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QIAamp® PowerFecal® DNA Kit Handbook

For the isolation of DNA from stool, gut material and biosolids

Sample to Insight

QIAGEN

Contents

Kit Contents

QIAamp PowerFecal DNA Kit	**(50)**
Catalog no.	**12830-50**
Number of preps	**50**
Bead Tubes, Dry Garnet 0.7 mm	50
PowerBead Solution	42 ml
MB Spin Columns	50
Solution C1	6.6 ml
Solution C2	15 ml
Solution C3	15 ml
Solution C4	72 ml
Solution C5	30 ml
Solution C6	9 ml
Collection Tubes (2 ml)	4 x 50
Quick Start Protocol	1

Storage

The QIAamp PowerFecal DNA Kit reagents and components can be stored at room temperature (15–25°C) until the expiration date printed on the box label.

Intended Use

All QIAamp products are intended for molecular biology applications. These products are 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 C5 contains alcohol and is flammable.

WARNING: Do not use bleach to clean the inside of the QIAvac® 24 Plus Manifold.

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

PowerBead Solution and Solution C4 contain guanidine salts, which can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilt, clean with a 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 QIAamp PowerFecal DNA Kits is tested against predetermined specifications to ensure consistent product quality.

Introduction

The QIAamp PowerFecal DNA Kit is designed for fast and easy purification of both microbial and host genomic DNA from stool and feces. Based on the DNeasy® PowerSoil® Kit, the QIAamp PowerFecal DNA Kit uses the same Inhibitor Removal Technology® (IRT) for stool that has worked so well for soil. IRT is very effective at removing inhibitory substances commonly found in stool, such as polysaccharides, heme compounds and bile salts. The result is highly pure DNA that is ready to use in demanding downstream applications.

Principle and procedure

We recommend starting with 0.25 grams of stools or biosolids. Each sample is homogenized in a 2 ml bead beating tube containing garnet beads. Cell lysis of host cells, as well as microbial cells, is facilitated by both mechanical collisions between beads and chemical disruption of cell membranes, ensuring efficient extraction from even the toughest microorganisms. The IRT is then used to remove common substances in fecal samples that interfere with PCR. Total genomic DNA is captured on a silica membrane in a spin column format. DNA is then washed and eluted from the membrane. The isolated DNA is ready for PCR analysis and other downstream applications, including qPCR and next generation sequencing analysis.

High-throughput options

We offer a vacuum-based protocol for faster processing without centrifugation for the DNA-binding and column-washing steps using MB Spin Columns. The QIAvac 24 Plus Manifold (cat. no. 19413) allows for processing of up to 24 MB Spin Column preps at a time. For additional high-throughput options, we offer the DNeasy UltraClean 96 Microbial Kit (cat. no. 10196-4) for processing up to 2 x 96 samples using a centrifuge capable of spinning two stacked 96-well blocks (13 cm x 8 cm x 5.5 cm) at 2500 x *g*. For 96-well homogenization of bacteria, we offer the TissueLyser II and Plate Adapter Set (cat. no. 85300 and 11990, respectively).

Automated nucleic acid purification on the QIAcube

Purification of DNA using the QIAamp PowerFecal DNA Kit can be automated on the QIAcube®. The innovative QIAcube uses advanced technology to process QIAGEN spin columns, enabling seamless integration of automated, low-throughput sample prep into your laboratory workflow. Sample preparation using the QIAcube follows the same steps as the manual procedure (i.e., lyse, bind, wash and elute), enabling you to use the QIAamp PowerFecal DNA Kit for purification of high-quality DNA.

If automating the QIAamp PowerFecal DNA Kit on the QIAcube, the instrument may process fewer than 50 samples due to dead volumes, evaporation and additional reagent consumption by automated pipetting. QIAGEN only guarantees 50 sample preps with manual use of the QIAamp PowerFecal DNA Kit.

For more information about the automated procedure, see the relevant protocol sheet available at **www.qiagen.com/MyQIAcube**. Up-to-date protocol sheets can be downloaded free of charge, or may be obtained by contacting QIAGEN Technical Services at **support.qiagen.com**.

