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QIAmini PowerFecal® Pro DNA/RNA Kit Instructions for Use

For automated purification of total nucleic acid, DNA, or RNA from stool material using QIAmini

Sample to Insight

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Kit Contents

QIAmini PowerFecal Pro DNA/RNA Kit **Catalog no.** **Number of reactions**	**(48)** **591205** **48**
PowerBead Pro Tubes	2 mL (50)
Solution CD1*	40 mL
Solution CD2	15 mL
QIAmini PowerFecal Pro DNA/RNA Cartridges*	48
QIAmini Rod Covers	12
Quick-Start Protocol	1

* Contains chaotropic salt. Not compatible with disinfecting agents containing bleach. See Safety Information.

Shipping and Storage

The QIAmini PowerFecal Pro DNA/RNA Kit is shipped at ambient temperature. Upon receipt, store Solution CD2 at 2–8°C. Store all other kit components dry at room temperature (15–25°C).

When stored properly, buffers and reagent cartridges are stable until the expiration date on the kit label.

Intended Use

The QIAmini 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.

CAUTION Do not use bleach, or acidic solutions directly to the sample preparation waste.

Solution CD1 and buffers in the QIAmini PowerFecal Pro DNA/RNA Cartridges contain chaotropic salts, which can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilled, clean with a suitable laboratory detergent and water. If the spilled liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

If liquid containing potentially infectious agents is spilled on QIAmini, refer to the instrument user manual for decontamination instructions.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of the QIAmini PowerFecal Pro DNA/RNA Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

The QIAmini PowerFecal Pro DNA/RNA Kit comprises a new proprietary one-step lysis and Inhibitor Removal Technology® for a more streamlined automatic handling of stool and gut samples. The procedure allows a fully automated processing of samples after lysis to reduce manual steps and hands-on time significantly for more convenience.

Principle and procedure

The QIAmini PowerFecal Pro DNA/RNA Kit efficiently lyses microbial cells and removes inhibitory substances commonly found in stool (e.g., polysaccharides, heme compounds, and bile salt) using a novel combined one-step lysis and inhibitor removal procedure. The lysis buffer, Solution CD1, IRT buffer, Solution CD2, and an organic phase separation agent (e.g., phenol–chloroform–isoamyl alcohol) ensure both effective mechanical and chemical lysis using bead beating tubes and efficient inhibitor depletion during centrifugation after lysis and disruption. The supernatant is placed on the QIAmini instrument and either total NA, DNA-only, or RNA-only can be extracted in a fully automated workflow.

The workflow is optimized for microbial nucleic acid extraction but is not selective; all nucleic acid, including the generally smaller proportion deriving from the host, will be purified. Nucleic acids are eluted in 100 µL RNase-free water. For the extraction of DNA-only or RNA-only, the corresponding enzyme: RNase A or RNase-free DNase I Set need to be purchased separately.

Note that the use of phenol–chloroform–isoamyl alcohol is crucial for the depletion of downstream inhibitors. At the same time, it also deactivates RNases that can be released during disruption, allowing the extraction of high-quality RNA. For detailed information about alternatives to phenol–chloroform–isoamyl alcohol, see "Appendix: Phenol-free Lysis and IRT".

The recommended amount of starting material is 50 mg of stool. For typical samples, this amount provides yields of 10–25 µg DNA and 20–90 µg RNA. The maximum amount that can be processed in the QIAmini PowerFecal Pro DNA/RNA Kit is 100 mg for DNA-only and total NA extraction and 50 mg of stool for RNA-only extraction. Attempting to process larger amounts could lead to adverse effects such as overloading binding capacity or inefficient washing, which will decrease the final yield and quality of nucleic acid. These recommendations are based on the total amount of stool material, not total volume. For example, 50 mg of stool in 200 µL of transport buffer can still be processed.

Automation

QIAmini can perform all steps following lysis and disruption of the sample. This automation is based on magnetic particle technology and includes nucleic acid binding, optional enzymatic digestion, washing, and elution. Up to eight samples can be processed in a single run.

