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MagAttract® Microbial DNA Kit Handbook

For hands-free isolation of DNA from microbial and food cultures using automated processing and liquid handling systems

Sample to Insight

QIAGEN

Contents

Kit Contents

MagAttract Microbial DNA Kit	**(384)**
Catalog no.	**27200-4**
Number of preps	**4 x 96**
PowerBead DNA Plates, Glass 0.1 mm	4
SwiftMag Beads	22 ml
Solution MBL	2 x 150 ml
Solution IRS	2 x 44 ml
Solution EB	51 ml
RNase A Solution	4 x 2 ml
Collection Plates (1 ml)	4
Collection Plates (2 ml)	4
Sealing Tape	2 x 16
Elution Sealing Mats	4
Quick Start Protocol	1

Storage

RNase A Solution can be stored at room temperature (15–25°C) for 1 year. For storage longer than 1 year or if ambient temperatures often exceed 25°C, we recommend keeping the RNase A Solution at 2–8°C.

All other reagents and components of the MagAttract Microbial DNA Kit can be stored at room temperature (15–25°C) until the expiration date printed on the box 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 Microbial DNA Kits is tested against predetermined specifications to ensure consistent product quality.

Introduction

The MagAttract Microbial DNA Kit is optimized for use with the Thermo Scientific™ KingFisher™ Flex, the KingFisher Duo and the Eppendorf® ep*Motion*® 5075 TMX platforms.

The MagAttract Microbial DNA Kit can be used for automated isolation of high-quality genomic DNA from pure microbial cultures, food cultures and swabs. A variety of microorganisms, including bacterial spores, fungal types and food samples such as meats, cheeses and dairy products, chocolate, fruits, vegetables and juices have been tested successfully with this kit.

Principle and procedure

The MagAttract Microbial DNA Kit protocol is designed to isolate DNA from up to 450 µl of lysate. The protocol uses Inhibitor Removal Technology® (IRT) to remove PCR-inhibiting compounds associated with food cultures, including lipids and polysaccharides. A novel, proprietary magnetic bead system is used to isolate nucleic acids, which results in inhibitor-free DNA that is ready to use in the most demanding downstream applications, including PCR, qPCR and next-generation sequencing (NGS).

Pelleted cells are resuspended in lysis solution and RNase A Solution, added to a 96 well bead beating plate and homogenized rapidly and thoroughly. Cell lysis occurs by a combination of mechanical and chemical methods. IRT provides efficient contaminant removal and improved DNA yields. Total genomic DNA is captured on magnetic beads in the presence of ethanol, which eliminates the use of any chaotropic salts. DNA captured on the beads is washed and then eluted using a low salt buffer (10 mM Tris, pH 8). The eluted DNA is ready for qPCR, NGS, and other downstream applications.

The MagAttract Microbial DNA Kit requires the use of a specialized plate shaker for 96 well homogenization of samples in blocks. We recommend the TissueLyser II (cat. no. 85300) and Plate Adapter Set (cat. no. 11990). For low through-put platforms such as the KingFisher Duo, homogenization may also be performed in 2 ml bead tubes using a Vortex-Genie® 2 or a

high-powered bead beater such as the PowerLyzer® 24 Homogenizer (cat. no. 13155). With both of these methods, lysates need to be transferred individually to appropriate 96 well plates for automation on the KingFisher or ep*Motion* platforms.

The order of placement of components and reagents for the platform portion of the protocol will be described in the downloaded software specific to the platform being used.

