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AllPrep® PowerFecal® Pro DNA/RNA Kit Handbook

For simultaneous purification of microbial DNA and RNA from the same stool sample

Sample to Insight

QIAGEN

Contents

Kit Contents

AllPrep PowerFecal Pro DNA/RNA Kit	**(50)**
Catalog no.	**80254**
Number of preps	**50**
PowerBead Pro Tubes	50
Solution CD1	40 ml
Solution CD2	15 ml
Solution CD3	35 ml
MB Spin Columns	50
MB RNA Spin Columns	50
Collection Tubes 2 ml	150
Solution EA	72 ml
Solution C5	60 ml
RNase free water	14 ml
Microcentrifuge Tubes, 2 ml	100
Elution Tubes, 1.5 ml	100
Quick-Start Protocol	1

Shipping and Storage

Solution CD2 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, the AllPrep PowerFecal Pro DNA/RNA Kit is good until the expiration date printed on kit box lid.

Intended Use

The AllPrep PowerFecal Pro DNA/RNA Kit is intended for the simultaneous purification of microbial DNA and RNA from the same stool sample. The AllPrep PowerFecal Pro DNA/RNA Kit is intended for research use only. This product is not intended for the diagnosis, prevention, or treatment of a disease.

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

Safety Information

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

WARNING 	Solution EA and Solution C5 are flammable.

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

Solutions CD1, CD3, and 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 AllPrep PowerFecal Pro DNA/RNA Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

The AllPrep PowerFecal Pro DNA/RNA Kit comprises a novel and proprietary method for isolating both DNA and RNA from the same stool or gut sample. 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 DNA and RNA that can be used immediately in downstream applications, including PCR, RT-PCR, qPCR, and next-generation sequencing (NGS) (16S, metagenome, and metatranscriptome).

Principle and procedure

The AllPrep PowerFecal Pro DNA/RNA Kit is designed to purify microbial DNA and RNA simultaneously from the same stool sample, while separating the DNA and RNA into separate eluate fractions. The AllPrep PowerFecal Pro DNA/RNA Kit integrates QIAGEN's technology for selective binding of double-stranded DNA with well-established RNeasy® technology in addition to incorporating IRT during initial sample lysis.

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 an MB Spin Column. This column, in combination with a high-salt buffer, allows selective and efficient binding of genomic DNA. The DNA is then washed and eluted and is ready for PCR analysis and other downstream applications, including qPCR and NGS.

Ethanol is added to the flow-through from the MB Spin Column to provide appropriate binding conditions for RNA. The sample is then transferred to an MB RNA Spin Column, where total RNA binds to the membrane. The RNA is then washed and eluted in RNase-free water ready for downstream applications including RT-PCR, qPCR and NGS.

