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AllPrep® PowerFecal® 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 DNA/RNA Kit	**(50)**
Catalog no.	**80244**
Number of preps	**50**
AllPrep DNA MinElute® Spin Columns	50
RNeasy® Mini Spin Columns	50
Microbial Lysis Tubes	50
Collection Tubes (1.5 ml)	150
Collection Tubes (2 ml)	300
Solution PM1	55 ml
Solution IRS	15 ml
Solution C4	30 ml
Buffer RW1	45 ml
Buffer RPE	11 ml
Buffer AW1	19 ml
Buffer AW2	13 ml
RNase-Free Water	10 ml
Buffer EB	15 ml
RNase A Solution	0.25 ml
Quick-Start Protocol	1

Storage

Upon arrival, open the kit and store AllPrep DNA MinElute Spin Columns at 2–8°C. The remaining kit components can be stored at room temperature (15–25°C). Under these conditions, the AllPrep PowerFecal DNA/RNA Kit can be stored up to 12 months without showing any reduction in performance and quality.

Intended Use

The AllPrep PowerFecal DNA/RNA Kit is intended for the simultaneous purification of microbial DNA and RNA from the same stool sample. The AllPrep PowerFecal 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.

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

Buffer AW1 and Buffer RW1 contain guanidine 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 AllPrep PowerFecal DNA/RNA Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

The AllPrep PowerFecal DNA/RNA Kit is designed for efficient sequential purification of microbial DNA and RNA from stool and feces. The AllPrep PowerFecal DNA/RNA Kit utilizes Inhibitor Removal Technology® (IRT), also found in other kits, such as the RNeasy PowerMicrobiome Kit. IRT is very effective at removing inhibitory substances commonly found in stool, such as polysaccharides, heme compounds and bile salts. The result is high-purity DNA and RNA that is ready to use in the most demanding downstream applications.

Principle and procedure

The AllPrep PowerFecal 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 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 0.1–0.2 g 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 AllPrep DNA MinElute Spin Column. This column, in combination with a high-salt buffer, allows selective and efficient binding of genomic DNA. DNA is then washed and eluted, and is ready for PCR analysis and other downstream applications, including qPCR and next-generation sequencing (NGS).

Ethanol is added to the flow-through from the AllPrep DNA MinElute Spin Column to provide appropriate binding conditions for RNA. The sample is then transferred on an RNeasy Mini Spin Column, where total RNA binds to the membrane. RNA is then washed and eluted in RNase-Free Water ready for downstream applications including PCR, qPCR and NGS.