Figure 1. The QIAcube instrument.

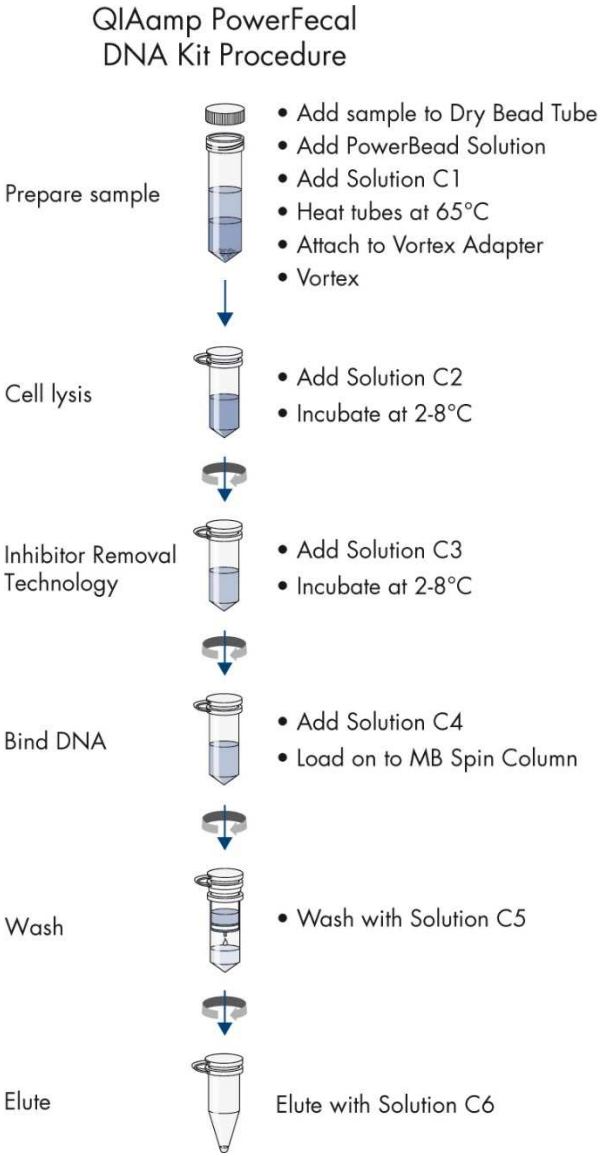

Figure 2. QIAamp PowerFecal DNA Kit procedure.

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.

- Microcentrifuge (13,000 x *g*)
- Pipettes (60 µl–750 µl)
- Vortex-Genie® 2 Vortex
- Vortex Adapter for 24 (1.5–2.0 ml) tubes (cat. no. 13000-V1-24)
- 100% ethanol (for the QIAvac 24 Plus Manifold protocol)

Protocol: Experienced User

Important points before starting

- Perform all centrifugation steps at room temperature (15–25°C).
- If Solution C1 has precipitated, heat at 60°C until precipitate dissolves.
- Shake to mix Solution C4 before use.

Procedure

1. Add 0.25 g of stool or biosolid to the Bead Tube provided.
   Note: For fecal samples that are especially high in lipids, polysaccharides and protein (e.g. meconium or some bird feces) smaller amounts of starting material (~0.10 g) may improve DNA yield and purity.
2. Add 750 µl of PowerBead Solution to the Bead Tube.
3. Add 60 µl of Solution C1 and invert several times or vortex briefly.
4. Heat the tubes at 65°C for 10 min.
5. Secure tubes horizontally using a Vortex Adapter (cat. no. 13000–V1–24). Vortex at maximum speed for 10 min.
6. Centrifuge the tubes at 13,000 x *g* for 1 min.
7. Transfer the supernatant to a clean 2 ml Collection Tube (provided). Expect between 400 to 500 µl of supernatant.
8. Add 250 µl of Solution C2 and vortex briefly to mix. Incubate at 2–8°C for 5 min.
   Note: You can skip the 5 min incubation. However, if you have already validated the PowerFecal extractions with the incubation we recommend you retain the step.
9. Centrifuge the tubes at 13,000 x *g* for 1 min.
10. Avoiding the pellet, transfer up to 600 µl of supernatant to a clean 2 ml Collection Tube.
11. Add 200 µl of Solution C3 and vortex briefly. Incubate at 2–8°C for 5 min.
    Note: You can skip the 5 min incubation. However, if you have already validated the PowerFecal extractions with the incubation we recommend you retain the step.

