.5 and 2 ml tubes)
- Vortex-Genie<sup>®</sup> 2
- Equipment for sample disruption and homogenization (see "Disruption and homogenization of starting material", page 13). Depending on the method chosen, one of the following is required:
  - O Vortex adapter for 24 (1.5–2 ml) tubes (cat. no. 13000-V1-24)
  - O PowerLyzer® 24 Homogenizer (cat.no. 13155)
  - TissueLyser II (cat. no. 85300) with adapter sets for use with the PowerBead Pro
    Tubes (TissueLyser Adapter Set 2 x 24, cat. no. 69982, and 2ml Tube Holder, cat.
    no. 11993, in conjunction with Plate Adapter Set, cat.no. 11990)

### Important Notes

#### Sample storage and preservation

The yield and integrity of nucleic acids 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. The main components of stool are water (between 65–85%), bacterial cells, undigested food and fiber, bile, and bilirubin (which is derived from dead red blood cells). To a lesser degree, cellular components that have been shed from the walls of the gastrointestinal tract can also be found in stool. Because of the relatively high content of dead and decaying bacterial and human cells, nucleic acids isolated from stool typically appear to have some level of degradation using standard analysis methods.

To optimize the quality of nucleic acids from stool, process the sample as quickly as possible after collection. The PowerProtect DNA/RNA reagent enables stabilizes of stool samples at room temperature. See the relevant handbook for processing recommendations. Freezing the samples at –65 to –90°C will also preserve the quality of nucleic acids. If freezing at ultralow temperatures is not possible, freezing at –20°C is an alternative. Freezing in small aliquots avoids subjecting the bulk sample to freeze–thaw cycles, which can increase the lysis of cells and degradation of nucleic acids. Frozen samples should be processed rapidly by adding phenol–chloroform–isoamyl alcohol and CD1 lysis buffer to the bead tube before the sample has fully thawed. Homogenize immediately to saturate the cellular nucleic acids in the protective lysis buffer. For fresh (non-frozen) samples, rapid homogenization in lysis buffer is especially critical to isolate the highest quality nucleic acids.

#### Disruption and homogenization of starting material

Efficient disruption and homogenization of the staring material is an absolute requirement for all nucleic acid purification procedures.

Most samples do not require homogenization using a high-velocity bead beater. However, if the microorganism of interest requires stronger homogenization than provided by a vortex with the Vortex Adapter for 24 (1.5–2 ml) tubes (cat. no. 13000-V1-24), or if using a bead beater is desired, the RNeasy PowerFecal Pro Kit contains bead tubes suitable for high-powered bead beating and may be used in conjunction with the PowerLyzer 24 Homogenizer (110/220V) (cat. no. 13155) or the TissueLyser II (cat. no. 85300) using a 2 ml Tube Holder Set (cat. no. 11993).

Use of the PowerLyzer 24 instrument (cat.no. 13155) allows the simultaneous disruption of up to 24 samples.

For convenient high-throughput 96-well homogenization, we offer the TissueLyser II (cat. no. 85300) and Plate Adapter Set (cat. no. 11990). In conjunction with PowerBead Pro Plates (cat. no. 19301), the instrument provides high-throughput processing for simultaneous, rapid, and effective disruption of up to 2 x 96 samples in only a few minutes.

# Protocol: Experienced User

#### Important points before starting

- Ensure that the PowerBead Pro Tubes rotate freely in the centrifuge without rubbing.
- Prepare DNase I stock enzyme 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 aliquot and store at -30 to -15°C for long-term storage. Avoid freezing-thawing more than three times. To prepare DNase I Solution, thaw and combine 5 μl DNase I stock enzyme with 45 μl DNase Digestion Solution per prep. DNase I is sensitive to physical denaturation; do not vortex resuspended DNase I.
- Perform all centrifugation steps at room temperature (15–25°C).
- If preparing RNA for the first time, read Appendix: General Remarks on Handling RNA, page 22.

#### Procedure

- 1. Spin the PowerBead Pro Tube briefly to ensure that the beads have settled at the bottom.
- Add 50–100 mg of stool, 650 μl of Solution CD1, and 100 μl phenol–chloroform–isoamyl alcohol (25:24:1, pH 6.5–8.0) or QIAzol Lysis Reagent (cat. no. 79306) to the PowerBead Pro Tube and vortex briefly to mix.

**Note**: If you want to process wastewater, we recommend using 10–40 ml of wastewater centrifuged for 2 h at  $4500 \times g$  and  $4^{\circ}$ C and transferring the pellet into the PowerBead Pro Tube.

