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MagAttract® PowerSoil® DNA EP Kit Handbook

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

QIAGEN

Contents

Kit Contents

MagAttract PowerSoil DNA EP Kit	**(384)**
Catalog no.	**27100-4-EP**
Number of preps	**4 x 96**
PowerBead DNA Plates, Garnet	4
PowerBead Solution	2 x 200 ml
Solution SL	2 x 15 ml
Solution IR	200 ml
ClearMag® Binding Solution	2 x 200 ml
ClearMag Zorb Reagent	9 ml
ClearMag Wash Solution	765 ml
Solution EB	51 ml
RNase A Solution	2 ml
Collection Plates (1 ml)	2 x 4
Collection Plates (2 ml)	4
Microplate (96 Well)	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 components of the MagAttract PowerSoil DNA EP 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 directly to the sample preparation waste.**

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

Introduction

The MagAttract PowerSoil DNA EP Kit is optimized for use with the Eppendorf® ep*Motion*® 5075 TMX platform.

The MagAttract PowerSoil DNA EP 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, such as compost, sediment and manure. 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 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. 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 Set (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 MagAttract PowerSoil DNA EP Kit is optimized for use with the Eppendorf ep*Motion* 5075 TMX platform to isolate DNA 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. A heating block is optional, but recommended. We also highly recommend using the QIAGEN 96 well Magnet Type A (cat. no. 36915) with large open-platform robots for best results. However, other magnetic separators that efficiently pull the magnetic beads away from the center of the well may be also used.

The plastic plates provided with the MagAttract PowerSoil DNA EP Kit have thin plastic walls that permit the efficient conductivity of magnetic field, which allows for faster and more complete separation of the magnetic beads from solution.

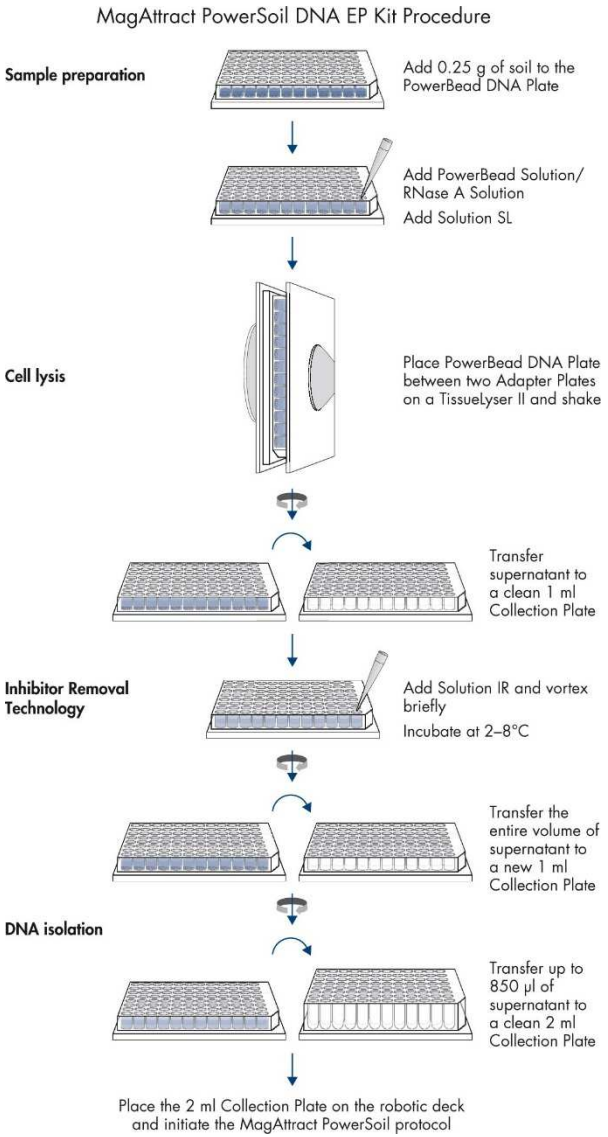

Figure 1. MagAttract PowerSoil DNA EP Kit procedure.

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 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 µl–850 µl)
- Single-channel pipettors (5 µl–1000 µl)
- For 96 well plates: Mechanical Shaker and Plate Adapter Sets (cat. nos. 11996 and 11990 respectively)
- Vortex-Genie 2 Vortex
- Multi-channel pipettor reagent reservoirs for 10–150 ml.
- 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 blocks. 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

- Add 4 µl RNase A Solution to each 750 µl of PowerBead Solution. Each 96 well plate will require exactly 72 ml of this mixture. To allow for pipetting variations and overage for the reagent reservoir, we suggest adding 400 µl of the RNase A Solution to 75 ml of the PowerMag® Bead Solution for every 96 well plate you plan to process.
- If Solution SL has precipitated, heat at 60°C until the precipitate has dissolved. Mix gently. Solution SL can be used while it is still warm.

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/RNase A Solution to each well of the PowerBead DNA Plate.
3. Add 60 µl of Solution SL to each well. Secure the Square Well Mat (from Step 1) tightly to the PowerBead DNA Plate.
4. **Note**: A proper seal of the mat is critical to prevent loss of sample and leakage.
5. Place each PowerBead Plate between 2 Adapter Plates (cat. no.11990) and place on a TissueLyser II (cat. no. 85300). Reference the protocol provided with the Adapter Plates for proper placement. Shake at speed 20 for 10 min.
6. After the first 10 min cycle, remove the block and rotate it so that the side closest to the machine body is now furthest from the machine. Shake again at speed 20 for 10 min.
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 Square Well Mat and transfer the supernatant to a clean 1 ml Collection Plate (provided).

9. Add 450 µl of Solution IR to each well and apply Sealing Tape to the 1 ml Collection Plate. Vortex horizontally for 5 s and incubate at 2–8°C for 10 min. Centrifuge the plate at 4500 x *g* for 6 min at room temperature. Remove and discard Sealing Tape.
10. Avoiding the pellet, transfer the entire volume of supernatant to a new 1 ml Collection Plate (provided). Apply Sealing Tape and centrifuge at 4500 x *g* for 6 min. Remove and discard Sealing Tape.
11. Avoiding any residual pellet, transfer no more than 850 µl of supernatant to a 2 ml Collection Plate (provided).
    Note: You may place 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.
12. Place the 2 ml Collection Plate containing the supernatant on the ep*Motion* robotic deck as indicated in the ep*Motion* program worktable.
13. 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.
14. For each 96 well plate to be processed, add 11 ml of Solution EB 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.
15. Vortex the bottle containing ClearMag Beads (Zorb Reagent) until beads are resuspended. 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.
16. 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 epMotion program worktable.
17. Initiate the ep*Motion* 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.
18. Upon completion, cover the wells of the 96 Well Plate with an Elution Sealing Mat (provided). The 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 EP 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 EP 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 –20°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 either –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 EP Kit (384)	For 384 preps: Hands-free isolation of DNA from soil using automated processing and liquid handling systems	27100-4-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 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
96 well Magnet Type A	For the separation of magnetic beads in wells of 96 well plates and 2 x 96 well Microplates FB	36915

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.

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Limited License Agreement for MagAttract PowerSoil DNA EP Kit

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

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