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MagAttract® PowerSoil® DNA KF Kit Handbook

For hands-free isolation of DNA from soil using automated processing and liquid-handling systems

Sample to Insight

QIAGEN

Contents

Kit Contents

MagAttract PowerSoil DNA KF Kit	**(384)**
Catalog no.	**27000-4-KF**
Number of preps	**384**
PowerBead DNA Plates, Garnet	4
PowerBead Solution	2 x 200 ml
SL Solution	2 x 15 ml
Solution IR	200 ml
ClearMag® Binding Solution	200 ml
ClearMag Zorb Reagent	9 ml
ClearMag Wash Solution	2 x 320 ml
Solution EB	51 ml
RNase A Solution	2 ml
Collection Plates (1 ml)	2 x 4
Sealing Tape	2 x 16
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 components of the MagAttract PowerSoil DNA KF Kit can be stored at room temperature 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 to directly to the sample preparation waste.**

PowerBead Solution contains 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 MagAttract PowerSoil DNA KF Kits is tested against predetermined specifications to ensure consistent product quality.

Introduction

The MagAttract PowerSoil DNA KF Kit is optimized for use with the Thermo Scientific™ KingFisher® Flex and KingFisher Duo platforms.

The MagAttract PowerSoil DNA KF Kit can be used for automated isolation of microbial DNA from all types of soil as well as other environmental samples high in humic acids, like compost, sediment and manure. The kit can be used to process as many as 384 samples of up to 0.25 grams each. The method 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 that is ready to use in demanding downstream applications, including PCR, qPCR and next-generation sequencing (NGS).

Principle and procedure

Environmental 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. Humic acids are removed using IRT. Prepared lysates are transferred to the KingFisher Flex or KingFisher Duo platforms where total genomic DNA is captured on specialized magnetic beads in the presence of buffers that avoid the use of chaotropic salts and ethanol. DNA is washed on the beads and then eluted using a 10 mM Tris (pH 8) buffer.

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 or interfere with NGS.

This 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 Sets (cat. no. 11990). 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).

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 KingFisher 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.

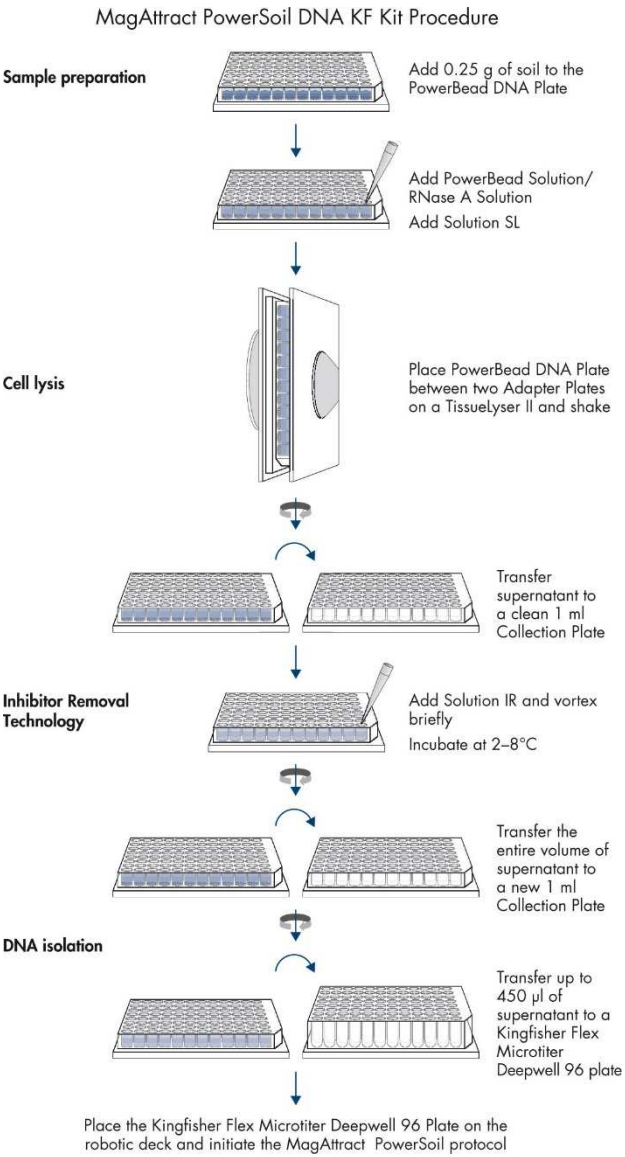

Figure 1. MagAttract PowerSoil DNA KF Kit procedure.