 Secure the PowerBead Pro Tube horizontally on a Vortex Adapter for 24 (1.5–2 ml) tube (cat. no. 13000-V1-24). Orient the tube caps to point toward the center of the vortex adapter. Vortex at maximum speed for 10 min. **Note**: If using the vortex adapter for more than 12 preps simultaneously, increase the vortex time by 5-10 min.

For more information about other bead beating methods, see the "Protocol: Detailed" section on page 17.

- 4. Centrifuge the PowerBead Pro Tube at 15,000 x g for 1 min.
- 5. Transfer the supernatant to a clean 2 ml microcentrifuge tube (provided).

**Note**: Expect a volume of  $500-600 \, \mu l$ . The supernatant may still contain some stool particles.

- 6. Add 200  $\mu$ l Solution CD2 and vortex for 5 s. Centrifuge at 15,000 x g for 1 min at room temperature.
- 7. Avoiding the pellet, transfer 300 µl of supernatant to a clean 2 ml microcentrifuge tube (provided).

**Note**: If desired, up to  $650 \, \mu$ l supernatant can be used and mixed with Solution EA in a ratio of 1:1. If the volume of the mixture exceeds  $700 \, \mu$ l, centrifuge successive aliquots in the same MB RNA Spin Column. Discard the flow-through after each centrifugation.

- 8. Add 300  $\mu l$  of Solution EA. Vortex briefly to mix.
- 9. Load 600  $\mu$ l supernatant-EA mix into an MB RNA Spin Column and centrifuge at 15,000  $\times$  g for 1 min. Discard the flow-through.
- 10. Add 650  $\mu$ l Solution EA and centrifuge at 15,000 x g for 1 min.
- 11. Place the MB RNA Spin Column into a clean 2 ml Collection Tube (provided). Add 50 µl DNase I Solution to the center of the Spin Column (prepared by mixing 45 µl DNase Digestion Solution and 5 µl DNase I stock enzyme; see "Important points before starting").

- 12. Incubate at room temperature for 15 min. Add 650  $\mu$ l Solution EA and centrifuge at 15,000 x g for 1 min.
- 13. Discard the flow-through. Add 500  $\mu$ l Solution C5. Centrifuge at 15,000 x g for 1 min.
- 14. Discard the flow-through and place the MB RNA Spin Column into a clean 2 ml Collection Tube (provided). Centrifuge at  $20,000 \times g$  (or full speed) for 1 min.
- 15. Place the MB RNA Spin Column into a clean 1.5 ml Elution Tube (provided).
- 16. Add 100 µl RNase free water to the center of the white filter membrane.
- 17. Incubate at room temperature for at least 1 min.
- 18. Centrifuge at  $15,000 \times g$  for 1 min. Discard the MB RNA Spin Column. The RNA is now ready for any downstream application.

### Protocol: Detailed

#### Procedure

- 1. Spin the PowerBead Pro Tube briefly to ensure that the beads have settled at the bottom.
- Add 50–100 mg of stool, 650 μl of Solution CD1, and 100 μl phenol–chloroform–isoamyl alcohol (25:24:1, pH 6.5–8.0) or QIAzol Lysis Reagent (cat. no. 79306) to the PowerBead Pro Tube and vortex briefly to mix.
- Secure the PowerBead Pro Tube horizontally on a Vortex Adapter for 24 (1.5–2 ml) tube (cat. no. 13000-V1-24). Orient tube caps to point toward the center of the vortex adapter. Vortex at maximum speed for 10 min.

**Note**: If using the vortex adapter for more than 12 preps simultaneously, increase the vortex time by 5–10 min.

#### Optional: Other bead beating methods can also be performed:

3a. Use a PowerLyzer 24 Homogenizer. The PowerBead Pro Tubes must be properly balanced in the tube holder of the PowerLyzer 24 Homogenizer. We recommend homogenizing the stool at 2000 rpm for 30 s, pausing for 30 s, then homogenizing again at 2000 rpm for 30 s.

**Note**: Homogenizing samples at higher speeds (up to 4000 rpm) may increase yields but result in more degraded RNA.

3b. Use a TissueLyser II. Place the PowerBead Pro Tube into the TissueLyser Adapter Set 2 x 24 (cat. no. 69982) or 2 ml Tube Holder (cat. no. 11993) and Plate Adapter Set (cat. no. 11990). Fasten the adapter into the instrument and shake for 5 min at speed 25 Hz. Reorient the adapter so that the side that was closest to the machine body is now furthest from it. Shake again for 5 min at a speed of 25 Hz.