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

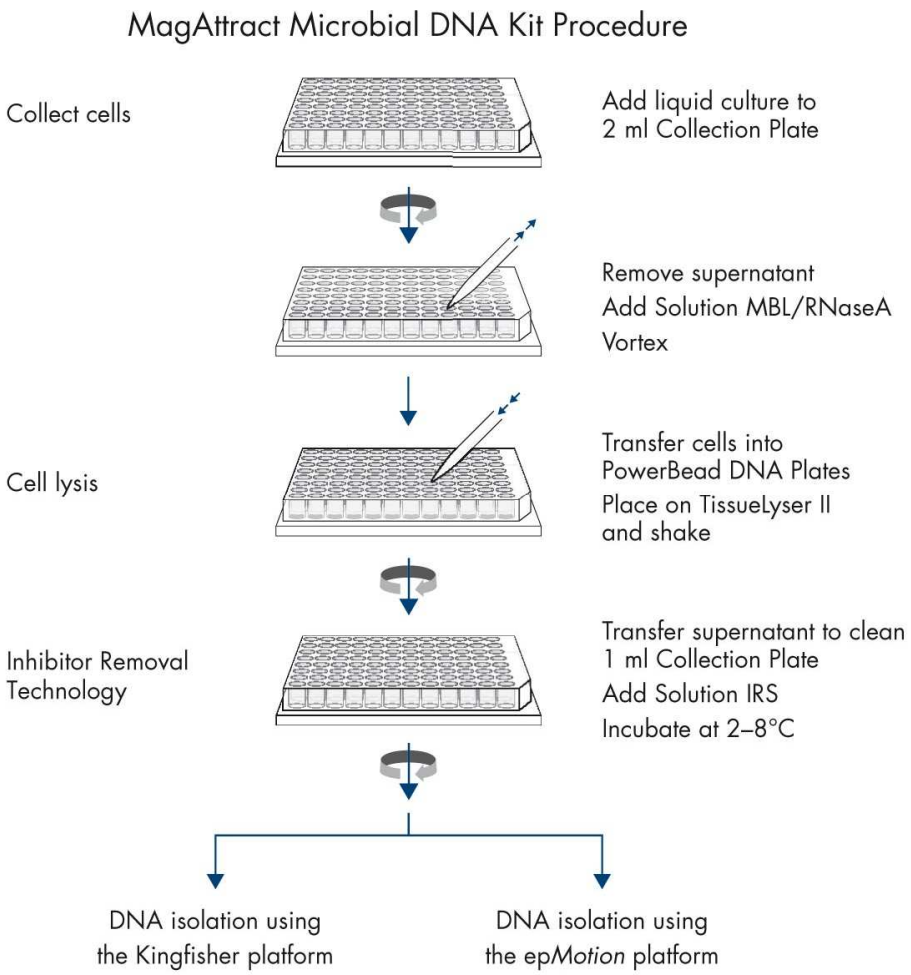

Figure 1. MagAttract Microbial DNA Kit procedure.

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.

- 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)
  Note: A 12 channel pipettor is required if multichannel pipetting is desired when using the KingFisher Duo 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).
- Single-channel pipettors (5–500 µl)
- Mechanical shaker for 96 well plates
  Note: We recommend the TissueLyser II (cat. no. 85300) and Plate Adapter Set (cat. no. 11990).
- 100% ethanol
- Vortex-Genie 2 Vortex
- **Optional:** Vortex Adapter for vortexing 1.7 or 2 ml tubes (cat. no. 13000-V1-24)
- Please contact your Thermo Fisher Scientific representative for KingFisher plastic disposables or your Eppendorf representative for ep*Motion* plastic disposables specific to the platform being used
- Kingfisher users will require reagent reservoirs for 5–300 ml

- Kingfisher Duo user will require appropriate 96 well storage plates capable of holding 450 µl and plate seals
- Eppendorf ep*Motion* users will require the following plastics (not provided):
  - 96 Well MASTERBLOCK® 2 ml Plates (Greiner [cat. no. 780280]; 4 plates required)
  - 96 Well MASTERBLOCK Elution Sealing Mats (Greiner [cat. no. 381070]; 4 mats required)
  - 96 Well Microplates (Greiner [cat. no. 650201]; 8 plates required)

Protocols

Important points before starting

- Warm Solution MBL at 60°C for 10 minutes before use. Use while still warm.
- Before starting, add 9 µl of the provided RNase A Solution per 1 ml of warmed Solution MBL. To allow for pipetting variations and overage for the reagent reservoir, add 315 µl of the RNase A Solution to 35 ml of Solution MBL. For KingFisher Duo applications add 350 µl of Solution MBL followed by 3 µl of RNase A Solution.
- You will need 333 ml of 100% ethanol for each full 96 well plate processed on the KingFisher platform and 363 ml of 100% ethanol for the ep*Motion* platform. The KingFisher Duo requires 36 ml of 100% ethanol for each 12 wells processed.
- Before first use, centrifuge PowerBead DNA Plates at 4500 x *g* for 3 minutes. Remove and discard Elution Sealing Mats.

Procedure

1. Dispense 1.8 ml of liquid culture into each well of a 2 ml Collection Plate and cover with Sealing Tape. Centrifuge at 4500 x *g* for 12 min.
2. Discard Sealing Tape and remove media without disturbing the cell pellet.
   Note: Excessive amounts of residual media will dilute the lysis chemistry; only minimal amounts of residual media should be left in each well.
3. Add 350 µl of Solution MBL/RNase A and apply fresh Sealing Tape (provided). Resuspend the cell pellet completely by high-speed vortexing. Centrifuge briefly to ensure that the cell suspension is at the bottom of the wells. Avoid re-pelleting the cells.
4. Centrifuge a PowerBead DNA Plate, Glass 0.1 mm at 4500 x *g* for 3 min. Remove the Elution Sealing Mat and discard. Transfer cell suspensions into PowerBead DNA Plate.
5. Seal the PowerBead DNA Plate with a new Elution Sealing Mat (provided).