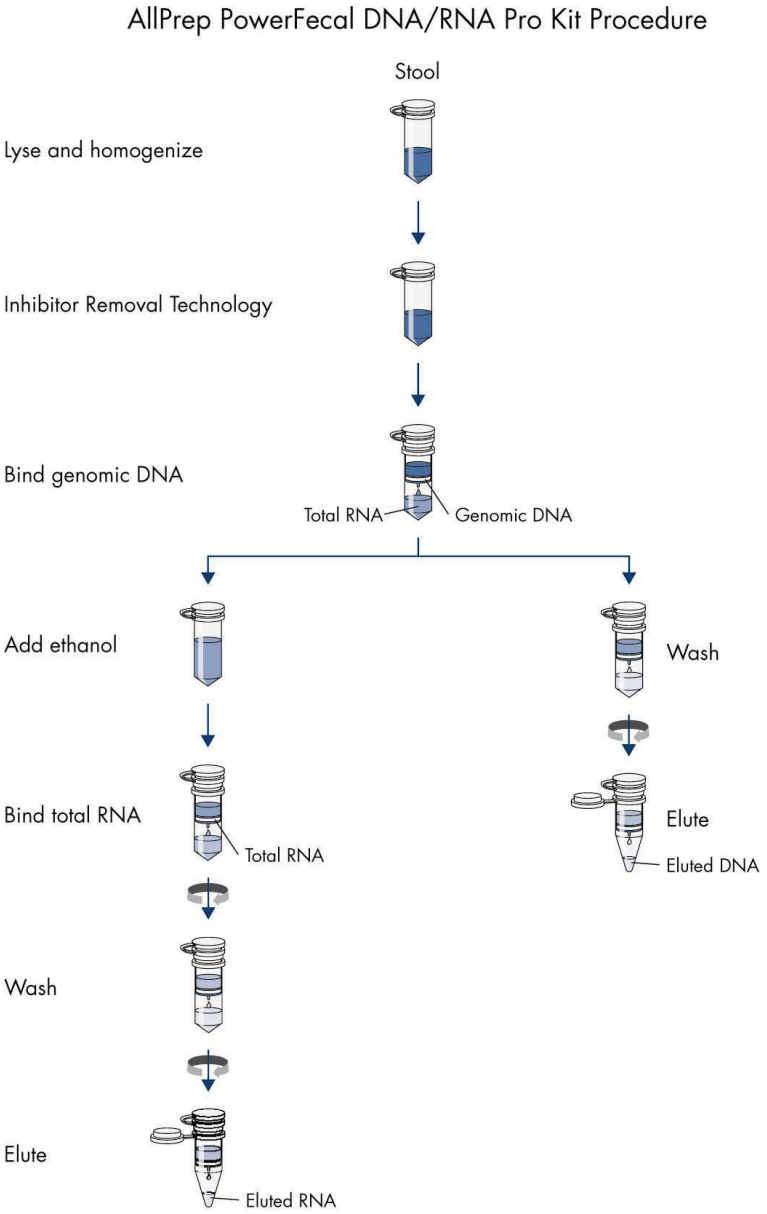

Figure 1. AllPrep PowerFecal Pro DNA/RNA procedure.

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)
- Ethanol (90–100%)*
- 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® 2
- Equipment for sample disruption and homogenization (see "Disruption and homogenization of starting material", page 11). Depending on the method chosen, one of the following is required:
    - Vortex Adapter for 24 (1.5–2 ml) tubes (cat. no. 13000-V1-24)
    - 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 2 ml Tube Holder, cat. no. 11993, in conjunction with Plate Adapter Set, cat. no. 11990)

* Do not use denatured alcohol, which contains other substances, such as methanol or methylethylketone.

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 (from 65 to 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 in order 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 RNA 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 Power Bead 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.
- If Solution CD3 has precipitated, heat at 60°C until precipitate dissolves.
- 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

Sample pretreatment

1. Spin the PowerBead Pro Tube briefly to ensure that the beads have settled at the bottom.

2. 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) to the PowerBead Pro Tube and vortex briefly to mix.

3. 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.

   For more information about other bead beating methods, see the "Protocol: Detailed" section on page 15.

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

Note: Expect a supernatant volume of 500–600 µl. The supernatant may still contain some stool particles.

5. Add 200 µl Solution CD2 and vortex for 5 s. Centrifuge at 15,000 x *g* for 1 min at room temperature.

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

   Note: It is feasible to use higher supernatant volumes, please refer to the "Protocol: Detailed" section on page 15 for detailed information.

7. Add 300 µl of Solution CD3. Vortex briefly to mix.

DNA binding

8. Load 600 µl supernatant–CD3 mix into a MB DNA Spin Column (white) and centrifuge at 15,000 x *g* for 1 min collecting the flow-through in a 2 ml tube for RNA purification, then place the spin column in a new 2 ml collection tube.

RNA binding

9. Add 300 µl 96–100% ethanol to the flow-through from step 8 and mix by pipetting up and down.

   Note: If you used a higher volume of the supernatant in step 6, you would have to adjust the binding conditions according to the instructions in the "Protocol: Detailed" section on page 15.

10. Transfer up to 700 µl of the mix to a MB RNA Spin Column (pink) placed in a 2 ml collection tube. Centrifuge at 15,000 x *g* for 1 min. Discard the flow-through.

    Note: If the volume of the mixture exceeds 700 µl, centrifuge successive aliquots in the same MB RNA Spin Column. Discard the flow-through after each centrifugation.

DNA and RNA washing and elution

11. Add 650 µl Solution EA to an MB DNA Spin Column (white) and an MB RNA Spin Column (pink) and centrifuge at 15,000 x *g* for 1 min. Discard the flow-through.