12. Centrifuge the tubes at 13,000 x *g* for 1 min.
13. Avoiding the pellet, transfer the supernatant to a clean 2 ml Collection Tube (provided). Do not transfer more than 750 µl at this step.
14. Add 1200 µl of Solution C4 to the supernatant and vortex for 5 s.
15. Load 650 µl of supernatant onto an MB Spin Column and centrifuge at 13,000 x *g* for 1 min. Discard the flow-through and repeat until all the supernatant has been processed.
    Note: Each sample processed will require a total of three loads.
16. Add 500 µl of Solution C5 and centrifuge for 1 min at 13,000 x *g*.
17. Discard the flow-through and centrifuge again for 1 min at 13,000 x *g*.
18. Carefully place the MB Spin Column in a clean 2 ml Collection Tube (provided).
    Note: Avoid splashing any Solution C5 onto the MB Spin Column.
19. Add 100 µl of Solution C6 to the center of the white filter membrane. Alternatively, you may use sterile, DNA-free, PCR-grade water (cat. no. 17000-10) or TE buffer.
    Note: Eluting with 100 µl of Solution C6 will maximize DNA yield. For more concentrated DNA, a **minimum** of 50 µl of Solution C6 can be used.
20. Centrifuge at 13,000 x *g* for 1 min and discard the MB Spin Column. The DNA in the tube is now ready for downstream applications.
    Note: We recommend storing DNA frozen (–20°C to –80°C) as Solution C6 does not contain EDTA. To concentrate DNA, see the Troubleshooting Guide.

Protocol: Detailed

Important points before starting

- Perform all centrifugation steps at room temperature (15–25°C)
- If Solution C1 has precipitated, heat at 60°C until precipitate dissolves
- Shake to mix Solution C4 before use

Procedure

1. Add up to 0.25 g of stool or biosolid to the Bead Tube provided.
   Note: For fecal samples that are especially high in lipids, polysaccharides and protein (e.g. meconium or some bird feces) smaller amounts of starting material (~0.10 g) may improve DNA yield and purity.
2. Add 750 µl of PowerBead Solution to the Bead Tube.
   Note: Once the sample is loaded into a Dry Bead Tube, the next step is homogenization and lysis. The garnet beads and PowerBead Solution will help disperse the soil particles.
3. If Solution C1 has precipitated, heat at 60°C until precipitate dissolves. Add 60 µl of Solution C1 and invert several times or vortex briefly.
   Note: Solution C1 contains SDS and other disruption agents required for complete cell lysis. In addition to aiding cell lysis, SDS is an anionic detergent that breaks down fatty acids and lipids associated with the cell membrane of several organisms. If it gets cold, it will form a white precipitate in the bottle. Heating to 60°C will dissolve the SDS but will not harm it or the other disruption agents. Solution C1 can be used while it is still warm. Vortexing mixes the components in the Bead Tube and begins to disperse the sample.
4. Heat the tubes at 65°C for 10 min.
   Note: Fecal samples contain a complex array of polysaccharides, lipids, salts and cells. Heating the samples increases the reaction rate between the lysis buffer and these substances and aids cell lysis.
5. Secure tubes horizontally using a Vortex Adapter (cat. no. 13000–V1–24). Vortex at maximum speed for 10 min.

Note: Vortexing is critical for complete homogenization and cell lysis. Cells are lysed by a combination of chemical agents from steps 1–4 and mechanical shaking introduced at this step. By randomly shaking the beads in the presence of disruption agents, collision of the beads with microbial cells will cause the cells to break open. Use of the vortex adapter will maximize homogenization, which can lead to higher DNA yields. Avoid using tape, which can become loose and result in reduced homogenization efficiency, inconsistent results and reduced yields.