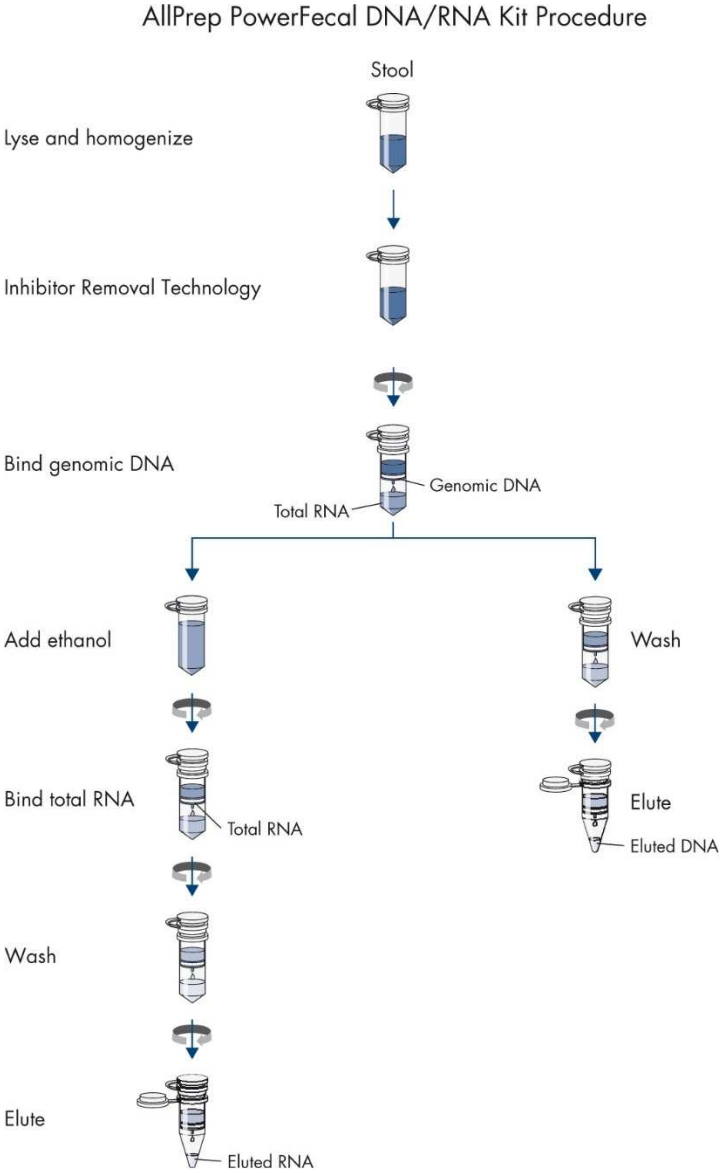

Figure 1. AllPrep PowerFecal DNA/RNA procedure.

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.

- Ethanol (96–100%)*
- Dithiothreitol (DTT), 1 M
- Pipette tips (pipette tips with aerosol barriers for preventing cross-contamination are recommended)
- Disposable gloves
- Heating block or water bath
- Refrigerator or ice bath at 2–8°C
- Microcentrifuge (with rotor for 1.5 and 2 ml tubes)
- Vortexer
- Equipment for sample disruption and homogenization (see “Disruption and homogenization of starting material”, page 10). Depending on the method chosen, one of the following is required:
  - Vortex Adapter for vortexing 1.7 or 2 ml tubes (cat. no. 13000-V1-24)
  - TissueLyser II (cat. no. 85300)
  - PowerLyzer® 24 Homogenizer (cat. no. 13155)
  - FastPrep®-24 instrument (MP Biomedicals cat no. 116004500)

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

Important Notes

Preparation of reagents

Buffers AW1, AW2 and RPE

Buffer AW1, Buffer AW2 and Buffer RPE are each supplied as a concentrate. Before using for the first time, add the appropriate amount of ethanol (96–100%) as indicated on the bottle.

Buffer AW1, Buffer AW2 and Buffer RPE are stable for 1 year when stored closed at room temperature (15–25°C).

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 at –65 to –90°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 (about 8%). 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 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. 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 nucleic acids from stool, process the sample as quickly as possible after collection. It may be preferable to freeze the samples at –65 to –90°C in small aliquots to avoid freeze/thaw cycles of the bulk sample, which can increase the lysis of cells and degradation of nucleic acids. Samples should be processed rapidly by adding DDT and PM1 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. Disruption and homogenization are two distinct steps:

- Disruption: Complete disruption of plasma membranes and cell walls of microbial cells is absolutely required to release all the nucleic acids contained in the sample. Different samples require different methods to achieve complete disruption. Incomplete disruption results in significantly reduced nucleic acid yields.
- Homogenization: Homogenization is necessary to reduce the viscosity of the lysates produced by disruption. Homogenization shears high-molecular-weight cellular components to create a homogeneous lysate. Incomplete homogenization results in inefficient binding of DNA and RNA and therefore significantly reduced yield and purity of nucleic acids. Excessive homogenization, on the other hand, results in shorter genomic DNA fragments.

Disruption and homogenization using the TissueLyser system

In bead milling, samples can be disrupted by rapid agitation in the presence of beads and lysis buffer. Disruption and simultaneous homogenization occur by the shearing and crushing action of the beads as they collide with the cells. Disruption efficiency is influenced by:

- Size and composition of beads
- Ratio of buffer to beads
- Amount of starting material
- Speed and configuration of the TissueLyser
- Disintegration time

Disruption parameters must be determined empirically for each application. For guidelines on disruption and homogenization of stool using the TissueLyser system, refer to the TissueLyser Handbook. For other bead mills, please refer to suppliers' guidelines for further details.

