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MagAttract® PowerWater® DNA/RNA Kit Handbook

Automated isolation of nucleic acids from filtered air and water samples

Sample to Insight

QIAGEN

Contents

Kit Contents

MagAttract PowerWater DNA/RNA Kit	**(384)**
Catalog no.	**27800-4-EP**
Number of preps	**4 x 96**
PowerWater DNA Bead Tubes	8 x 50
Solution MBL	2 x 424 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
2 ml Collection Plates	3 x 4
96 Well Microplates	4
Sealing Tape	2 x 16
Elution Sealing Mats	4
Quick Start Protocol	1

Storage

All components of the MagAttract PowerWater DNA/RNA 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 PowerWater DNA/RNA Kits is tested against predetermined specifications to ensure consistent product quality.

Introduction

The MagAttract PowerWater DNA/RNA Kit is a magnetic bead-based nucleic acid isolation kit optimized for use with the Eppendorf® ep*Motion*® 5075 TMX platform. It is also compatible with other automated platforms with similar features.

The MagAttract PowerWater DNA/RNA Kit can be used for automated isolation of microbial DNA and RNA from any liquid or air sample that is concentrated on a filter membrane. Both 25 mm and 47 mm filter membranes made of a variety of materials can be processed with this kit, which 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 without binding residual contaminants. The result is inhibitor-free DNA and RNA that is ready to use in demanding downstream applications, including PCR, qPCR, RT-qPCR and next-generation sequencing (NGS).

The MagAttract PowerWater DNA/RNA Kit has been used to isolate DNA and RNA from a variety of different membrane types used for air and water sampling (Table 1).

Table 1. Membrane types used with the MagAttract PowerWater DNA/RNA Kit

Membrane type	Air	Water
Mixed cellulose ester (MCE)	✔	✔
Cellulose acetate (CA)	✔	✔
Cellulose nitrate (CN)	✔	✔
Polyethersulfone (PES)	✔	✔
Glass fiber (GF/F)*	✔	✔
Polycarbonate (PC)	✔	✔
Nylon	✔	
Gelatin*	✔	

* The use of glass fiber filter (GF/F) and gelatin membranes requires modifications to the protocol to ensure optimal recovery of DNA and RNA. Please read the notes accompanying protocol steps and refer to Appendix B: Using GF/F or Gelatin Membranes.

Principle and procedure

Individual 25 mm or 47 mm filter membranes are placed into PowerWater DNA Bead Tubes that fit in the 5 ml Tube Adapter Set (cat. no. 11980) for rapid and thorough homogenization using the TissueLyzer II (cat. no. 85300). Cell lysis occurs by a combination of mechanical and chemical methods. Inhibitory compounds are removed using IRT. Total nucleic acids are captured on specialized magnetic beads in the presence of buffers that avoid the use of chaotropic salts and ethanol. DNA and RNA is washed on the beads and then eluted using RNase-Free Water.

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 PowerWater DNA/RNA Kit is optimized for use with the Eppendorf 5075 TMX platform to isolate DNA and RNA from up to 850 µl of lysate per well in the 2 ml Collection Plates (provided). This kit requires the use of a 96 well plate shaker on the robotic deck.

For optimal sample processing, this kit requires the use of a specialized plate shaker to facilitate the bead beating process in the PowerWater DNA Bead Tubes. We recommend the TissueLyser II and 5 ml Tube Adapter Sets. The 5 ml Tube Adapter Set can accommodate up to 16 bead tubes each, which allows for the simultaneous processing of 32 samples. To centrifuge all 32 samples at the same time, we recommend 5 ml Tube Centrifuge Blocks (cat. no. 11981) that fit into a 96 well plate bucket.

The plastic blocks recommended for use with the MagAttract PowerWater DNA/RNA Kit are provided. These blocks 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 software protocol.

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.