6. Centrifuge the tubes at 13,000 x *g* for 1 min.
7. Transfer the supernatant to a clean 2 ml Collection Tube (provided). Expect between 400 to 500 µl of supernatant.
   Note: The exact volume will depend on the absorbency of your starting material and is not critical for the procedure to be effective.
8. Add 250 µl of Solution C2 and vortex for 5 s. Incubate at 2–8°C for 5 min.
   Note: You can skip the 5 min incubation. However, if you have already validated the PowerSoil extractions with the incubation we recommend you retain the step. Solution C2 has Inhibitor Removal Technology (IRT). It contains a reagent that can precipitate non-DNA organic and inorganic material including polysaccharides, cell debris and proteins. It is important to remove contaminating organic and inorganic matter that may reduce DNA purity and inhibit downstream DNA applications.
9. Centrifuge the tubes at 13,000 x *g* for 1 min.
10. Avoiding the pellet, transfer up to 600 µl of supernatant to a clean 2 ml Collection Tube (provided).
    Note: The pellet at this point contains non-DNA organic and inorganic material including polysaccharides, cell debris and proteins. For best DNA yields and quality, avoid transferring any of the pellet.
11. Add 200 µl of Solution C3 and vortex briefly. Incubate at 2–8°C for 5 min.
    Note: You can skip the 5 min incubation. However, if you have already validated the PowerSoil extractions with the incubation we recommend you retain the step. Solution C3 has Inhibitor Removal Technology (IRT), and is a second reagent to precipitate additional non-DNA organic and inorganic material including

polysaccharides, cell debris and proteins. It is important to remove contaminating organic and inorganic matter that may reduce DNA purity and inhibit downstream DNA applications.

12. Centrifuge the tubes at 13,000 x *g* for 1 min.
13. Avoiding the pellet, transfer up to 750 µl of supernatant into a clean 2 ml Collection Tube (provided).
    Note: The pellet at this point contains non-DNA organic and inorganic material including polysaccharides cell debris and proteins. For best DNA yields and quality, avoid transferring any of the pellet.
14. Add 1200 µl of Solution C4 to the supernatant and vortex for 5 s.
    Note: Solution C4 is a high-concentration salt solution. Since DNA binds tightly to silica at high salt concentrations, this will adjust the DNA solution salt concentrations to allow binding of DNA, but not non-DNA organic and inorganic material that may still be present at low levels, to the MB Spin Columns.
15. Load 650 µl of supernatant onto an MB Spin Column and centrifuge at 13,000 x *g* for 1 min. Discard the flow-through and repeat until all the supernatant has been processed.
    Note: Each sample processed will require a total of three loads. In the high salt solution, DNA is selectively bound to the MB Spin Column, while contaminants pass through.
16. Add 500 µl of Solution C5 and centrifuge for 30 s at 13,000 x *g*.
    Note: Solution C5 is an ethanol-based wash solution used to further clean the DNA that is bound to the MB Spin Column. This wash solution removes residual salt, and other contaminants while allowing the DNA to stay bound to the MB Spin Column.
17. Discard the flow-through and centrifuge again for 1 min at 13,000 x *g*.
    Note: The flow-through fraction is non-DNA organic and inorganic waste removed from the silica MB Spin Column membrane by the ethanol wash solution. The second spin removes residual Solution C5 (ethanol wash solution). It is critical to remove all traces of wash solution because the ethanol in Solution C5 can interfere with many downstream DNA applications such as PCR, restriction digests and gel electrophoresis.
18. Carefully place the MB Spin Column in a clean 2 ml Collection Tube (provided).
    Note: Avoid splashing any Solution C5 onto the MB Spin Column.