Protocol: Simultaneous Purification of Microbial DNA and RNA from the Same Stool Sample

Notes before starting

- Warm Solution PM1 to use at 55°C for 10 min. Use Solution PM1 while still warm.
- Buffers AW1, AW2 and RPE are supplied as concentrates. Add the appropriate amounts of ethanol (96–100%) as indicated on the bottles (see Important Notes, page 10). Mix well after adding ethanol.
- Prepare 80% ethanol in water.
- For each prep, place a RNeasy Mini Spin Column in a Collection Tube (2 ml).
- If preparing RNA for the first time, read Appendix B: General Remarks on Handling RNA, page 20.
- Perform all steps of the procedure at room temperature. During the procedure, work quickly.

Procedure

1. Place 100–200 mg of stool into the Microbial Lysis Tube (provided).
   Note: This protocol is optimized for use with 100–200 mg stool. If the sample is liquid, pipet up to 200 µl sample into the Microbial Lysis Tube. Cut the end of the pipette to make pipetting easier. If the sample is frozen, use a scalpel or spatula to scrape frozen stool into a Microbial Lysis Tube on ice. Obtaining precise aliquots of stool samples is difficult, thus some variance in starting material is expected. For example, if trying to obtain a 200 mg stool sample, samples of 180–220 mg are considered within normal variance and this does not adversely affect results.
2. Add 650 µl Solution PM1 and 25 µl DTT (not supplied) to the lysis tube. Tightly cap the lid.
   Note: Prior to use, Solution PM1 must be warmed at 55°C for 10 min to dissolve precipitates (see Notes before starting, above). Use Solution PM1 while still warm.

3. Lyse bacterial cells. The following three options have been verified as compatible with the AllPrep PowerFecal DNA/RNA Kit.

   A) Place the bead tube in a TissueLyser II for 2 min 30 s at 30 Hz. Incubate the homogenate at room temperature for 5 min. Lyse again for 2 min 30 s at 30 Hz.

   B) Place the bead tube horizontally on a vortexer using a Vortex Adapter. Vortex at maximum speed for 5 min.

   C) Place the bead tube into a FastPrep-24 instrument for 45 s at a velocity of 6 m/s. Incubate the homogenate at room temperature for 5 min. Lyse again for 45 sec at 6 m/s.

4. Centrifuge at ≥18,000 x *g* for 1 min at room temperature.
5. Avoiding the deposit, transfer the supernatant to a clean 1.5 ml Collection Tube.
   Note: Expect a total volume of ~400–500 µl supernatant at this step.
6. Add 150 µl Solution IRS to the transferred supernatant, close the lid gently and vortex briefly to mix. Incubate at 2–8°C for 5 min.
7. Centrifuge at ≥13,000 x *g* for 1 min at room temperature.
8. Avoiding the pellet, transfer 300 µl of the supernatant to a clean 2 ml Collection Tube.
   Note: Expect a total volume of ~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 10–12, increasing the overall sample preparation time and reducing ease of handling.
9. Shake to mix Solution C4 before use. Add 400 µl Solution C4 to the transferred supernatant and mix well by pipetting. Do not centrifuge.
   Note: If more than 300 µl supernatant was transferred at step 8, add 1.4 volumes of Solution C4 to the supernatant and mix well by pipetting.
10. Transfer the 700 µl mix to an AllPrep DNA MinElute Spin Column placed in a 2 ml Collection Tube. Centrifuge at ≥8000 x *g* for 30 s at room temperature. After centrifugation, place the AllPrep DNA MinElute Spin Column in a new 2 ml Collection Tube and store it in a refrigerator at 2–8°C for DNA purification in steps 19–23. Use the flow-through for RNA purification in steps 11–18. Proceed to step 11.

Note: Verify that no liquid remains on top of the membrane. If the mixture has not completely passed through the AllPrep DNA MinElute Spin Column membrane, centrifuge the spin column again at ≥13,000 x *g* for 1 min.

Optional: If more than 300 µl supernatant was transferred at step 8, the AllPrep DNA MinElute Spin Column must be loaded twice. First, transfer 700 µl of the mix to the AllPrep DNA MinElute Spin Column and centrifuge at ≥8000 x *g* for 30 s at room temperature. After centrifugation, place the DNA AllPrep Spin Column in a clean 2 ml Collection Tube. Transfer the remaining mix to the AllPrep DNA MinElute Spin Column and centrifuge at ≥8000 x *g* for 30 s at room temperature. Place the AllPrep DNA MinElute Spin Column in a clean 2 ml Collection Tube and store in a refrigerator at 2–8°C for DNA purification in steps 19–23. Use the two flow-through for RNA purification in steps 11–18. Proceed to step 11.