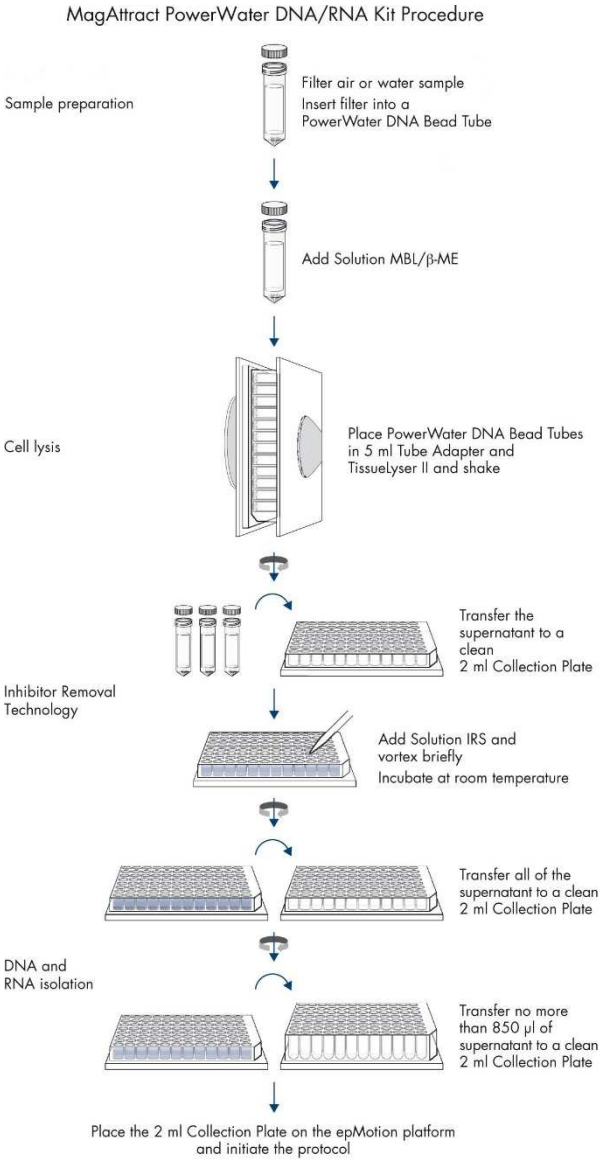

Figure 1. MagAttract PowerWater DNA/RNA Kit procedure.

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.

- Disposable/reusable filter funnels or air sampling unit
- Filter membranes (if using a reusable filter funnel)
- Centrifuge capable of handling 5 ml Tube Centrifuge Blocks (cat. no. 11981) and two 96 well blocks (13 cm x 8.5 cm x 6.0 cm) at 4500 x *g*
  Note: If you have a centrifuge with a maximum speed less than 4500 x *g*, refer to the Troubleshooting Guide.
- Multi-channel pipettors (50–1000 µl)
- Single-channel pipettors
- 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).
- TissueLyser II (cat. no. 85300) and 5 ml Tube Adapter Set (cat. no. 11980)
- Vortex-Genie® 2 Vortex with 3 inch platform
- β-mercaptoethanol (β-ME)
- Please contact your Eppendorf representative for the ep*Motion* plastic disposables specific to your platform.
- 96 well plate shaker

Protocol

Important points before starting

- Warm Solution MBL at 60°C for 15–20 minutes before use to dissolve precipitates.
- To extract both DNA and RNA or only RNA, add 25 ml of β-mercaptoethanol (β-ME) per 975 µl of Solution MBL. You will need 98 ml of Solution MBL/β-ME per 96 samples (1 ml/sample + 2 ml to account for loss during pipetting).
- To extract only DNA, add 9 µl of RNase A (cat. no. 19101) per ml of Solution MBL. You will need 98 ml of Solution MBL/RNase A per 96 samples; β-ME is not required.