19. Add 100 µl of Solution C6 to the center of the white filter membrane. Alternatively, you may use sterile DNA-free PCR-grade water (cat. no. 17000-10) or TE buffer.
    Note: Eluting with 100 µl of Solution C6 will maximize DNA yield. For more concentrated DNA, a **minimum** of 50 µl of Solution C6 can be used. Placing Solution C6 (sterile elution buffer) in the center of the small white membrane will make sure the entire membrane is wet. This will result in a more efficient and complete release of the DNA from the MB Spin Column. As Solution C6 passes through the MB Spin Column silica membrane, DNA that was bound in the presence of high salt is selectively released by Solution C6 (10 mM Tris), which lacks salt.
20. Centrifuge at 13,000 x *g* for 1 min and discard the MB Spin Column. The DNA is now ready for downstream applications.
    Note: We recommend storing DNA frozen (–20°C to –80°C) as Solution C6 does not contain EDTA. To concentrate DNA, see the Troubleshooting Guide.

Protocol: QIAvac 24 Plus Vacuum Manifold

Important point before starting

- Perform all centrifugation steps at room temperature (15–25°C)
- If Solution C1 has precipitated, heat at 60°C until precipitate dissolves
- Shake to mix Solution C4 before use
- For each sample lysate, use one MB Spin Column. Keep the MB Spin Column in the attached 2 ml Collection Tube and continue using the Collection Tube as a MB Spin Column holder until needed for the Vacuum Manifold protocol.
- Label each Collection Tube top and MB Spin Column to maintain sample identity. If the MB Spin Column becomes clogged during the vacuum procedure, switch to the centrifugation protocol.
- You will need to provide 100% ethanol for step 8 of this protocol.

Procedure

1. Connect the QIAvac 24 Plus to the vacuum source using the QIAvac Connecting System (for more details, refer to the QIAvac 24 Plus Handbook, Appendix A, page 16).
2. Insert a VacValve into each Luer slot of the QIAvac 24 Plus that is to be used. Close unused Luer slots with Luer plugs or close the inserted VacValve.
3. Insert a VacConnector into each VacValve. Perform this step directly before starting the purification to avoid exposure of VacConnectors to potential contaminants in the air.
4. Place an MB Spin Column into each VacConnector on the manifold.
5. Transfer 650 µl of lysate (after step 14 of centrifugation protocol) to the MB Spin Column.
6. Turn on the vacuum source and open the VacValve of the port. Hold the tube in place when opening the VacValve to keep the spin filter steady. Allow the lysate to pass through the MB Spin Column.

7. After the lysate has passed through the column completely, load again with 650 µl of lysate. Continue until all of the lysate has been loaded onto the MB Spin column. Close the VacValve of that port.
   Note: If the MB Spin Columns are filtering slowly, close the ports to samples that have completed filtering to increase the pressure to the other columns.
8. Add 800 µl of 100% ethanol to completely fill the MB Spin Column. Open the VacValve while holding the column steady. Allow the ethanol to pass through the column completely. Close the VacValve.
9. Add 500 µl of Solution C5 to each MB Spin Column. Open the VacValve and apply a vacuum until Solution C5 has passed through the column completely. Continue to pull a vacuum for another minute to dry the membrane. Close each port.
10. Turn off the vacuum source and open an unused port to vent the manifold. If all the ports are in use, break the vacuum at the source. Make certain that all vacuum pressure is released before performing the next step. It is important to turn off the vacuum at the source to prevent backflow into the columns.
11. Remove the MB Spin Column and place in the original labeled 2 ml Collection Tube. Place into the centrifuge and spin at 13,000 x *g* for 1 min to completely dry the membrane.
12. Transfer the MB Spin Column into a new 2 ml Collection Tube and add 100 µl of Solution C6 to the center of the white filter membrane. Alternatively, sterile DNA-free PCR-grade water (cat. no. 17000-10) may be used.
13. Centrifuge at 13,000 x *g* for 1 min at room temperature.
14. Discard the MB Spin Column. The DNA is now ready for downstream applications.
    Note: We recommend storing DNA frozen (–20°C to –80°C) as Solution C6 does not contain EDTA. To concentrate DNA, see the Troubleshooting Guide.