Total RNA purification

11. Add 700 µl 80% ethanol to the flow-through from step 10, and mix well by pipetting. Do not centrifuge.

    Note: Precipitates may be visible after addition of ethanol. This does not affect the procedure.

    Optional: If more than 300 µl supernatant was transferred at step 8, add 1 volume of 80% ethanol to each of the two flow-throughs. Mix well by pipetting.

12. Transfer up to 700 µl of the mix to an RNeasy Mini Spin Column placed in a 2 ml Collection Tube. Close the lid gently, and centrifuge at ≥8000 x *g* for 30 s. Discard the flow-through. Centrifuge successive aliquots in the same RNeasy Mini Spin Column. Discard flow-through after each centrifugation. Reuse the 2 ml Collection Tube in step 13.

13. Add 350 µl Buffer RW1 to the RNeasy Mini Spin Column. Close the lid gently, and centrifuge at >8000 x *g* for 30 s. Discard the flow-through. Reuse the 2 ml Collection Tube in step 14.

    Optional: If performing an optional on-column DNase digestion (see Appendix A: Optional On-Column DNase Digestion with the RNase-Free DNase Set, page 18), follow steps 1–4 instead of step 13.

14. Add 500 µl Buffer RPE to the RNeasy Mini Spin Column. Close the lid gently, and centrifuge at ≥8000 x *g* for 30 s. Discard the flow-through. Reuse the 2 ml Collection Tube in step 15.
    Note: Buffer RPE is supplied as a concentrate. Ensure that ethanol is added to Buffer RPE before use (see Important Notes, page 9).
15. Add 500 µl Buffer RPE to the RNeasy Mini Spin Column. Close the lid gently and centrifuge at >18,000 x *g* for 2 min. Discard the Collection Tube and the flow-through.
16. Place the RNeasy Mini Spin Column in a clean 2 ml Collection Tube. Close the lid gently and centrifuge at ≥18,000 x *g* for 1 min.
    Note: Perform this step to eliminate any possible carryover of Buffer RPE or if residual flow-through remains on the outside of the RNeasy Spin Column after step 15.
17. Place the RNeasy Mini Spin Column in a clean 1.5 ml Collection Tube. Add 30 µl RNase-Free Water directly to the Spin Column membrane. Close the lid gently, and centrifuge at ≥8000 x *g* for 1 min.
18. Repeat step 17 for increased RNA yield.

Genomic DNA purification

19. Add 500 µl Buffer AW1 to the AllPrep DNA MinElute Spin Column from step 10. Close the lid gently, and centrifuge at ≥8000 x *g* for 1 min. Discard the flow-through. Reuse the 2 ml Collection Tube in step 20.
    Note: Verify that no liquid remains on top of the membrane. If the mixture has not completely passed through the AllPrep DNA MinElute Spin Column membrane, centrifuge again at ≥13,000 x *g* for 1 min.
    Note: Buffer AW1 is supplied as a concentrate. Ensure that ethanol is added to Buffer AW1 before use (see Important Notes, page 9).
    Optional: If RNA-free genomic DNA is required, add 500 µl Buffer AW1 and 4 µl RNase A (25 mg/ml) to the AllPrep DNA MinElute Spin Column. Incubate for 2 min at room temperature before continuing with step 20.
20. Add 500 µl Buffer AW2 to the AllPrep DNA MinElute Spin Column. Close the lid gently and centrifuge at ≥18,000 x *g* for 2 min.

Note: Buffer AW2 is supplied as a concentrate. Ensure that ethanol is added to Buffer AW2 before use (see "Important Notes", page 9).

21. Place the AllPrep DNA MinElute Spin Column in a clean 2 ml Collection Tube and discard the old Collection Tube with the flow-through. Close the lid gently, and centrifuge at ≥18,000 x *g* for 1 min.
    Note: Perform this step to eliminate any possible carryover of Buffer AW2 or if residual flow-through remains on the outside of the AllPrep DNA MinElute Spin Column after step 20.
22. Place the AllPrep DNA MinElute Spin Column in a clean 1.5 ml Collection Tube. Add 30 µl Buffer EB directly to the spin column membrane, and close the lid gently. Incubate at room temperature for 1 min, then centrifuge at ≥8000 x *g* for 1 min.
23. Repeat step 22 for increased DNA yield.