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 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 (100–850 µl)
  Note: The Kingfisher Duo applications require a 12-channel pipettor if multi-channel pipetting is desired when using that platform.
- Single-channel pipettors (5–1000 µl)
- For 96-well plates: Mechanical Shaker and Plate Adapter Sets (cat. nos. 11996 and 11990, respectively)
- Vortex-Genie 2 Vortex
- **Optional:** (for KingFisher Duo applications): Vortex Adapter for 24 (1.5–2 ml) tubes (cat. no. 13000-V1-24)
- Please contact your Thermo Fisher Scientific representative for specific KingFisher Flex and KingFisher Duo consumables.
- Multi-channel pipettor reagent reservoirs for 10–150 ml.
- 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 blocks. Examples of appropriate tips are Thermo Scientific ART™ (cat. no. 2179-HR), Eppendorf (cat. no. 0030077750) and Rainin™ (cat. no. RT-1000F).

Protocol: KingFisher Flex

Important points before starting

- Before starting, add 400 µl of RNase A Solution to 75 ml of PowerMag Bead Solution for every 96-well plate you plan to process.
- If SL Solution has precipitated, heat at 60°C until precipitate dissolves.

Procedure

1. Carefully peel off the Square Well Mat that covers the PowerBead DNA Plate and set aside. Add 0.25 g of soil sample to each well of the PowerBead DNA Plate.
   Note: This is an appropriate stopping point. You can store the PowerBead DNA Plate at 2–8 °C covered with the Square Well Mat.
2. Add 750 µl of PowerBead Solution/RNase A Solution to each well of the plate.
3. Add 60 µl of SL Solution to each well. Secure the Square Well Mat tightly.
   Note: A proper seal of the mat is critical to prevent loss of sample and leakage.
4. Place the PowerBead DNA Plate with mat securely fastened between 2 Adapter Plates (cat. no. 11990) on a Plate Shaker or TissueLyser II (cat. no. 85300).
5. Shake at speed 20 Hz for 10 min. Re-orient plates so that the side that was closest to the machine body is now farthest from it and shake again at speed 20 Hz for 10 min.
6. Centrifuge the plate at room temperature for 6 min at 4500 x *g*.
7. Carefully remove and discard the Square Well Mat. Transfer supernatant to a clean 1 ml Collection Plate.
   Note: The supernatant may still contain some soil particles.
8. Add 450 µl of Solution IR to each well. Apply Sealing Tape. Vortex horizontally for 5 s. Incubate at 2–8°C for 10 min. Centrifuge at room temperature for 6 min at 4500 x *g*.
   Note: You can skip the 10 min incubation. However, if you have already validated MagAttract PowerSoil extractions with this incubation, we recommend you retain this step.

9. Remove and discard Sealing Tape. Avoiding the pellet, transfer the entire volume of supernatant to a new 1 ml Collection Plate.
10. Apply Sealing Tape. Centrifuge at room temperature for 6 min at 4500 x *g*.
11. Taking care to avoid any residual pellet, transfer no more than 450 µl of supernatant from each well to a clean KingFisher Deep Well 96 Plate.
    Note: If you wish to use the remaining 400 µl of supernatant in each well, transfer to another KingFisher Deep Well 96 Plate and store at 2–8°C until they can be processed.
12. Resuspend ClearMag Beads by vortexing. For each 96-well plate to be processed, add 2 ml of the resuspended ClearMag Beads to 45 ml of ClearMag Binding Solution and mix well. Immediately transfer to a multi-channel pipette reservoir.
    Note: Maintain the ClearMag Beads in suspension to ensure uniform distribution.
13. Add 470 µl of the ClearMag Beads/ClearMag Binding Solution to each well containing lysate in a KingFisher Microtiter Deep Well 96 Plate.
14. Place the plate on the robotic deck at the specified location indicated in the program.
15. Add 500 µl of ClearMag Wash Solution to each well of three clean KingFisher Microtiter Deep Well 96 plates. Place on the robotic deck at the specified locations indicated in the program.
16. Add 100 µl of EB Solution to each well of a clean KingFisher 96 KF plate and place on the robotic deck at the specified location. Initiate the robotic program.
17. Upon completion of the robotic program, cover the wells of the KingFisher 96 KF plate with an appropriate storage seal. DNA is now ready for downstream applications.

Protocol: KingFisher Duo

Important points before starting

- Before starting, add 400 µl of RNase A Solution to 75 ml of PowerMag Bead Solution for every 96-well plate you plan to process.
- If the SL Solution has precipitated, heat at 60°C until precipitate dissolves.