Magnetic-particle technology combines the speed and efficiency of silica-based nucleic acid purification with the convenient handling of magnetic particles. Total nucleic acid, DNA-only, or RNA-only is isolated from lysates in one step through its binding to the silica surface of the particles in the presence of a chaotropic salt. The particles are subsequently separated from the lysates using a magnet. Additional washing steps remove any residual contaminants. Finally, the nucleic acids are efficiently eluted.

Equipment and Reagents 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. These are available online in convenient and compact PDF format at **www.qiagen.com/safety** where you can find, view, and print the SDS kit for each QIAGEN kit and kit component.

The following materials are not included in the kit and are to be supplied by the user:

- **For RNA-only protocol**:
  - RNase-free DNase Set (50) (cat. no. 79254)
  - Isopropanol
- **For DNA-only protocol**: RNase A (17,500 U) (cat. no. 19101)
- **For all protocols**: Phenol–chloroform–isoamyl alcohol (25:24:1) pH 6.5–8
- Microcentrifuge (with rotor for 2 mL tubes)
- 1.5 mL tubes
- Equipment for sample disruption and homogenization, one of the following:
  - Vortex-Genie® 2 and Vortex Adapter for 24 (1.5–2 mL) tubes (cat. no. 13000-V1-24)
  - TissueLyser III (cat. no. 9003240) with adapter sets for use with the PowerBead Pro Tubes (TissueLyser Adapter Set 2 × 24 [cat. no. 69982] or 2 mL Tube Holder [cat. no. 11993], in conjunction with the Plate Adapter Set [cat. no. 11990])

Important Notes

Handling and storage of starting material

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 component of stool are water (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. Host DNA can also be found in these samples but is normally a minor component compared to microbial DNA. Nucleic acids isolated from stools typically appear to have some level of degradation using standard analysis methods because of the relatively high content of dead and decaying cells.

To optimize the quality of nucleic acids from stool, process the sample as quickly as possible after collection. The PowerProtect DNA/RNA reagent (cat. nos. 14800 and 14810) enables stabilization of stool samples at room temperature. See the *PowerProtect DNA/RNA Handbook* (**www.qiagen.com/HB-3043**) for processing recommendations. Freezing the samples at –65°C to –90°C also preserves 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 lysis buffer CD1, inhibitor removal buffer CD2, and phenol–chloroform–isoamyl alcohol 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.

Disrupting and homogenizing starting material

Efficient disruption and homogenization of the staring material is an absolute requirement for all nucleic acid purification procedures. The QIAmini PowerFecal Pro DNA/RNA Kit contains bead tubes suitable for homogenization by a vortex in conjunction with the Vortex Adapter for 24 (1.5–2 mL) tubes (cat. no. 1300-V1-24), or high-powered bead beating using the TissueLyser III (cat. no. 9003240) in conjunction with a 2 mL Tube Holder Set (cat. no. 11993).

Preparation of buffers

Preparing DNase I stock solution for RNA-only protocol

Prepare the DNase I stock solution by dissolving the lyophilized DNase I (1500 Kunitz units) in 550 µL RNase-free water. In some cases, the vial of DNase may appear to be empty. This is due to lyophilized enzyme sticking to the septum. 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.

Insoluble material may remain when dissolving DNase. This does not affect DNase performance. Due to the production process, insoluble material may be present in the lyophilized DNase. However, rigorous QC tests are carried out to ensure that DNase activity remains consistent from lot to lot.

Note: Do not vortex reconstituted DNase I as it is sensitive to physical denaturation. Mixing should only be carried out by gently inverting the vial.

For long-term storage of DNase I, remove the stock solution from the vial, divide it into single aliquots, and store at –30°C to –15°C for up to 9 months. Thawed aliquots can be stored at 2–8°C for up to 4 weeks. Do not refreeze the aliquots after thawing.

