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MagAttract® PowerMicrobiome® DNA/RNA EP Kit Handbook

For hands-free isolation of nucleic acids from stool and gut material using an automated processing or liquid handling system

Sample to Insight

QIAGEN

Contents

Kit Contents

MagAttract PowerMicrobiome DNA/RNA EP Kit	**(384)**
Catalog no.	**27500-4-EP**
Number of preps	**4 x 96**
PowerBead DNA Plates, Glass 0.1 mm	4
Solution MBL	2 x 150 ml
Solution IRS	2 x 44 ml
ClearMag® Binding Solution	2 x 200 ml
ClearMag Zorb Reagent	9 ml
ClearMag Wash Solution	765 ml
RNase-Free Water	50 ml
Collection Plates (2 ml)	3 x 4
96 Well Microplates	3 x 4
Sealing Mats	12
Sealing Tape	2 x 16
Elution Sealing Mats	4
Quick Start Protocol	1

Storage

All components of the MagAttract PowerMicrobiome DNA/RNA EP Kit can be stored at room temperature (15–25°C) until the expiration date printed on the label.

Intended Use

All MagAttract 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.

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

Solution MBL contains guanidine salts, which can form highly reactive compounds when combined with bleach. If liquid containing this buffer 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 MagAttract PowerMicrobiome DNA/RNA EP Kits is tested against predetermined specifications to ensure consistent product quality.

Introduction

The MagAttract PowerMicrobiome DNA/RNA EP Kit is a magnetic bead-based nucleic acid isolation kit optimized for use with the Eppendorf® ep*Motion*® 5075 TMX platform.

The MagAttract PowerMicrobiome DNA/RNA EP Kit can be used for the automated isolation of microbial RNA and DNA from all stool, gut and similar sample types and other difficult environmental samples containing high inhibitor content, such as bile, bilirubin, digested food and humic acids. The kit can be used to process up to 0.25 grams of sample and employs Inhibitor Removal Technology® (IRT) to remove PCR inhibitors released during the extraction process. Additionally, a novel, proprietary magnetic bead system is used to isolate nucleic acids from the IRT-treated lysate without binding residual contaminants. The result is inhibitor-free DNA and RNA that is ready to use in demanding downstream applications, including PCR, qPCR, qRT-PCR and next-generation sequencing (NGS).

Principle and procedure

Microbiome samples are added to a 96 well bead beating plate for rapid and thorough homogenization. Cell lysis occurs by a combination of mechanical and chemical methods. Inhibitory compounds are removed using IRT. Nucleic acids are captured on specialized magnetic beads in the presence of buffers that avoid the use of chaotropic salts and ethanol. RNA and DNA is washed on the beads and then eluted using RNase-free water.

Note: Quantification of DNA using PicoGreen® will yield values approximately 15% lower than the actual yield due to the presence of residual wash solution in the DNA. The wash solution does not inhibit PCR, cDNA synthesis, qRT-PCR or interfere with NGS or other downstream applications.

The MagAttract PowerMicrobiome DNA/RNA EP Kit requires the use of a specialized plate shaker to facilitate the bead beating process in the PowerBead DNA Plates. We recommend the TissueLyser II (cat. no. 85300) and Plate Adapter Set (cat. no. 11990).

The MagAttract PowerMicrobiome DNA/RNA EP Kit can be used to isolate nucleic acids from up to 850 µl of lysate per well in the 2 ml Collection Plate (provided). This kit requires the use of a plate shaker on the robotic deck.

The plastic plates provided with the MagAttract PowerMicrobiome DNA/RNA EP Kit have thin plastic walls that permit the efficient conduction of magnetic fields, which allows for faster and more complete separation of the magnetic beads from solution.

The order of placement of components and reagents on the robotic deck are described in the downloaded software.

Other open platform robots may be used with this kit. However, you may need to contact your local field application scientist for the manufacturer of your robot for help in adapting this protocol to your system.