**Note**: Vortexing/shaking is critical for complete homogenization and cell lysis.

Cells are lysed by a combination of chemical agents from step 1 and mechanical shaking introduced at this step. Randomly shaking the beads in the presence of

disruption agents will cause the beads to collide with microbial cells and lead to the cells breaking open.

4. Centrifuge the PowerBead Pro Tube at  $15,000 \times g$  for 1 min.

**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. Transfer the supernatant to a clean 2 ml microcentrifuge tube (provided).

**Note**: Expect a volume of  $500\text{--}600~\mu l$ . The supernatant may still contain some stool particles.

6. Add 200  $\mu$ l Solution CD2 and vortex for 5 s. Centrifuge at 15,000 x g for 1 min at room temperature.

**Note**: Solution CD2 is the Inhibitor Removal Solution that completes the IRT process and removes the contaminants from the sample that would cause problems with PCR and other downstream applications.

7. Avoiding the pellet, transfer 300 µl of supernatant to a clean 2 ml microcentrifuge tube (provided).

**Note**: If desired, up to 650  $\mu$ l supernatant can be used and mixed with Solution EA in a ratio of 1:1. If the volume of the mixture exceeds 700  $\mu$ l, centrifuge successive aliquots in the same MB RNA Spin Column. Discard the flow-through after each centrifugation.

- 8. Add 300 µl of Solution EA. Vortex briefly to mix.
- Load 600 µl supernatant-EA mix into an MB RNA Spin Column and centrifuge at 15,000 x g for 1 min. Discard the flow-through.
- 10. Add 650  $\mu$ l Solution EA, and centrifuge at 15,000 x g for 1 min.

- 11. Place the MB RNA Spin Column into a clean 2 ml Collection Tube (provided). Add 50 µl DNase I Solution to the center of the Spin Column (prepared by mixing 45 µl DNase Digestion Solution and 5 µl DNase I stock enzyme; see "Important points before starting").
- 12. Incubate at room temperature for 15 min. Add 650  $\mu$ l Solution EA and centrifuge at 15,000 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 EA inactivates the DNase enzyme and removes it from the column membrane along with digested DNA.

- 13. Discard the flow-through. Add 500  $\mu$ l Solution C5. Centrifuge at 15,000 x g for 1 min.
- 14. Discard the flow-through and place the MB RNA Spin Column into a clean 2 ml Collection Tube (provided). Centrifuge at 20,000 x g (or full speed) for 1 min.
  Note: The final dry spin ensures all ethanol is cleared from the membrane so that the elution will be efficient.
- 15. Place the MB RNA Spin Column into a clean 1.5 ml Elution Tube (provided).
- 16. Add 100 µl RNase-free water to the center of the white filter membrane.
  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.
- 17. Incubate at room temperature for at least 1 min.
- 18. Centrifuge at  $15,000 \times g$  for 1 min. Discard the MB RNA Spin Column. The RNA is now ready for any downstream application.

# 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

D		

a) RNA degradation

The optimal method for storing stool samples prior to RNA isolation is to freeze at -90 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.

The use of phenol–chloroform–isoamyl alcohol (25:24:1) buffered to pH 6.7–8.0 assists in protecting the integrity of the RNA during homogenization of the sample. Add 100 µl phenol–chloroform–isoamyl alcohol to the PowerBead Pro Tube 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 CD2 to 300  $\mu$ l to remove more inhibitors. Alternatively, use less sample if the purity is not improved with the addition of more Solution CD2.

c) Total NA isolation

If total nucleic acid is to be isolated in one eluate, the DNase digestion step can be omitted.

#### Stool processing

a) Amount of stool to process

The RNeasy PowerFecal Pro Kit is designed to process up to 0.2 g of stool. For inquiries regarding the use of larger sample amounts, please contact Technical Support for suggestions.

#### Comments and suggestions

b)	Stool sample is high in water content	Remove contents from the PowerBead Pro Tube (beads) and transfer into another sterile microcentrifuge tube (not provided). Add stool/soil sample to PowerBead Pro Tube and centrifuge at room temperature for 30 s at $10,000 \times g$ . Remove as much liquid as possible with a pipette tip. Add beads back to PowerBead Pro Tube.
c)	Stabilized stool samples	If using stool samples preserved in PowerProtect DNA/RNA, refer to the <i>PowerProtect DNA/RNA Handbook</i> .
Alter	native lysis methods <u>and othe</u>	er sample types
a)	Cells are difficult to lyse	After adding Solution CD1 and prior to the bead-beating step, incubate at 65°C for 10 min. Resume protocol from step 2.
b)	Wastewater processing	For wastewater processing, we recommend using 10–40 ml of wastewater centrifuged for 2 h at 4500 x $g$ and 4°C and transferring the pellet into the PowerBead Pro Tube and follow the instructions in the protocol.
c)	Sample type	The RNeasy PowerFecal Pro Kit is recommended for sludge, pelleted samples derived after ultracentrifugation or other concentrated samples.