Procedure

1. Filter air or water sample through a 25 mm or 47 mm membrane.
   Note: If you are using glass fiber filter (GF/F) or gelatin membranes, please refer to Appendix B: Using GF/F or Gelatin Membranes before continuing.
2. Using two sets of sterile forceps, pick up the filter membrane at opposite edges and roll the filter into a cylinder with the top side facing inward.
3. Insert the rolled filter membrane into a 5 ml PowerWater DNA Bead Tube.
4. Add 1 ml of warmed Solution MBL/ β-ME to each PowerWater DNA Bead Tube (if isolating only DNA, add 1 ml of warmed Solution MBL/RNase A).
   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.
5. Place 16 PowerWater DNA Bead Tubes into each 5 ml Tube Adapter (cat. no. 11980) and place on a TissueLyzer II (cat. no. 85300). Refer to the protocol provided with the 5 ml Tube Adapter Set for proper placement. Shake at speed 20 Hz for 5 min.
   Note: If using GF/F, shake at speed 10 Hz for 5 min.
6. After the 5 min cycle, rotate the 5 ml Tube Adapter assemblies so that the side closest to the machine body is now furthest from it. Shake again at speed 20 Hz for 5 min.
   Note: If using GF/F shake at speed 10 Hz for 5 more min.

Note: The 5 ml Tube Adapter assemblies need to be rotated to ensure uniform bead beating for all PowerWater DNA Bead Tubes. For assistance with loading/unloading the 5 ml Tube Adapter assemblies, please contact QIAGEN Technical Support at **support.qiagen.com**.

7. Centrifuge PowerWater DNA Bead Tubes at 4500 x *g* for 1 min at room temperature.
   Note: You will need 5 ml Tube Centrifuge Blocks (cat. no. 11981).
   Note: If using GF/F, transfer the entire content of the PowerWater DNA Bead Tube to a 10 ml syringe barrel. Place the syringe barrel in a 15 ml tube and centrifuge for 3 min at 4000 x *g* to recover the supernatant.
8. Transfer to a clean 2 ml Collection Plate (provided). Push the pipette tip through the beads into the bottom of the PowerWater DNA Bead Tube to recover as much supernatant as possible.
   Note: The supernatant may still contain some biosolid particles.
9. Add 200 µl of Solution IRS to each well and apply Sealing Tape. Vortex horizontally for 5 s to ensure that the solution is mixed well. Incubate at room temperature for 5 min.
10. Centrifuge at 4500 x *g* for 6 min at room temperature. Remove and discard Sealing Tape.
11. Avoiding the pellet, transfer all of the supernatant to a new 2 ml Collection Plate (provided).
12. Apply Sealing Tape. Centrifuge at 4500 x *g* for 6 min to clear any residual particulates.
13. Avoiding the pellet, transfer no more than 850 µl of supernatant to a new 2 ml Collection Plate (provided). Place the 2 ml Collection Plate containing the supernatant on the ep*Motion* robotic deck as indicated in the ep*Motion* program worktable.
    Note: If you are using gelatin filters, add 30 µl of Proteinase K (20 mg/ml) and incubate at 65°C for 10 min.
    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.

14. For each 96 well plate to be processed, add 174 ml of ClearMag Wash Solution into an Eppendorf 30 ml reservoir placed in an Eppendorf tub holder located 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 of ClearMag Beads to 85 ml of ClearMag Binding Solution in a mixing vessel (user provided). Vortex well to mix.
17. Transfer all of the 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 plate 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
Sample processing		
a)	Stabilizing samples for storage and during processing	Filter membranes will remain stable if stored dry. When possible, store PowerWater DNA Bead Tubes containing filter membranes without Buffer MBL at –15 to –30°C.
b)	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.
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.

		Comments and suggestions
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 minutes to overnight. Centrifuge at 13,000 x *g* for 15 minutes. Decant all liquid and wash the DNA pellet with ice-cold 70% ethanol. Centrifuge at 10,000 x *g* for 10 min to re-pellet the DNA. Decant and remove residual ethanol in a speed vac, a dessicator or air dry. Resuspend precipitated DNA in sterile water or sterile 10 mM Tris. **Note:** This procedure must be done after transferring the eluted samples to individual microcentrifuge tubes.
c)	Storing DNA and RNA	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 the microplates at 2–8°C will result in the loss of liquid due to evaporation.
Alternative lysis methods		
a)	Difficult to lyse cells	When working with organisms that have proven to be difficult to lyse using mechanical or chemical methods, incubate at 65°C for 10 min after adding Solution MBL (step 4). However, this could result in some degradation of RNA depending on which microorganisms are present. After the incubation, proceed with the mechanical lysis step using the TissueLyzer II (Step 5).