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
Fecal processing		
a)	Amount of fecal or stool sample to process	The QIAamp PowerFecal DNA Kit is designed to process 0.25 grams of fecal or stool samples per prep. For fecal samples with very high levels of proteins, lipids and polysaccharides, starting with less material (0.10 grams) may improve the yield and purity of the extracted DNA.
b)	Fecal sample is wet	Add fecal sample to Bead Tube and centrifuge at 10,000 x *g* for 30 seconds at room temperature. Remove as much liquid as possible with a pipet tip. Resume protocol from Step 2.
DNA		
a)	DNA does not amplify	Make sure to check DNA yields by gel electrophoresis or spectrophotometer reading. An excess amount of DNA will inhibit a PCR reaction. Diluting the template DNA should not be necessary with DNA isolated using the QIAamp PowerFecal DNA Kit; however, it should still be attempted. If DNA will still not amplify after trying the steps above, then PCR optimization (changing reaction conditions and primer choice) may be needed.
b)	Concentrating eluted DNA	The final volume of eluted DNA will be 100 µl. The DNA may be concentrated by adding 10 µl of 5 M NaCl and inverting 3–5 times to mix. Next, add 200 µl of 100% cold ethanol and invert 3–5 times to mix. Centrifuge at 10,000 x *g* for 5 minutes at room temperature. Decant all liquid. Remove residual ethanol in a speed vac, a dessicator or air dry. Resuspend precipitated DNA in sterile water or sterile 10 mM Tris.
c)	DNA floats out of a well when loading a gel	This usually occurs because residual Solution C5 remains in the final sample. Prevent this by being careful in step 18 and not transferring liquid onto the bottom of the spin filter basket. Ethanol precipitation (described in "Concentrating eluted DNA") is the best way to remove residual Solution C5.

		Comments and suggestions
d)	Storing DNA	DNA is eluted in Solution C6 (10 mM Tris) and must be stored at –20°C to –80°C to prevent degradation. DNA can be eluted in TE without loss, but the EDTA may inhibit downstream reactions such as PCR and automated sequencing. DNA may also be eluted with sterile DNA-free PCR-grade water (cat. no. 17000-10).
Alternative lysis methods		
a)	Reduction of shearing of DNA	After adding Solution C1, vortex 3–4 seconds, then heat to 70°C for 5 minutes. Vortex 3–4 seconds. Heat another 5 minutes. Vortex 3–4 seconds. This alternative procedure will reduce shearing but may also reduce yield.

Ordering Information

Product	Contents	Cat. no.
QIAamp PowerFecal DNA Kit (50)	For 50 preps: Isolate DNA from stool, gut material and biosolids.	12830-50
Vortex Adapter, Genie, holds 24 (1.5-2.0 ml) tubes	For vortexing 1.7, 2, 5, 15 and 50 ml tubes using the Vortex-Genie 2 Vortex.	13000-V1-24
Ceramic Bead Tubes, 1.4 mm (50)	Ready to use bead tubes for rapid and reliable biological sample lysis from a wide variety of starting materials.	13113-50
Glass Bead Tubes, 0.5 mm (50)	Ready to use bead tubes for rapid and reliable biological sample lysis from a wide variety of starting materials.	13116-50
QIAvac 24 Plus Manifold	Vacuum Manifold for processing 1–24 spin columns; includes QIAvac 24 Plus Vacuum Manifold, Luer Plugs, and Quick Couplings	19413
TissueLyser II	For medium- to high-throughput sample disruption for molecular analysis	85300
Related Products		
DNeasy PowerSoil HTP 96 Kit (384)	For 4 x 96 preps: High-throughput isolation of DNA from soil samples in less than one day.	12955-4
DNeasy PowerMax® Soil Kit (10)	For 10 preps: Isolate microbial DNA from large quantities of soil; great for samples with low microbial load.	12988-10
RNeasy® PowerMicrobiome® Kit (50)	50 preps: Isolate total RNA from stool and gut material.	26000-50

MagAttract® PowerMicrobiome DNA/RNA EP Kit	Hands-free isolation of nucleic acids from stool and gut material using an automated processing or liquid handling system.	27500-4-EP

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.

Notes

Limited License Agreement for QIAamp PowerFecal DNA Kit

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

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