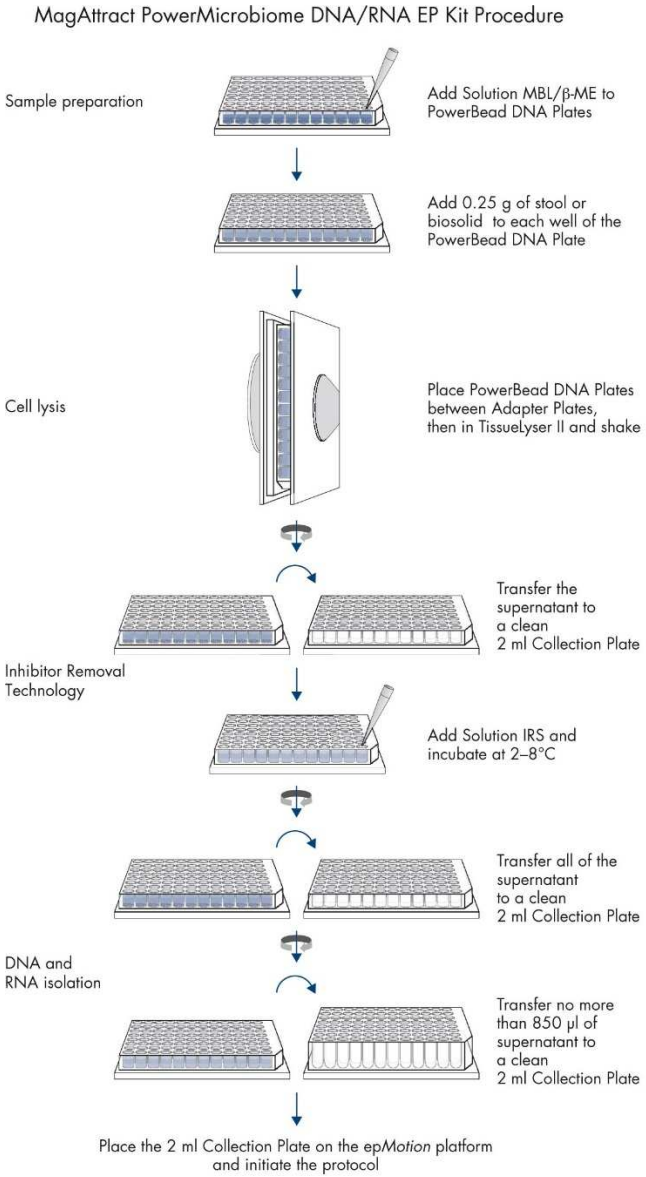

Figure 1. MagAttract PowerMicrobiome DNA/RNA EP Kit procedure.

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.

- Centrifuge capable of handling two 96 well blocks (13 cm x 8.5 cm x 6 cm) at 4500 x *g*
  Note: If your centrifuge has a maximum speed less than 4500 x *g*, please refer to the Troubleshooting Guide.
- Multi-channel pipettors (50–1000 µl)
- Mechanical shaker for 96 well plates
  Note: We recommend the TissueLyser II (cat. no. 85300) and Plate Adapter Set (cat. no. 11990).
- Vortex-Genie® 2 Vortex with 3 inch platform
- β-mercaptoethanol (β-ME)
- **Optional:** Phenol:chloroform:isoamyl alcohol (25:24:1; pH 6.5–8)
- 96 well plate shaker
- Please contact your Eppendorf representative for the ep*Motion* plastic disposables specific to your platform.
- Appropriate tips for multi-channel pipettors to be used in the lysate preparation steps
  Note: These tips must fit in the round wells of the 1 ml Collection Plates. Examples of appropriate tips are Thermo Scientific™ ART™ (cat. no. 2179-HR), Eppendorf (cat. no. 0030077750) and Rainin™ (cat. no. RT-1000F).

Protocol

Important points before starting

- Warm Solution MBL at 60°C for 15–20 minutes before use to dissolve precipitates.
- Add 25 µl of β-mercaptoethanol (β-ME) per 1 ml of Solution MBL. You will need 64 ml of Solution MBL/β-ME per 96 samples.

Procedure

1. Centrifuge a PowerBead DNA Plate, Glass 1.0 mm, at 4500 x *g* for 1 min. Carefully peel off the Elution Sealing Mat that covers the PowerBead DNA Plate and discard.
2. Add 650 µl of warmed Solution MBL/β-ME to each well of the PowerBead DNA Plate.
   Note: Solution MBL contains SDS, which can precipitate at room temperature. Heating at 60°C will dissolve the SDS. Solution MBL can be used while it is warm.
   Optional: To enhance recovery and integrity of RNA, add 100 µl of phenol:chloroform:isoamyl alcohol (25:24:1; pH 6.5–8) to the PowerBead DNA Plate wells pre-loaded with 650 µl of Solution MBL/β-ME before filling with stool samples.
3. Add 0.25 g of sample to each well of the PowerBead DNA Plate.
   Note: This is the most time-consuming step of the protocol. Care must be taken to avoid cross-contamination between sample wells. Using an Anti-Static Polypropylene Weighing Funnel (TWD Scientific® cat. no. ASWF1S) can make it easier to weigh and add some sample types to each well without spilling into adjacent wells.
4. Seal the PowerBead DNA Plate well with a Sealing Mat. Vortex horizontally for 5 s ensuring that the solution/sample is mixed well.
   Note: A proper seal is critical to prevent sample loss and leakage that might damage the TissueLyser II.
   Note: This is an appropriate stopping point. You can store the PowerBead DNA Plate covered with a Sealing Mat at 2–8°C or at –15 to –30°C.