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
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 and Solution PM1 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 RNeasy Spin Column membrane	Repeat RNA elution, but incubate the RNeasy Spin Column on the benchtop for 10 minutes with RNase-Free Water before centrifuging.
e)	DNA still bound to AllPrep DNA MinElute Spin Column membrane	Repeat DNA elution, but incubate the AllPrep DNA MinElute Spin Column on the benchtop for 10 minutes with Buffer EB before centrifuging. Alternatively, heat Buffer EB to 70°C prior to DNA elution.
f)	Ethanol carryover	During the second wash with Buffers RPE/AW2, be sure to centrifuge at ≥8000 x *g* for 2 minutes at 15–25°C to dry the spin column membrane. Perform the optional centrifugation to dry the RNeasy Spin Column membrane if any flow-through is present on the outside of the column.
Low A_{260}/A_{280} ratio for purified nucleic acids		
a)	Inefficient removal of inhibitory substances due to insufficient mixing with Solution IRS	Repeat the nucleic acid purification procedure with a new sample. Be sure to briefly vortex the sample after addition of Solution IRS.

		Comments and suggestions
b)	Buffer AW1, Buffer AW2 or Buffer RPE prepared incorrectly	Check that buffer concentrates were diluted with correct volumes of pure ethanol. Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone. Repeat the procedure with a new sample.
High A_{260}/A_{280} ratio for purified nucleic acids		
	High level of residual RNA in DNA	Add 20 µl RNase A (25 mg/ml) to the eluate and incubate for 10 minutes at room temperature (15–25°C).
DNA or RNA does not perform well in downstream applications		
	Inefficient removal of inhibitory substances	In most cases, no inhibition will be observed when amplifying nucleic acids purified using the AllPrep PowerFecal 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. Be sure to briefly vortex the sample after addition of Solution IRS. Take care to not transfer any of the pellet after Solution IRS treatment.
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 DNA/RNA procedure or later handling (see Appendix B: General Remarks on Handling RNA, page 20, for guidelines on handling RNA.

Appendix A: Optional On-Column DNase Digestion with the RNase-Free DNase Set

The RNase-Free DNase Set (cat. no. 79254) provides efficient on-column digestion of DNA during RNA purification. The DNase is efficiently removed in subsequent wash steps.

Note: Standard DNase buffers are not compatible with on-column DNase digestion. Use of other buffers may affect the binding of RNA to the RNeasy membrane, reducing RNA yield and integrity.

Lysis and homogenization of the sample and binding of RNA to the RNeasy membrane are performed according to the standard protocols. After washing with a reduced volume of Buffer RW1, the RNA is treated with DNase I while bound to the RNeasy membrane. The DNase I is removed by a second wash with Buffer RW1. Washing with Buffer RPE and elution of RNA are then performed according to the standard protocol.

Important points before starting

- Do not vortex the reconstituted DNase I. DNase I is especially sensitive to physical denaturation. Mixing should only be carried out by gently inverting the tube.

Things to do before starting

- Prepare DNase I stock solution before using the RNase-Free DNase Set for the first time. Dissolve the lyophilized DNase I (1500 Kunitz units) in 550 µl of RNase-Free Water provided. To avoid loss of DNase I, do not open the vial. Inject RNase-Free Water into the vial using an RNase-free needle and syringe. Mix gently by inverting the vial. Do not vortex.

- For long-term storage of DNase I, remove the stock solution from the glass vial, divide into single-use aliquots, and store at –15 to –30°C for up to 9 months. Thawed aliquots can be stored at 2–8°C for up to 6 weeks. Do not refreeze the aliquots after thawing.

Procedure

Prepare and load samples onto the RNeasy Spin Column as indicated in the protocol. Instead of performing the first wash step (step 13 of the protocol on page 14), follow steps 1–4.