6. Place each PowerBead DNA Plate (with Elution Sealing Mat securely affixed) between two Adapter Plates (cat. no. 11990). Place on a TissueLyzer II (cat. no. 85300). Refer to the protocol provided for placement of Adapter Plates. Shake at speed 20 Hz for 5 min.
7. 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 5 min.
8. Centrifuge the PowerBead DNA Plate at 4500 x *g* for 6 min.
9. Remove and discard Elution Sealing Mat. Avoiding the glass beads, transfer the supernatant to a clean 1 ml Collection Plate.
   Note: Supernatant may still contain some beads.
10. Add 100 µl of Solution IRS to the wells of the plate and cover with Sealing Tape. Vortex for 5 s. Incubate at 2–8 °C for 10 min, then centrifuge the plate at 4500 x *g* for 9 min.
    Note: This step removes inhibitors that may be present and glass beads that may have carried over.
11. Open the protocol specific to your platform. For the KingFisher Flex protocol, go to page 13, for the ep*Motion* protocol, go to page 14 and for the KingFisher Duo protocol, go to page 15.

KingFisher Flex

Continued from step 11 on page 12

12. Avoiding the pellet, transfer up to 450 µl of supernatant to the appropriate wells on a KingFisher Microtiter Deep Well 96 Plate (user provided).
    Note: You may keep the supernatant in the plate at 2–8°C for several hours if you need to stop during the protocol or if you can only process one 96 well plate at a time.
13. Resuspend the SwiftMag Beads by vortexing the bottle. Add 5 ml of the resuspended SwiftMag Beads to 45 ml of 100% ethanol in an appropriate vessel (user provided) for each plate to be processed. Immediately transfer to a multi-channel reservoir.
14. Mix well. Add 500 µl of SwiftMag Beads/ethanol to each well of the KingFisher Microtiter Deep Well 96 Plate containing lysate.
    Note: Work quickly; SwiftMag Beads/ethanol will slowly settle over time. Maintain the beads in suspension for uniform distribution to each well.
15. Place the KingFisher Microtiter Deep Well 96 Plate containing the lysate and SwiftMag Beads/ethanol on the deck as indicated in the display on the instrument.
16. Add 1 ml of 100% ethanol into each corresponding well of three KingFisher Microtiter Deep Well 96 Plates (user provided) and place on the deck as indicated in the display.
17. Place 100 µl of Solution EB into each corresponding well of a KingFisher 96 KF plate and place on the deck as indicated.
18. Initiate the KingFisher PowerMag® Microbial DNA Isolation protocol program.
19. Upon completion, cover the wells of the KingFisher 96 KF Plate with an appropriate storage seal (user provided). The DNA is now ready for downstream applications.
    Note: We recommend storing DNA frozen (–15 to –30°C or –65 to –90°C) as Solution EB does not contain EDTA.

Eppendorf ep*Motion*

Continued from step 11 on page 12

12. Avoiding the pellet, transfer up to 450 µl of supernatant to the appropriate wells on a Greiner 96 Well 2 ml Plate (Greiner cat. no. 780280; see page 10 for details) and place at the appropriate location on the deck as indicated by the DNA isolation robotic program.
    Note: You may keep the supernatant in the plate at 2–8°C for several hours if you need to stop during the protocol or if you can only process one 96 well plate at a time.
13. For each plate to be processed, place two Greiner 96 Well Microplates (Greiner cat. no. 650201; see page 10 for details) at the appropriate locations on the deck as indicated in the ep*Motion* software. These plates will be referred to as MTP1 and MTP2 by the software.
14. For each plate to be processed, place 318 ml of 100% ethanol into an Eppendorf 400 ml reservoir placed at the appropriate location on the deck as indicated in the ep*Motion* software.
15. For each plate to be processed, place 11 ml of Solution EB 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* software.
16. Resuspend the SwiftMag Beads by vortexing the bottle. Add 5 ml of the resuspended SwiftMag Beads to 45 ml of 100% ethanol in an appropriate vessel (user provided) for each plate to be processed. Mix well and place the entire volume into an Eppendorf 100 ml reservoir placed in an Eppendorf tub holder located at the appropriate location on the deck as indicated in the ep*Motion* software.
17. Initiate the ep*Motion* PowerMag Microbial DNA Isolation protocol program.
    Note: Start the program immediately as SwiftMag Beads/ethanol will slowly settle over time.

18. Upon completion, cover the wells of the Greiner 96 Well Microplate (MTP 2) with a Greiner Elution Sealing Mat (Greiner cat. no. 381070; see page 10 for details). The DNA is now ready for downstream applications.
    Note: We recommend storing DNA frozen (–15 to –30°C or –65 to –90°C) as Solution EB does not contain EDTA.