12. Add 500 µl Solution C5. Centrifuge at 15,000 x *g* for 1 min.

13. Discard the flow-through and place the MB RNA and DNA Spin Columns into clean 2 ml collection tubes (provided). Centrifuge at 20,000 x *g* (or full speed) for 1 min.

14. Place the MB RNA and DNA Spin Columns into clean 1.5 ml Elution Tubes (provided).

15. Add 100 µl RNase-free water to the center of the white filter membrane.

16. Incubate at room temperature for at least 1 min.

17. Centrifuge at 15,000 x *g* for 1 min. Discard the MB RNA and DNA Spin Columns. The RNA and DNA are now ready for any downstream applications.

Protocol: Detailed

Procedure

Sample pretreatment

1. Spin the PowerBead Pro Tube briefly to ensure that the beads have settled at the bottom.

2. 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) to the PowerBead Pro Tube and vortex briefly to mix.

3. 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.

   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 x *g* for 1 min. Transfer the supernatant to a clean 2 ml microcentrifuge tube (provided).

   Note: Expect a supernatant volume of 500–600 µl. The supernatant may still contain some stool particles.

   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 DNA and RNA from both human and bacterial cells.

5. Add 200 µl 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, which completes the IRT process and removes the contaminants from the sample that would cause problems with PCR and other downstream applications.

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

   Note: Expect a total volume of approximately 400–500 µl supernatant at this step.

   Optional: Users who want to maximize nucleic acid yield may transfer up to 450 µl supernatant at this step. However, volumes greater than 300 µl will require additional pipetting at protocol steps 7–10, increasing the overall sample preparation time and reducing ease of handling.

7. Add 300 µl of Solution CD3. Vortex briefly to mix.

Optional: If more than 300 µl supernatant was transferred at step 6, add 1 volume of the Solution CD3 to the supernatant and vortex briefly to mix.

DNA binding

8. Load 600 µl supernatant–CD3 mix into a MB DNA Spin Column (white) and centrifuge at 15,000 x *g* for 1 min collecting the flow-through in a 2 ml tube for RNA purification, then place the spin column in a new 2 ml collection tube.

   Optional: If more than 300 µl supernatant was transferred at step 6, the MB DNA Spin Column must be loaded twice. First, transfer 600 µl of the mix to the column and centrifuge at 15,000 x *g* for 1 min. Transfer the flow-through to a clean 2 ml tube; it will be used for RNA purification. Transfer the remaining mix to the MB DNA Spin Column and centrifuge at 15,000 x *g* for 1min. Place the MB DNA Spin Column in a clean 2 ml collection tube and store at room temperature for DNA purification in steps 11–17. Transfer the second flow-through to the 2 ml collection tube containing the first flow-through for RNA purification.

RNA binding

9. Add 300 µl 96–100% ethanol to the flow-through from step 8 and mix by pipetting up and down.

   Optional: If you used a higher volume of the supernatant in step 6, add 0.5 volumes of 96–100% ethanol to the flow-through from step 8 and mix by pipetting up and down.

10. Transfer up to 700 µl of the mix to an MB RNA Spin Column (pink) placed in a 2 ml collection tube. Centrifuge at 15,000 x *g* for 1 min. Discard the flow-through.

    Note: If the volume of the mixture exceeds 700 µl, centrifuge successive aliquots in the same MB RNA Spin Column. Discard the flow-through after each centrifugation.

DNA and RNA washing and elution

11. Add 650 µl Solution EA to an MB DNA Spin Column (white) and an MB RNA Spin Column (pink) and centrifuge at 15,000 x *g* for 1 min. Discard the flow-through.

12. Add 500 µl Solution C5. Centrifuge at 15,000 x *g* for 1 min.

13. Discard the flow-through and place the MB RNA and DNA Spin Columns into clean 2 ml collection tubes (provided). Centrifuge at 20,000 x *g* (or full speed) for 1 min.

    Note: Perform this step to eliminate any possible carryover of Buffer C5 or if residual flow-through remains on the outside of the Spin Column after step 12.