# Appendix: 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 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 non-disposable 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, which 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.

#### 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.

#### Non-disposable plasticware

Non-disposable plasticware should be treated before use to ensure that it is RNase-free. Plasticware should be thoroughly rinsed with 0.1 M NaOH\*, 1 mM EDTA\* followed by RNase-free water. Alternatively, chloroform-resistant plasticware can be rinsed with chloroform\* to inactivate RNases.

#### 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 RNases. Alternatively, glassware can be treated with DEPC\* (diethyl pyrocarbonate). Fill glassware with 0.1% DEPC\* (0.1% in water), allow to stand overnight (12 hours) at 37°C, and then autoclave or heat to 100°C for 15 minutes to eliminate residual DEPC.

#### Electrophoresis tanks

Electrophoresis tanks should be cleaned with detergent solution (e.g., 0.5% SDS\*), thoroughly rinsed with RNase-free water, and then rinsed with ethanol<sup>†</sup> and allow to dry.

<sup>\*</sup> 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.

<sup>&</sup>lt;sup>†</sup> Plastics used for some electrophoresis tanks are not resistant to ethanol. Take proper care and check the supplier's instructions.

#### 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<sub>2</sub>. 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.

<sup>\*</sup> 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 PowerFecal Pro Kit (50)	For the isolation of microbial RNA from stool and gut material	78404
Related Products		
PowerProtect DNA/RNA (500 ml)	For the stabilization of the microbial community profile and expression profile in stool samples	14800
PowerProtect DNA/RNA (1000 ml)	For the stabilization of the microbial community profile and expression profile in stool samples	14810
QIAzol Lysis Reagent (200 ml)	For RNA protection	79306
PowerBead Pro Tubes (2 ml) (50)	For disruption of stool and all soil samples in 2ml format	19301
TissueLyser II	Bead mill (100–120/220–240 V, 50/60 Hz) for medium- to high-throughput sample disruption for molecular analysis; requires the TissueLyser Adapter Set 2 x 24 or 2 ml Tube Holder Set	85300
TissueLyser Adapter Set 2 x 24	Two sets of adapter plates and 2 racks for use with 2 ml microcentrifuge tubes on the TissueLyser II	69982
2ml Tube Holder Set	For sample homogenization in 2 ml bead tubes on the TissueLyser II	11993

Vortex Adapter for 24 (1.5–2.0 ml) tubes	For vortexing 1.5 ml or 2 ml tubes using the Vortex-Genie 2 Vortex	13000-V1-24
PowerLyzer 24 Homogenizer (110/220 V)	For complete lysis and homogenization of any biological sample	13155
QIAcube Connect — for fully autom QIAGEN spin-column kits	nated nucleic acid extraction with	
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

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.

<sup>\*</sup> 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.

# Document Revision History

Date	Changes
04/2022	Initial release.

Notes

Notes

Notes

#### Limited License Agreement for RNeasy PowerFecal Pro 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.
- 3. This kit and its components are licensed for one-time use and may not be reused, refurbished, or resold.
- 4. QIAGEN specifically disclaims any other licenses, expressed or implied other than those expressly stated.
- 5. The purchaser and user of the kit agree not to take or permit anyone else to take any steps that could lead to or facilitate any acts prohibited above. QIAGEN may enforce the prohibitions of this Limited License Agreement in any Court, and shall recover all its investigative and Court costs, including attorney fees, in any action to enforce this Limited License Agreement or any of its intellectual property rights relating to the kit and/or its components.

For updated license terms, see www.qiagen.com.

Trademarks: QIAGEN®, Sample to Insighi®, QIAcube®, QIAzol®, DNase Max®, RNeasy®, Powerfecal®, Powerlyzer®, PowerMicrobiome®, DNeasy®, Inhibitor Removal Technology®, Powerlyzer® (QIAGEN Group); Yorkex-Genie® (Scientific Industries, Inc.). Registered names, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law.

04/2022 HB-2918-001 © 2022 QIAGEN, all rights reserved.

