October 2023

RNeasy® PowerMax® Soil Pro Kit Handbook

For the isolation of microbial RNA from soil

Sample to Insight

Contents

Kit Contents

Kit Catalog no. Number of preps	(n) 47082 10
PowerMax Bead Pro Tubes	10
Solution CD1	200 mL
Solution CD2	75 mL
MB Maxi Spin Columns	10
Collection Tubes 50 mL	40
Solution EA	525 mL
Solution C5	175 mL
RNase free water	14 mL
RDD Buffer	10 mL
DNase I (lyophilized)	2 tubes
Quick-Start Protocol	1

Shipping and Storage

Solution CD2, Buffer RDD 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.

Intended Use

The RNeasy PowerMax Soil Pro Kit is intended for the purification of microbial RNA from soil samples. The RNeasy PowerMax Soil Pro 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.

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 RNeasy PowerMax Soil Pro Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

The RNeasy PowerMax Soil Pro Kit comprises a novel and proprietary method for isolating total RNA from samples high in PCR inhibitors, like soil samples. The kit uses QIAGEN's second-generation Inhibitor Removal Technology® (IRT), and is intended for use with samples containing inhibitory substances commonly found in soil samples with high humic acid content, including difficult soil types such as compost, sediment, and manure. 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. RNAseq or metatranscriptome).

Principle and procedure

The recommended starting material is 5 to 15 g of soil. Each sample is homogenized in a 50 mL bead beating tube containing a mixture of lysis beads. Lysis of 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 soil samples that interfere with PCR and other downstream applications. The lysate is then passed through a MB Maxi Spin Column. DNA is removed using on-column DNase followed by a wash solution. This removes the enzyme and any digested nucleic acids. The RNA eluted in RNase-free water is ready to use in any downstream application.

Note: Soil samples should be preserved as soon as possible after collection to protect the integrity of the RNA.

Workflow

Lyse in PowerMax Bead Pro Tube
Solution CD1 + Solution CD2 +
Phenol-chloroform-isoamyl alcohol

Mechanical Disruption
TissueLyser III or Vortex Adapter

IRT
Solution CD2

Bind to MB Maxi Spin Column
Solution EA

Wash
Solution EA

DNase digestion
(on column)

Washes
Solution EA/C5/80%EtOH

Elution
RNase-free water

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:Isoamylalcohol (25:24:1; pH 6.5–8.0)
- Pipettes and disposable pipette tips with aerosol barriers (1000 µL – 10 mL)
- Disposable gloves
- Centrifuge (with rotor for 50 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:
    - TissueLyser III (cat. no. 9003240) for use with the 50 mL Tube Adapter Set (cat. no. 11960)
    - Vortex adapter for 2 (50 mL) tubes (cat. no. 13000-V1-50)

Important Notes

Sample storage and preservation

To optimize the quality of nucleic acids from soil, process the sample as quickly as possible after collection. Freezing the samples at –90°C to –65°C will 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, CD1 lysis buffer and CD2 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 Vortex Adapter for 50 mL tubes (cat. no. 13000-V1-50), or if using a bead beater is desired, the RNeasy PowerMax Soil Pro Kit contains bead tubes suitable for high-powered bead beating and may be used in conjunction with the TissueLyser III (cat. no. 9003240) using a 50 mL Tube Adapter Set (cat no. 11960).

Protocol: Experienced User

Notes before starting

- 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 100 µL portions and store at –30°C to –15°C for long-term storage. Avoid freeze/thaw more than three times. To prepare DNase I Solution, thaw and combine 100 µL DNase I stock enzyme with 900 µL RDD Buffer 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 21.
- Prepare fresh 80% ethanol use RNase-Free Water (not provided).

1. Add 5–15 g of soil, 16 mL of Solution CD1, 2 mL of Solution CD2 and 2 mL phenol–chloroform–isoamyl alcohol (25:24:1, pH 6.5–8.0) to the PowerMax Bead Pro Tube and vortex briefly to mix.
2. Secure the PowerMax Bead Pro Tube in the 50 mL Tube Adapter Set (cat. no. 11960). Fasten the adapter into the TissueLyser III instrument and shake for 10 min at speed 25 Hz.

   For more information about other bead beating methods, see the "Protocol: Detailed" section on page 14.
3. Centrifuge the PowerMax Bead Pro Tube at 2500 x *g* for 10 min.
4. Transfer the supernatant to a clean 50 mL collection tube (provided).

   Note: Expect a volume of 15 mL. The supernatant may still contain some soil particles.
5. Add 5 mL Solution CD2 and vortex for 5 s. Centrifuge at 5500 x *g* for 10 min.
6. Avoiding the pellet, transfer up to 20 mL of the supernatant to a clean 50 mL collection tube (provided).