5. Place each PowerBead DNA Plate (with Sealing Mat securely affixed) between two Adapter Plates (cat. no. 11990). Place on a TissueLyser II (cat. no. 85300) and shake at speed 20 Hz for 10 min.
6. Remove plates and re-orient them so that the side closest to the machine body is now furthest from it. Shake again at speed 20 Hz for 10 min.
   Note: The block needs to be rotated to ensure uniform bead beating for all the wells.
7. Centrifuge the PowerBead DNA Plate at 4500 x *g* for 6 min at room temperature.
8. Carefully and without splashing, remove and discard the Sealing Mat and transfer the supernatant to a new 2 ml Collection Plate (provided).
   Note: The supernatant may still contain some biosolid particles.
9. Add 150 µl of Solution IRS to each well of the 2 ml Collection Plate and apply Sealing Tape. Vortex horizontally for 5 s until solution is mixed well and incubate at 2–8°C for 10 min.
10. Centrifuge the plate at 4500 x *g* for 6 min at room temperature. Remove and discard Sealing Tape.
11. Avoiding the pellets, transfer the entire volume of supernatant to a new 2 ml Collection Plate (provided). Apply Sealing Tape and centrifuge at 4500 x *g* for 6 min to clear any residual particulates that may have carried over. Remove and discard Sealing Tape.
    Note: For wells at the center of the plate, it may help to draw a line on the pipette tip to mark how far to insert the tip without touching the pellet.
12. Avoiding any residual pellet, transfer no more than 850 µl of supernatant to a new 2 ml Collection Plate (provided).
    Note: You may keep the supernatant in the 2 ml Collection Plate at 2–8°C for several hours if you need to stop or if you can only process one 96 well plate at a time.
13. Place the 2 ml Collection Plate containing the supernatant on the ep*Motion* robotic deck as indicated in the ep*Motion* program worktable.
14. For each 96 well plate to be processed, add 174 ml of ClearMag Wash Solution into an Eppendorf 400 ml reservoir placed at the appropriate location on the deck as indicated in the ep*Motion* program worktable.

15. For each 96 well plate to be processed, add 11 ml of RNase-Free Water into an Eppendorf 30 ml reservoir placed in an Eppendorf tub holder at the appropriate location on the deck as indicated in the ep*Motion* program worktable.
16. Vortex the bottle containing ClearMag Beads (Zorb Reagent) to resuspend the beads. For each 96 well plate to be processed, add 2 ml ClearMag Beads to 85 ml of ClearMag Binding Solution in a mixing vessel (user provided). Vortex well to mix.
17. Transfer the entire volume of ClearMag Binding Solution/ClearMag Beads into an Eppendorf 100 ml reservoir placed in an Eppendorf tub holder located at the appropriate location on the deck as indicated on the ep*Motion* program worktable.
18. Initiate the protocol.
    Note: Start the protocol immediately to avoid settling of the beads. If there is a delay of more than 3 min, re-agitate the beads.
19. Upon completion, cover the wells of the 96 Well Microplate (provided) with an Elution Sealing Mat (provided). DNA and/or RNA are now ready for downstream applications.
    Note: We recommend storing DNA and RNA frozen (–15 to –30°C and –65 to –90°C respectively).