14. Place the MB RNA and DNA Spin Columns into clean 1.5 ml Elution Tubes (provided).

15. Add 100 µl RNase-free water to the center of the white filter membrane.

    Note: Eluting with 100 µl RNase-free water will maximize DNA/RNA yield. For more concentrated DNA/RNA, a minimum of 50 µl RNase-free water can be used.

16. Incubate at room temperature for at least 1 min.

17. Centrifuge at 15,000 x *g* for 1 min. Discard the MB RNA and DNA Spin Columns. The RNA and DNA are now ready for any downstream applications.

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
Low nucleic acid yield		
a)	Sample stored under unfavorable conditions	Samples should be stored at –65 to –90°C.
b)	Insufficient disruption and homogenization	Repeat the DNA purification procedure with a new sample. Be sure to mix the sample, PCI and Solution CD1 until the sample is thoroughly homogenized.
c)	Too much starting material	Overloading the spin columns significantly reduces nucleic acid yields. Reduce the amount of starting material.
d)	RNA still bound to MB RNA Spin Column membrane	Repeat RNA elution, but incubate the MB RNA Spin Column on the benchtop for 10 min with RNase-Free Water before centrifuging.
e)	DNA still bound to MB Spin Column membrane	Repeat DNA elution, but incubate the MB Spin Column on the benchtop for 10 minutes with RNase-Free Water before centrifuging. Alternatively, heat RNase-Free Water to 70°C prior to DNA elution.
f)	Solution C5 carryover	During the second wash with Solution C5, be sure to centrifuge at 20,000 x *g* (or full speed) for 1 min at 15–25°C to dry the spin column membrane. Perform the optional centrifugation to dry the Spin Column membrane if any flow-through is present on the outside of the column.
Stool Processing		
a)	Amount of stool to process	The AllPrep PowerFecal Pro DNA/RNA 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 x *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 *PowerProtect DNA/RNA Handbook*.
DNA/RNA		
a)	Inefficient removal of inhibitory substances	In most cases, no inhibition will be observed when amplifying nucleic acids purified using the AllPrep PowerFecal Pro DNA/RNA Kit. However, if the template nucleic acids do not perform well in downstream applications (which may occur with extreme sample types), dilution of the template (suggested dilution 1:10) prior to downstream processing should be attempted. If dilution is not successful in recovering the sample, repeat the nucleic acid purification procedure with a new sample, increase the amount of Solution CD2 to 300 µl to remove more inhibitors. Alternatively, use less sample if the purity is not improved with the addition of more Solution CD2. Take care to not transfer any of the pellet after Solution CD2 treatment.
b)	Storing DNA/RNA	DNA and RNA is eluted in RNase-free Water and must be stored at –30 to –15°C or –90 to –65°C to prevent degradation.
DNA fragmented		
a)	Sample stored under unfavorable conditions	Samples should be stored at –65 to –90°C.
b)	Homogenization was too vigorous	The length of the purified DNA depends strongly on the homogenization conditions. If longer DNA fragments are required, keep the homogenization time to a minimum or use a gentler homogenization method if possible (e.g., use a vortex adapter instead of a TissueLyser system).
RNA degraded		
a)	Sample stored under unfavorable conditions	Samples should be stored at –65 to –90°C.
b)	RNase contamination	RNases are ubiquitous and stable laboratory contaminants and can be potentially introduced to a sample during use. Be certain not to introduce any RNases during the AllPrep PowerFecal Pro DNA/RNA procedure or later handling (see Appendix: General Remarks on Handling RNA, page 22, for guidelines on handling RNA).

		Comments and suggestions
Alternative lysis methods		
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)	Reduction of shearing of DNA	After adding Solution CD1, vortex for 3-4 seconds, then heat to 70°C for 5 min. Repeat once. This alternative procedure will reduce shearing but may also reduce yield.

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† and allow to dry.

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

† 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_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.

* 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.
AllPrep PowerFecal Pro DNA/RNA Kit (50)	For the isolation of total DNA and RNA from stool and gut material	80254
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
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 two racks for use with 2 x 24 samples in 2 ml tubes	69982
2 ml Tube Holder Set	For homogenization of up to 2 x 48 samples 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

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
04/2022	Initial release.

Notes

Notes

Notes

Limited License Agreement for AllPrep PowerFecal Pro DNA/RNA 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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