Procedure

1. Carefully peel off the Square Well Mat that covers the PowerMag Bead DNA Plate and set aside. Add 0.25 g of soil sample to each well of the PowerBead DNA Plate.
   Note: This is an appropriate stopping point. You can store the PowerBead DNA Plate at 4°C covered with the Square Well Mat.
2. Add 750 µl of PowerBead Solution/RNase A Solution to each well of the plate.
3. Add 60 µl of SL Solution to each well. Secure the Square Well Mat tightly.
   Note: A proper seal of the mat is critical to prevent loss of sample and leakage
4. Place the PowerBead DNA Plate with mat securely fastened between 2 adapter plates (cat. no. 11990) on a Plate Shaker or TissueLyser II (cat. no. 85300).
5. Shake at speed 20 Hz for 10 min. Re-orient plates so that the side that was closest to the machine body is now farthest from it and shake again at speed 20 Hz for 10 min.
6. Centrifuge the plate at room temperature for 6 min at 4500 x *g*.
7. Carefully remove and discard the Square Well Mat. Transfer supernatant to a clean 1 ml collection plate.
   Note: The supernatant may still contain some soil particles.
8. Add 450 µl of IRT Solution to each well. Apply sealing tape. Vortex horizontally for 5 s. Incubate at 2–8°C for 10 min. Centrifuge at room temperature for 6 min at 4500 x *g*.
   Note: You can skip the 10 min incubation. However, if you have already validated the MagAttract PowerSoil extractions with this incubation, we recommend you retain this step.

9. Remove and discard sealing tape. Avoiding the pellet, transfer the entire volume of supernatant to a new collection plate.
10. Apply sealing tape. Centrifuge at room temperature for 6 min at 4500 x *g*.
11. Taking care to avoid any residual pellet, transfer no more than 450 µl of supernatant from each well to a clean KingFisher Deep Well 96 Plate.
    Note: If you wish to use the remaining 400 µl of supernatant in each well, transfer to another KingFisher Deep Well 96 Plate and store at 2–8°C until they can be processed.
12. Transfer lysate from up to 12 wells to the first long row (A) of a clean KingFisher Deep Well 96 Plate.
13. Add 450 µl of ClearMag Binding Solution to each well containing lysate in row A.
14. Resuspend ClearMag Beads by vortexing. Immediately add 20 µl of the resuspended ClearMag Beads to each well containing lysate/ClearMag Binding Solution mixture.
    Note: Maintain the ClearMag Beads in suspension to ensure uniform distribution.
15. Place a KingFisher Duo 12-tip comb into the second row (B) of the KingFisher Deep Well 96 Plate.
16. Add 500 µl of ClearMag Wash Solution to each well of the next three rows (C, D and E) of the KingFisher Deep Well 96 Plate and place on to the deck.
17. Add 100 µl of EB Solution to each well of a KingFisher Duo Elution Strip and place the strip on to the deck.
18. Initiate the KingFisher MO BIO PowerMag® Soil robotic program.
19. Upon completion of the robotic program, cover the wells of the KingFisher Duo Elution Strip with an appropriate storage seal. DNA is now ready for downstream applications.

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 soil to process	The MagAttract PowerSoil DNA KF Kit is designed to process 0.25 g of soil. For efficient 96 well homogenization, we do not recommend increasing the amount of soil processed.
b)	Soil sample is high in water content	Weigh the slurry and dispense into wells. We suggest restricting the starting weight of the slurry to 0.25 g. Increasing the amount of slurry used will increase volumes of subsequent steps.
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	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 MagAttract PowerSoil DNA KF 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.

		Comments and suggestions
b)	Concentrating eluted DNA	The final volume of eluted DNA will be 100 µl. The DNA 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 10,000 x *g* for 5 min at room temperature. Decant all liquid and wash the DNA pellet with cold 70% ethanol. Centrifuge at 10,000 x *g* for 10 min to pellet the DNA. 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 individually; eluted samples must be transferred to microcentrifuge tubes.
c)	Storing DNA	DNA is eluted in EB Solution (10 mM Tris) and must be stored at –15 to –30°C or –65 to –90°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). DNA that has been eluted with sterile water should be stored at –65°C to –90°C. 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	After adding SL Solution, incubate at 70°C for 10 min. After the incubation, proceed with the mechanical lysis step using the Plate Shaker (Step 4).
b)	Enhancing lysis using freeze-thaw cycles	Add samples to the PowerBead DNA Plate and maintain at –15 to –30°C or –65 to –90°C until the samples are completely frozen. Immediately float the PowerBead DNA Plate in a 65°C water bath. Repeat the freeze-thaw a second time and proceed with the addition of SL Solution. **Optional:** After the second freeze-thaw, SL Solution can be added along with Proteinase K Solution (cat. no. 1222-2) to improve lysis efficiency for some organisms.

Ordering Information

Product	Contents	Cat. no.
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
Related products		
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
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

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

Limited License Agreement for MagAttract PowerSoil DNA KF Kit

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

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