Working with QIAmini

The main features of QIAmini include the following:

- Purification of high-quality nucleic acids from a wide variety of sample types
- Flexible throughput, allowing processing of up to eight samples per run
- Simple, low-complexity automation of magnetic, particle-based bind–wash–elute workflows
- Pre-filled reagent cartridges enabling convenient load-and-go operation
- Compact footprint, designed to fit on any laboratory bench and allow rapid implementation
- User-friendly operation, making automated nucleic acid purification accessible even for occasional users
- Browser-based application for protocol management
- UV decontamination to help reduce sample carryover from run-to-run and allow pathogen decontamination on worktable surface

Note: UV decontamination helps reduce possible pathogen contamination on QIAmini worktable surfaces. The efficiency of inactivation must be determined for each specific organism and depends on factors such as sample type and contaminant load. QIAGEN cannot guarantee complete eradication of specific pathogens.

Protocol: QIAmini PowerFecal Pro DNA/RNA Kit

Important notes before starting

- A list of all imported protocols is available in the Run menu list view.
- For detailed protocol descriptions and the latest overview of all available QIAmini protocols, refer to the *QIAmini Protocol Overview* (**www.qiagen.com/HB-3870**), available for download separately.
- Download the appropriate protocol file from **www.qiagen.com/QIAmini** or from QIAmini Suite (**www.qiagen.com/QIAminiSuite**), and transfer to your QIAmini instrument.
- **RNA-only protocol**: Prepare DNase I stock enzyme by adding 550 µL of RNase-free water to the DNase I (RNase-free) lyophilized powder and mixing gently. Aliquot the DNase I stock enzyme in 85–90 µL portions and store at –30°C to –15°C for long-term storage. Avoid more than three freeze–thaw cycles. DNase I is sensitive to physical denaturation; do not vortex resuspended DNase I.
- Perform centrifugation steps at room temperature.
- If preparing RNA for the first time, read "Important Notes".
- Up to eight cartridges can be processed in one run.

Procedure

1. Spin the PowerBead Pro Tube briefly to ensure that the beads have settled at the bottom of the wells.
2. Add up to 100 mg of stool material, 450 µL of Solution CD1, 150 µL of Solution CD2, and 150 µL phenol–chloroform–isoamyl alcohol (25:24:1, pH 6.5–8.0) to the PowerBead Pro Tube (in that order), and vortex briefly to mix.

   Note: The absolute maximum amount of stool material that can be processed is 100 mg. The recommended starting amount is no more than 50 mg of stool, which provides high amounts of nucleic acids for most kind of stool samples. For RNA-only protocol, 50 mg of stool is also the maximum amount of starting material that should be processed.

   Note: As an alternative to phenol–chloroform–isoamyl alcohol, a mix of benzyl alcohol and chloroform can be used. For more information about alternatives to phenol–chloroform–isoamyl alcohol, see Appendix: Phenol-free Lysis and IRT.
3. Mechanically disrupt the samples using one of the following methods:
   - Secure the PowerBead Pro Tube horizontally on a Vortex Adapter for 24 (1.5–2 mL) tubes (cat. no. 13000-V1-24). Orient the tube caps toward the center of the adapter. Vortex at maximum speed for 10 min.
   - Use TissueLyser III. Place the PowerBead Pro Tube into the TissueLyser Adapter Set 2 × 24 (cat. no. 69982) or the 2 mL Tube Holder (cat. no. 11993) in conjunction with Plate Adapter Set (cat. no. 11990). Fasten the adapter into the instrument and disrupt samples for 5 min at 25 Hz. Reorient the adapter so that the side closest to the instrument body is now furthest away, and disrupt again for 5 min at 25 Hz.
4. Centrifuge the PowerBead Pro Tube at 15,000 × *g* for 5 min.
5. Use the supernatant for DNA-only, total NA, or RNA-only extraction.

6. For DNA-only protocol: Transfer 420 µL sample supernatant into a new 1.5 mL tube. Add 4 µL RNase A, mix by vortexing, and incubate for 2 min at room temperature.