KingFisher Duo

Continued from step 11 on page 12

12. Avoiding the pellet, transfer up to 450 µl of supernatant to the corresponding wells on an appropriate 96 well plate (user provided).
    Note: You may keep the supernatant in the plate at 2–8°C for several hours if you need to stop during the protocol.
13. Transfer lysate from up to 12 wells to the first long row (A) on a KingFisher Microtiter Deep Well 96 Plate.
14. Add 450 µl of 100% ethanol to each well in row A that contains lysate.
15. Resuspend SwiftMag Beads by vortexing the bottle. Immediately add 50 µl of the resuspended SwiftMag Beads to each well in row A containing lysate/ethanol mixture.
    Note: Work quickly; SwiftMag Beads/ethanol will slowly settle over time. Maintain the beads in suspension for uniform distribution to each well
16. Place a KingFisher Duo 12 tip comb into the second row (B) of the KingFisher Microtiter Deep Well 96 Plate.
17. Add 1 ml of 100% ethanol into each well of the next three rows (C, D and E) of the KingFisher Microtiter Deep Well 96 Plate and place on the deck.
18. Add 100 µl of Solution EB into each well of a KingFisher Duo Elution Strip and place on the deck.
19. Initiate the KingFisher Duo PowerMag Microbial DNA Isolation protocol program.
20. Upon completion, cover the wells of the KingFisher Duo Elution Strip with an appropriate storage seal (user provided). The DNA is now ready for downstream applications.
    Note: We recommend storing DNA frozen (–15 to –30°C or –65 to –90°C) as Solution EB does not contain EDTA.

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)	Sample contains cells that are difficult to lyse	Incubate at 70°C for 10 minutes after adding Solution MBL/RNase A Solution (step 3). Then continue with step 4.
b)	Enhance lysis using freeze-thaw cycles	Add samples to the PowerBead DNA Plate and maintain at either –20°C or at –70°C until the samples are completely frozen. Immediately float the PowerBead DNA Plate in a 65°C water bath. Repeat the freeze-thaw cycle.
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.
DNA		
a)	DNA does not amplify	Check DNA yields using PicoGreen®, gel electrophoresis or spectrophotometry. Typically, 10–100 ng of DNA template is added per reaction, although more or less may be needed depending on the reaction conditions, enzyme activity and copy number of the target sequence. If DNA does not amplify after altering the amount of template per reaction, PCR optimization (changing reaction conditions, validating primers or testing a different polymerase) may be needed.

		Comments and suggestions
b)	Quantifying DNA	PicoGreen measurements of DNA yields are more accurate compared to spectrophotometry, which detects low molecular weight, digested RNA that may be recovered during purification. We recommend running the isolated DNA on an agarose gel (0.8–1.2%) to visually compare with the yield analysis.
c)	Concentrating eluted DNA	The final volume of eluted DNA will be 100 µl. The nucleic acids may be concentrated by adding 5 µl of 3 M NaCl and inverting 3–5 times to mix. Next, add 200 µl of 100% ice-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. Wash the nucleic acid pellet with 70% cold ethanol and centrifuge at 13,000 x *g* for 10 minutes to re-pellet. Decant all liquid. Briefly dry residual ethanol in a speed vac or ambient air. Avoid over-drying the pellet or resuspension may be difficult. Resuspend precipitated nucleic acids in desired volume of PCR-Grade Water (cat. no. 17000-10) or 10 mM Tris. **Note:** This procedure must be done after transferring the eluted samples to individual microcentrifuge tubes.
d)	Storing DNA	DNA is eluted in Solution EB (10 mM Tris) and must be stored at –15 to –30°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 in sterile DNA-Free PCR-Grade Water (cat. no. 17000-10). DNA that has been eluted in sterile water should be stored at –65°C to –90°C. Prolonged storage in the Greiner 96 Well Microplate (MTP 2) at 2–8°C will result in the loss of liquid due to evaporation.
e)	DNA floats out of a well when loading a gel	This usually occurs because residual ethanol remains in the final sample. The beads need to be completely dried before elution to prevent ethanol carryover. Ethanol precipitation (described in "Concentrating eluted DNA") is the best way to remove residual ethanol.
f)	Eluted DNA has color	With samples that have high secondary byproduct content (e.g., red pepper, chocolate, coffee), there is the unlikely possibility that the eluted DNA may contain some color. If this occurs, increase amount of Solution IRS used to 150 µl (step 10). If the eluted DNA still has color, please contact QIAGEN Technical Services (**support.qiagen.com**).

Ordering Information

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

Product	Contents	Cat. no.
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
Plate Adapter Set	Set of four adapters required to assemble two 96 well plates onto the 96 Well Plate Shaker	11990
PowerLyzer 24 Homogenizer (110/220V)	For the most efficient and complete lysis and homogenization of any biological sample	13155

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 Microbial DNA 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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