Appendix A: Water and Air Sampling

The amount of water or air that can be filtered through a particular type of filter membrane varies depending on the sample source and the filter membrane material, diameter and pore size.

Clear water samples:

Water samples may vary from clear to highly turbid. Larger volumes of clear water can be processed because there is less chance of clogging the filters. Potable drinking water will generally allow for very high volumes depending on the quality and particulate count. In most cases, 100 ml to 10 liters can be processed, and some users report processing even higher volumes.

Turbid water samples

Turbid samples with high levels of suspended solids or sediments will tend to quickly clog filters with smaller pore size (0.22 micron). Using 0.45 micron filters is recommended for these types of samples. Prior to filtering, samples can be stored in a container to allow suspended solids to settle out. For samples where settling does not occur or is not desired, stacking filters with larger pore sizes on top of a filter membrane of the desired pore size is recommended.

A common setup is to stack a sterile 1 micron filter on top of a membrane with a smaller pore size (0.22 or 0.45 micron). This layering filters out large debris and allows the filter with smaller pore size to trap microorganisms. The layered filter system can be washed with sterile saline or sterile phosphate buffer to knock down some of the microorganisms trapped on the larger pore-size filters. Although this process is not 100% efficient, it will increase the overall yield of microbial DNA.

Air samples

Air is typically considered a low biomass sample and utilizes 25–35 mm filter membranes. Extended sampling times and sample heating during the protocol (see the section on

"Difficult to Lyse Cells" in the Troubleshooting Guide; page 15) may be required to improve nucleic acid yields. Membrane pore sizes typically range from 0.45 micron to 5 micron and are dependent on the filter membrane type and the air collection device. Depending on the air collection device and pump, smaller pore sizes may result in increased back pressure and reduced air flow. To reduce this problem select a pore size that is recommended for the air sampling device or results in optimal flow rate.

Appendix B: Using GF/F or Gelatin Membranes

Using GF/F membranes

Glass fiber filter (GF/F) membranes are highly absorbent and will retain most if not all of the Solution MBL/β-ME, regardless of membrane size (25 mm or 47 mm). To recover the supernatant after the mechanical lysis step, the membrane must be placed in a column that will retain the membrane but allow the supernatant to be centrifuged out for use in subsequent steps. To do this, remove the plunger from a 10 ml syringe barrel and discard. Carefully transfer the contents of the PowerWater DNA Bead Tube into the syringe barrel and place the barrel in a 15 ml tube. Centrifuge at 4,000 x *g* for 3 minutes. The supernatant should be at the bottom of the 15 ml tube while the membrane is still retained in the syringe barrel.

When GF/F membranes are mechanically homogenized with the same settings used for other types of filter membrane, DNA shearing can increase. To reduce DNA shearing, we recommend reducing the 96 well plate shaker setting to speed 10 (during steps 5 and 6 of the protocol).

Using gelatin membranes

Gelatin membranes are typically used for sampling microorganisms, including viral particles, that are viable and can be cultured. These membranes dissolve in aqueous buffers and on agar plates for culturing. To extract DNA and RNA from these membranes, a Proteinase K digestion prior to bead beating is required. After adding Solution MBL/β-ME, add 30 µl of Proteinase K (20 mg/ml) and incubate at 65°C for 10 minutes. Continue with Step 5 of the protocol.

Ordering Information

Product	Contents	Cat. no.
MagAttract PowerWater DNA/RNA Kit (384)	For 384 preps: Automated isolation of nucleic acids from filtered air and water samples	27800-4-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 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
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

Product	Contents	Cat. no.
PowerLyzer® 24 Bench Top Bead-Based Homogenizer (110/220 V)	For the most efficient and complete lysis and homogenization of any biological sample	13155
TissueLyser II	For medium- to high-throughput sample disruption for molecular analysis	85300
5 ml Tube Adapter Set	For sample homogenization using 5 ml bead tubes on a TissueLyzer II	11980
5 ml Tube Centrifuge Blocks	For easy centrifugation of MO BIO 5 ml bead tubes in standard 96 well centrifuge buckets	11981

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 PowerWater DNA/RNA Kit

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

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