Cartridge preparation

7. Invert the pre-filled cartridge(s) to resuspend magnetic beads. Flick down to remove reagents and bead suspension from under the foil.
8. Place the cartridge(s) into the cartridge frame. Make sure to keep the pull tab accessible. Ensure that the cartridge(s) are fully inserted into the cartridge frame.
9. **Important**: Carefully remove the sealing foil. Avoid splashing of reagents.
10. **For RNA-only protocol**: Pipet 75 µL Buffer RDD, 150 µL isopropanol, and 10 µL resuspended DNase I to the bead suspension in well 6 of the cartridge.
11. Transfer 420 µL sample supernatant (**DNA-only**: 420 µL sample supernatant mixed with 4 µL RNase A from step 6) into well 1 of the cartridge.

Procedure on QIAmini

1. Turn on QIAmini.
2. Select the desired protocol.

   Note: A list of all imported protocols is available in the Run menu list view.

   Note: For detailed protocol descriptions and the latest overview of all available QIAmini protocols, refer to the *QIAmini Protocol Overview* (**www.qiagen.com/HB- 3870**), available for download separately.
3. Open the instrument door. Load the cartridge frame holding the cartridge(s) into the instrument.

   Note: Ensure that the cartridge frame is placed in correct orientation. Press the cartridges down to fully engage with the heater block.
4. Slide rod cover(s) into the rod cover holder.

   Note: Press the clip down to allow full insertion of the rod cover holder.
5. Close the instrument door, press ▶, and confirm that consumables have been installed correctly to initiate the protocol run.
6. The display shows "End of run" when the run is completed. Select **Confirm** and press ⮌.
7. Open the instrument door. Remove and discard the rod cover(s). Then remove the cartridge frame holding the cartridge(s) and transfer the purified nucleic acids from well 5 of each cartridge into a new reaction tube (e.g., 1.5 mL tube). Discard the cartridge(s).

   Recommended: Clean the instrument and perform a UV decontamination of the instrument chamber.

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 **support.qiagen.com**).

	Comments and suggestions
General handling	
Loss of reagents from the cartridge when removing the seal	After inverting the reagent cartridges to resuspend the magnetic particles, make sure to flick down the cartridges to deposit the reagents at the bottom of the wells.
RNA degraded	
Inappropriate handling of starting material	Use phenol–chloroform–isoamyl alcohol or as an alternative benzyl alcohol and chloroform in lysis to protect RNA from degradation by RNases. Furthermore, ensure to process the sample material as soon as possible after collection or store it either at –90°C to –20°C or in an appropriate stabilization reagent such as PowerProtect DNA/RNA until nucleic acid extraction.
Low or no recovery of RNA and DNA	
Too much starting material	In subsequent preparations, reduce the amounts of starting material. It is essential to use the correct amount of starting material. See "Handling and storage of starting material.
Inefficient disruption and/or homogenization	See "Disrupting and homogenizing starting material" for a detailed description of homogenization methods".
Lysis without phenol–chloroform–isoamyl	Lysis without phenol–chloroform–isoamyl alcohol can result in reduced RNA and DNA yield. In subsequent preparation use phenol–chloroform–isoamyl alcohol to ensure extraction of highest possible yields

Appendix: Phenol-free Lysis and IRT

The use of phenol–chloroform–isoamyl alcohol in lysis is crucial for the depletion of downstream inhibitors and extraction of high amounts of nucleic acids with the QIAmini PowerFecal Pro workflow. At the same time, it also deactivates RNases which can be released during disruption, enabling the extraction of high-quality RNA.

However, if working with phenol is not desired, benzyl alcohol mixed with chloroform in a 14:1 ratio can be used as an alternative to phenol–chloroform–isoamyl–alcohol. Another phenol-free alternative is to follow a two-step lysis and IRT procedure.

Note: For both phenol-free protocols, RNA quality may not match that obtained using phenol–chloroform–isoamyl alcohol, and overall RNA and DNA yields may be reduced.

Procedure using benzyl alcohol and chloroform during lysis

1. Spin the PowerBead Pro Tube briefly to ensure that the beads have settled at the bottom.
2. Add up to 100 mg of stool, 450 µL of Solution CD1, 150 µL of Solution CD2, 140 µL of benzyl alcohol, and 10 µL of chloroform to the PowerBead Pro Tube in that order, and vortex briefly to mix.