1. Add 350 µl Buffer RW1 to the RNeasy Spin Column, and centrifuge for 15 s at >8000 x *g* to wash the membrane. Discard the flow-through. Reuse the Collection Tube.
2. Add 10 µl DNase I stock solution (see above) to 70 µl Buffer RDD. Mix by gently inverting the tube. Centrifuge briefly to collect residual liquid from the inside of the tube.
   Note: Buffer RDD is supplied with the RNase-Free DNase Set.
   Note: DNase I is especially sensitive to physical denaturation. Mixing should only be carried out by gently inverting the tube. Do not vortex.
3. Add the DNase I incubation mix (80 µl) directly to the RNeasy Spin Column membrane, and incubate at room temperature (15–25°C) for 15 min.
   Note: Be sure to add the DNase I incubation mix directly to the RNeasy Spin Column membrane. DNase digestion will be incomplete if part of the mix sticks to the walls of the O-ring of the spin column.
4. Add 350 µl Buffer RW1 to the RNeasy Spin Column, and centrifuge for 15 s at >8000 x *g*. Discard the flow-through. Continue with step14 of the protocol on page 14 (i.e., the first wash with Buffer RPE). Reuse the Collection Tube in step 14.

Appendix B: 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. In order 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 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.

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 and 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 DNA/RNA Kit (50)	For 50 preps: Simultaneous purification of genomic DNA and total RNA from stool samples	80244
Related products		
RNase-Free DNase Set (50)	For 50 preps: DNase digestion during RNA purification	79254
Pathogen Lysis Tubes S	With small beads for mechanical disruption of bacteria	19091
Pathogen Lysis Tubes L	With large beads for mechanical disruption of fungi, including yeast, and some bacteria	19092
TissueLyser II	For medium- to high-throughput sample disruption for molecular analysis*	85300
TissueLyser Adapter Set 2 x 24	For efficient disruption of up to 48 samples in parallel on the TissueLyser II	69982
TissueLyser Adapter Set 2 x 96	For efficient disruption of up to 192 samples in parallel on the TissueLyser II	69984
QIAamp DNA Stool Kit (50)	For 50 preps: Isolation of gDNA from stool samples	51604
QIAamp Fast DNA Stool Mini Kit (50)	For 50 preps: Isolation of DNA from stool	51504
QIAamp PowerFecal DNA Kit (50)	For 50 preps: Isolation of DNA from stool, gut material and biosolids	12830-50

* The TissueLyser II must be used in combination with the TissueLyser Adapter Set 2 x 24 or TissueLyser Adapter Set 2 x 96.

Product	Contents	Cat. no.
RNeasy PowerMicrobiome Kit (50)	For 50 preps: Isolate total RNA from stool and gut material	26000-50
DNeasy PowerSoil Kit (50)	For 50 preps: Isolate microbial DNA from all soil types	12888-50
DNeasy PowerSoil Kit (100)	For 100 preps: Isolate microbial DNA from all soil types	12888-100
RNeasy PowerSoil Total RNA Kit (25)	For 25 preps: Isolate high-quality RNA from all soil types	12866-25
QIAamp DNA Microbiome Kit (50)	For 50 preps: Isolation of bacterial microbiome DNA from mixed samples	51704
QIAseq FX DNA Library Kit (24)	Complete, all-enzymatic NGS library prep for high-complexity DNA sequencing applications compatible with any Illumina sequencer; includes a plate containing 24 adapters with different barcodes	180473
QIAseq FX DNA Library Kit (96)	Complete, all-enzymatic NGS library prep for high-complexity DNA sequencing applications compatible with any Illumina sequencer; includes a plate containing 96 adapters with different barcodes	180475
QIAseq Ultralow Input Library Kit (12)	For 12 reactions: Preparation of DNA libraries for next-generation sequencing applications that use Illumina instruments	180492
QIAseq Ultralow Input Library Kit (96)	For 96 reactions: Preparation of DNA libraries for next-generation sequencing applications that use Illumina instruments	180495

Product	Contents	Cat. no.
QIAseq 1-step Amplicon Library Kit (12)	For 12 reactions: For fast and efficient preparation of DNA libraries for use in next-generation sequencing applications	180412
QIAseq 1-step Amplicon Library Kit (96)	For 96 reactions: For fast and efficient preparation of DNA libraries for use in next-generation sequencing applications	180415

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.

Revision History

Document	Description of changes
HB-2190-001	Initial release.
HB-2190-002	Kit Contents volumes updated: • Solution IRS (15 ml instead of 11 ml) DNA storage temperatures updated to reflect appropriate temperature ranges. Editorial changes.

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