Note: The supernatant can be decanted when the pellet is solid.

7. Add equal volume of Solution EA to the supernatant. Vortex briefly to mix.
8. Load 15 mL binding mix (supernatant + Solution EA) into a MB Maxi Spin Column and centrifuge at 2500 x *g* for 2 min. Discard the flow-through.
9. Repeat step 8 until the whole binding mix is processed.
10. Add 15 mL Solution EA to the spin column and centrifuge at 2500 x *g* for 2 min. Discard the flow-through.
11. Add 1 mL DNase I Solution to the center of the Spin Column (prepared by mixing 900 µL RDD Buffer and 100 µL DNase I stock enzyme; see "Notes before starting").
12. Incubate at room temperature for 15 min. Add 10 mL Solution EA and centrifuge at 2500 x *g* for 2 min.
13. Discard flow-through. Add 10 mL Solution C5. Centrifuge at 2500 x *g* for 2 min.
14. Discard flow-through. Add 15 mL 80% EtOH. Centrifuge at 2500 x *g* for 3 min.
15. Discard flow-through and place the MB Maxi Spin Column into a clean 50 mL Collection Tube (provided). Centrifuge at 5500 x *g* for 5 min to dry.
16. Place the MB Maxi Spin Column into a clean 50 mL Collection Tube (provided). Add 1 mL RNase free water to the center of the white filter membrane. Incubate at room temperature for at least 1 min.
17. Centrifuge at 5500 x *g* for 5 min. Discard the MB Maxi Spin Column. Transfer the Eluate into a clean 1.5 mL LoBind Tube (not provided). The RNA is now ready for any downstream application.

Protocol: Detailed

1. Add 5–15 g of soil, 16 mL of Solution CD1, 2 mL of Solution CD2 and 2 mL phenol–chloroform–isoamyl alcohol (25:24:1, pH 6.5–8.0) to the PowerMax Bead Pro Tube and vortex briefly to mix.

   Note: The input of 5 g of soil would be sufficient for most kind of samples. The Solution CD1 is a buffer that disperses cells and soil particles. Solution CD2 is a precipitation reagent that removes non-NA organic and inorganic material including cell debris, proteins and humic acids. Phenol/chloroform/isoamyl alcohol maximizes lysing efficiency and yield. Lysed cell components are trapped in the solvent and proteins are denatured leaving the nucleic acid in solution. It also helps to save RNA against degradation.

2. Secure the PowerMax Bead Pro Tube in the 50 mL Tube Adapter Set (cat. no. 11960). Fasten the adapter into the TissueLyser III instrument and shake for 10 min at speed 25 Hz.

   Optional: Other bead beating methods can also be performed:

   Secure the PowerMax Bead Pro Tube horizontally on a Vortex Adapter for 2 (50 mL) tubes (cat. no. 13000-V1-50). Orient tube caps to point toward the centre of the vortex adapter. Vortex at maximum speed for 10 min.

   Using tape to attach tubes is not recommended.

   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.

3. Centrifuge the PowerMax Bead Pro Tube at 2500 x *g* for 10 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 bacterial, fungal and plant cells.

4. Transfer the supernatant to a clean 50 mL collection tube (provided).

   Note: Expect a volume of 15 mL. The supernatant may still contain some soil particles.

5. Add 5 mL Solution CD2 and vortex for 5 s. Centrifuge at 5500 x *g* for 10 min at.

   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 up to 20 mL of the supernatant to a clean 50 mL collection tube (provided).

   Note: The supernatant containing nucleic acids can be decanted when the pellet is solid. The pellet at this point contains non-NA organic and inorganic material including humic acids, cell debris, and proteins. For best RNA yields and quality, avoid transferring any of the pellet.

7. Add equal volume of Solution EA to the supernatant. Vortex briefly to mix.

   Note: Solution EA contains chemical components for optimal RNA-silica binding conditions.

8. Load 15 mL binding mix (supernatant + Solution EA) into a MB Maxi Spin Column and centrifuge at 2500 x *g* for 2 min. Discard the flow-through.

   Note: RNA is selectively bound to the silica membrane in the MB Maxi Spin Column. Contaminants pass through the filter membrane, leaving only RNA bound to the membrane.

9. Repeat step 8 until the whole binding mix is processed.

10. Add 15 mL Solution EA to the spin column and centrifuge at 2500 x *g* for 2 min. Discard the flow-through and place the MB Spin Column back into the same 50 mL Collection Tube.

    Note: At this step Solution EA is using as a wash buffer that removes protein and other non-aqueous contaminants from the MB Maxi Spin Column filter membrane.