   Note: The absolute maximum amount of stool that can be processed is 100 mg. QIAGEN recommends starting with no more than 50 mg of stool, which yields high amounts of nucleic acids for most stool sample types. For RNA-only protocol, 50 mg of stool is also the maximum amount of starting material that should be processed.
3. Proceed with step 3 of "Protocol: QIAmini PowerFecal Pro DNA/RNA Kit".

Two-step lysis and IRT procedure

1. Spin the PowerBead Pro Tube briefly to ensure that the beads have settled at the bottom.
2. Add up to 100 mg of stool and 650 µL of Solution CD1. Vortex briefly to mix.

   Note: The absolute maximum amount of stool that can be processed is 100 mg. We recommend starting with no more than 50 mg of stool, which will give high amounts of nucleic acids for most kind of stool samples.
3. Mechanically disrupt the samples using one of the following methods:
   - Secure the PowerBead Pro Tube horizontally on Vortex Adapter for 24 (1.5–2 mL) Tubes (cat. no. 13000-V1-24). Orient the tube caps toward the center of the adapter and vortex at maximum speed for 10 min.
   - Use a TissueLyser III. Place the PowerBead Pro Tube into the TissueLyser Adapter Set 2 × 24 (cat. no. 69982), 2 mL Tube Holder (cat. no. 11993), or Plate Adapter Set (cat. no. 11990). Shake for 5 min at 25 Hz. Reorient the adapter so that the side closest to the machine body is now furthest from it and shake again for 5 min at 25 Hz.
4. Perform the following steps:
   a. Centrifuge the PowerBead Pro Tube at 15,000 × *g* for 5 min.
   b. Transfer the supernatant to a clean 2 mL microcentrifuge tube (not provided).

      Note: Expect 500–600 µL. The supernatant may still contain some stool particles.
   c. Add 300 µL of Solution CD2 and vortex for 5 s.
   d. Centrifuge at 15,000 × *g* for 1 min.
5. Proceed with step 5 of "Protocol: QIAmini PowerFecal Pro DNA/RNA Kit"

Ordering Information

Product	Contents	Cat. no.
QIAmini PowerFecal Pro DNA/RNA Kit (48)	For the isolation of microbial total NA, DNA-only, or RNA only from stool samples	591205
Related products		
PowerBead Pro Tubes (50)	Bead tubes ready for rapid and reliable biological sample lysis from a wide variety of starting materials, 2 mL	19301
RNase Free DNase Set (50)	For the removal of genomic DNA contamination in RNA preparations	79254
RNAse A (17,500 U)	2.5 mL solution for removal of RNA	19101
TissueLyser		
TissueLyser III	Bead mill, 100–120/220–240 V, 50/60 Hz; requires the TissueLyser Adapter Set 2 × 24 or TissueLyser Adapter Set 2 × 96*	9003240
TissueLyser Adapter Set 2 × 24	Two sets of adapter plates and two racks for use with 2 × 24 samples in 2 mL microcentrifuge tubes on the TissueLyser III	69982
Plate Adapter Set	Two sets of adapter plates and two racks for use with two 96-well plates on the TissueLyser III	11990
2 mL Tube Holder Set	Only operational in conjunction with Plate Adapter Set	11993
PowerProtect		
PowerProtect DNA/RNA (500 mL)	Reagent for ambient temperature stabilization of nucleic acids in stool	14800
PowerProtect DNA/RNA (1000 mL)	Reagent for ambient temperature stabilization of nucleic acids in stool	14810

* The TissueLyser III must be used in combination with TissueLyser adapters.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit instructions for use or user manual. QIAGEN kit instructions for use and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Service or your local distributor.

Document Revision History

Date	Description
08/2026	Initial release

Limited License Agreement for QIAmini

Use of this product signifies the agreement of any purchaser or user of the product to the following terms:

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