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
Soil processing		
a)	Amount of sample to process	The MagAttract PowerMicrobiome DNA/RNA EP Kit is designed to process 0.25 g of sample. For efficient 96 well homogenization, we do not recommend increasing the amount of sample processed.
b)	Stabilizing samples for storage and during processing	Add 100 µl of phenol:chloroform:isoamyl alcohol (25:24:1; pH 6.5–8) to each well of the PowerBead DNA Plate pre-loaded with 650 µl of Solution MBL/β-ME (after step 2; before filling with samples) to quickly inactivate nucleases and stabilize samples during both sample addition and plate storage at –15 to –30°C overnight, if desired. If you don't want to use phenol:chloroform:isoamyl alcohol, pre-loading wells with Solution MBL/β-ME before adding samples and then storing overnight at –15 to –30°C overnight, if desired, will also offer additional protection during the time the samples are in the block at room temperature during filling.
c)	Using a centrifuge with a maximum speed less than 4500 x *g*	Multiply the protocol time and speed to determine the total force required (x *g*). Divide this total by the maximum speed of your centrifuge (round up if necessary). This will be the number of minutes your centrifuge will need to run to achieve the appropriate overall force. **Example:** 10 min at 4500 x *g* = 45,000. If your centrifuge has a maximum speed of 2500 x *g*, divide 45,000 by 2500 = 18 min of centrifugation.
Alternative lysis methods		
a)	Difficult to lyse cells	After adding Solution MBL and sample (step 3), incubate the PowerBead DNA Plate at 70°C for 10 min. After the incubation, proceed with step 4.

		Comments and suggestions
DNA		
a)	DNA does not amplify	Check DNA and RNA yields by gel electrophoresis or spectrophotometer reading. DNA template is typically added at 10 ng per reaction, although more or less may be needed depending on the reaction conditions, enzyme activity and copy numbers of the target sequences. If DNA does not amplify after altering the amount of template used, then PCR optimization (changing reaction conditions, validating primers or testing different polymerases) may be needed.
b)	Concentrating eluted DNA	The final volume of eluted DNA and RNA will be 100 µl. Nucleic acids may be concentrated by adding 5 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. Incubate at –15 to –30°C for at least 10 min to overnight. Centrifuge at 13,000 x *g* for 15 minutes. Decant all liquid and wash the pellet with ice-cold 70% ethanol. Centrifuge at 10,000 x *g* for 10 min to re-pellet nucleic acids. Decant and remove residual ethanol in a speed vac, a dessicator or air dry. Resuspend precipitated nucleic acids in sterile water or sterile 10 mM Tris. **Note:** This procedure must be done individually; eluted samples must be transferred to microcentrifuge tubes.
c)	Storing DNA	DNA and RNA is eluted in RNase-Free Water and must be stored at –15 to –30°C and –65 to –90°C respectively to prevent degradation. DNA and RNA can be eluted in 10 mM Tris buffer, pH 7, or TE without loss, but the EDTA in TE may inhibit downstream reactions such as PCR and automated sequencing. Prolonged storage in 96 Well Microplates at 2–8°C will result in loss of liquid due to evaporation.

Ordering Information

Product	Contents	Cat. no.
MagAttract PowerMicrobiome DNA/RNA EP Kit (384)	For 384 preps: Hands-free isolation of nucleic acids from stool and gut material using an automated processing or liquid handling system	27500-4-EP
Related products		
MagAttract PowerSoil® DNA KF Kit (384)	For 384 preps: Hands-free isolation of DNA from soil using automated processing and liquid handling systems	27000-4-KF
MagAttract PowerSoil DNA EP Kit (384)	For 384 preps: Hands-free isolation of DNA from soil using automated processing and liquid handling systems	27100-4-EP
MagAttract Microbial DNA Kit (384)	For 384 preps: Automated isolation of DNA from microbial and food cultures using automated processing and liquid handling systems	27200-4
MagAttract PowerMicrobiome DNA/RNA KF Kit (384)	For 384 preps: Hands-free isolation of nucleic acids from stool and gut material using an automated processing or liquid handling system	27600-4-EP
MagAttract PowerClean® DNA Kit (384)	For 384 preps: Automated removal of PCR inhibitors from previously purified DNA using magnetic bead technology	27900-4-KF
TissueLyser II	For medium- to high-throughput sample disruption for molecular analysis	85300

Product	Contents	Cat. no.
Plate Adapter Set	Set of four adapters required to assemble two 96 well plates onto the 96 Well Plate Shaker	11990

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

Notes

Limited License Agreement for MagAttract PowerMicrobiome DNA/RNA EP Kit

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

1. The product may be used solely in accordance with the protocols provided with the product and this handbook and for use with components contained in the kit only. QIAGEN grants no license under any of its intellectual property to use or incorporate the enclosed components of this kit with any components not included within this kit except as described in the protocols provided with the product, this handbook, and additional protocols available at www.qiagen.com. Some of these additional protocols have been provided by QIAGEN users for QIAGEN users. These protocols have not been thoroughly tested or optimized by QIAGEN. QIAGEN neither guarantees them nor warrants that they do not infringe the rights of third-parties.
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