11. Add 1 mL DNase I Solution to the center of the Spin Column (prepared by mixing 900 µL RDD Buffer and 100 µL DNase I stock enzyme; see "Notes before starting").
12. Incubate at room temperature for 15 min. Add 10 mL Solution EA and centrifuge at 2500 x *g* for 2 min.

    Note: The DNase in RDD Buffer 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 flow-through and place the MB Maxi Spin Column back into the same 50 mL Collection Tube. Add 10 mL Solution C5. Centrifuge at 2500 x *g* for 2 min.

    Note: Solution C5 is an ethanol-based wash solution used to further clean the RNA that is bound to the silica filter membrane in the MB Maxi Spin Column. This wash solution removes residual salt, humic acid, and other contaminants while allowing the RNA to stay bound to the silica membrane.
14. Discard flow-through and place the MB Maxi Spin Column back into the same 50 mL Collection Tube. Add 15 mL 80% EtOH. Centrifuge at 2500 x *g* for 3 min.
15. Discard flow-through and place the MB Maxi Spin Column into a clean 50 mL Collection Tube (provided). Centrifuge at 5500 x *g* for 5 min to dry.
    Note: The final dry spin ensures all ethanol is cleared from the membrane. It is important because ethanol can interfere with downstream RNA applications, such as RT-PCR, and gel electrophoresis.
16. Place the MB Maxi Spin Column into a clean 50 mL Collection Tube (provided). Add 1 mL RNase free water to the center of the white filter membrane. Incubate at room temperature for at least 1 min.

    Note: Eluting with 1 mL RNase-free water will maximize RNA yield. For more concentrated RNA, a minimum of 500 µL RNase-free water can be used.
17. Centrifuge at 5500 x *g* for 5 min. Discard the MB Maxi Spin Column. Transfer the Eluate into a clean 1.5 mL LoBind Tube (not provided). The RNA is now ready for any downstream application.

Note: We recommend storing the RNA frozen (–30°C to –15°C or –90°C to –65°C) to prevent RNA degradation.

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** (for contact information, visit **www.qiagen.com**).

	Comments and suggestions
RNA	
RNA degradation	The optimal method for storing soil samples prior to RNA isolation is to freeze at –90°C to –65°C and to avoid multiple freeze–thaw cycles. 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 2 mL phenol–chloroform–isoamyl alcohol to the PowerMax Bead 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.
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 A260/A230 readings are low, increase the amount of Solution CD2 in protocol step 5 to 6 mL to remove more inhibitors. Alternatively, use less sample if the purity is not improved with the addition of more Solution CD2.
Genomic DNA contamination	Depending on the quality of bacterial cells in the starting sample, it is possible to still have residual genomic DNA in the RNA even after the on-column DNase digest. To completely remove the genomic DNA, the DNase treatment using the RNase-Free DNase Set (cat. no. 79254 and 79256) will ensure removal of DNA without the addition of EDTA or heat inactivation of the enzyme.
Storing RNA	RNA is eluted in RNase-free water and must be stored at -30 to -15°C or –90°C to –65°C to prevent degradation.

	Comments and suggestions
Soil processing	
Amount of soil to process	The RNeasy PowerMax Soil Pro Kit is designed to process up to 15 grams of soil. For inquiries regarding the use of larger sample amounts, please contact Technical Support for suggestions. Also lower soil amounts can be processed with the RNeasy PowerMax Soil Pro Kit. Down to 250 mg of soil samples with a high microbial load would be sufficient for RNA extraction.
Soil sample stabilization	To preserve the quality of nucleic acids soil samples can be stored at –90°C to –65°C or –30 to –15°C. 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. For recommendation on soil stabilization, please contact our Technical Support.

Contact Information

For technical assistance and more information, please see our Technical Support Center at **www.qiagen.com/Support**, call 00800-22-44-6000, or contact one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit **www.qiagen.com**).

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. 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.

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

* 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.

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.

Ordering Information

Product	Contents	Cat. no.
RNeasy PowerMax Soil Pro Kit	For the isolation of microbial RNA from soil samples	47082
Relative Products		
RNase-Free DNase Set (50)	For the removal of genomic DNA contamination in RNA preparations	79254
RNase-Free DNase Set (250)	For the removal of genomic DNA contamination in RNA preparations	79256
TissueLyser III*	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 4 for 50 mL Tubes*	9003240
50 mL Tube Adapter Set	2 adapters for use with 50 mL tubes on the TissueLyser III	11960
Vortex Adapter for 2 (50mL) tubes	For vortexing 50mL tubes using the Vortex-Genie 2 Vortex.	13000-V1-50

* The TissueLyser III must be used in combination with the 50 mL Tube Adapter Set.

Document Revision History

Revision	Description
10/2023	Initial release

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Limited License Agreement for [Name] Kit

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