



September 2022

MagAttract[®] PowerSoil[®] Pro DNA Kit Handbook

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

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Kit Contents

MagAttract PowerSoil Pro DNA Kit (for use on KingFisher™)
Catalog no.
No. of preps

(384)
47109
384

Solution CD1	2 x 200 ml
RNase A (25 mg/ml)	1
Solution CD2	2 x 60 ml
MagAttract Suspension G	13 ml
QSB1 concentrate	95 ml
MW1 concentrate	180 ml
Solution C6	66 ml
Collection microtubes, racked (CMTRs)	4
Caps for collection microtubes (55 x 8)	1
Quick-Start Protocol	1

MagAttract PowerSoil Pro DNA EP Kit
Catalog no.
No. of preps

(384)
47119
384

Solution CD1	2 x 200 ml
RNase A (25 mg/ml)	1
Solution CD2	2 x 60 ml
MagAttract Suspension G	13 ml
QSB1 concentrate	95 ml
MW1 concentrate	180 ml
Solution C6	66 ml
Collection microtubes, racked (CMTRs)	4
Caps for collection microtubes (55 x 8)	1
MagAttract Suspension G	13 ml
Sarstedt® Deep-Well Block (2 ml)	4
Microplate	8
Elution Sealing Mats	4
Quick-Start Protocol	1

Scripts for KingFisher Flex and epMotion 5075 can be requested from Technical Service.

Shipping and Storage

Solution CD2 should be stored at 2–8°C upon arrival. All other reagents and kit components of the MagAttract PowerSoil Pro DNA Kit and MagAttract PowerSoil Pro DNA EP 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 	Buffer QSB1 and Buffer MW1 are flammable once alcohol is added.
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CAUTION 	DO NOT add bleach or acidic solutions directly to the sample preparation waste.
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Solution CD1, Buffer QSB1, and Buffer MW1 contain chaotropic salts, which can form highly reactive compounds when combined with bleach. If the 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 Pro DNA Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

The MagAttract PowerSoil Pro DNA Kit and MagAttract PowerSoil Pro DNA EP Kit allow automated high-throughput isolation of DNA from up to 384 soil samples in less than 1 day and are optimized for use with the Thermo Scientific® KingFisher Flex platform (MagAttract PowerSoil Pro DNA Kit) or Eppendorf® *epMotion*® 5075 platform TMX (MagAttract PowerSoil Pro DNA EP Kit).

The MagAttract PowerSoil Pro DNA Kit and MagAttract PowerSoil Pro DNA EP Kit comprise a novel and proprietary method for isolating microbial genomic DNA from environmental and stool samples. The kits use QIAGEN's second-generation Inhibitor Removal Technology® (IRT®) and are intended for use with stool samples or environmental samples containing high humic acid content, including difficult soil types such as compost, sediment, and manure. Other more common soil and stool types have also been used successfully with this kit. A novel, proprietary magnetic bead system is used to isolate nucleic acids from the IRT-treated lysate without binding residual contaminants. Improved IRT combined with more efficient bead beating and lysis chemistry yields high-quality DNA that can be used immediately in demanding downstream applications, including PCR, qPCR, and next-generation sequencing (NGS; 16S and whole genome).

Principle and procedure

The MagAttract PowerSoil Pro DNA Kit and MagAttract PowerSoil DNA EP Kit are effective at removing PCR inhibitors from soil and stool materials from even the most difficult types. Environmental or human samples are added to a 96-well bead beating plate or bead-beating tubes 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 and *epMotion* 5075 TMX platforms where total genomic DNA is captured on specialized magnetic beads in the presence of buffers. DNA is washed on the beads and then eluted and is ready for NGS, PCR, and other downstream applications.

The MagAttract PowerSoil DNA EP Kit (MagAttract PowerSoil Pro DNA Kit with additional EP accessories) is optimized for use with the Eppendorf epMotion 5075 TMX platform to isolate DNA from up to 450 μ l of lysate per well in a Sarstedt deep-well block (provided). This kit requires the use of a plate shaker on the robotic deck. We also highly recommend using the Magnum FLX[®] magnetic adapter 96-well plate for best results. However, other magnetic separators that efficiently pull the magnetic beads away from the center of the well may also be used. An epMotion 5075 without TMX can be upgraded with a TMX unit. For that, the right part of the worktable must be exchanged. Please contact the Eppendorf service for this modification.

Starting material

This protocol is intended primarily for the extraction of microbial genomic DNA from complex substrates, such as stool or soil. Typically, these samples contain high amounts of inhibitors that interfere with downstream enzymatic reactions and compounds that can degrade DNA. The MagAttract PowerSoil Pro DNA Kit and MagAttract PowerSoil Pro DNA EP Kit are specially designed for the removal of these substances and for the extraction of microbial genomic DNA that is free of proteins, nucleases, and other contaminants or inhibitors.

The recommended starting amount of stool sample is 100 mg. The maximum recommended starting material for stool samples is 200 mg; for dehydrated stool samples, start with no more than 100 mg. In general, highest yields are seen with 50–150 mg of input stool material, although this varies with the source. The recommended starting amount of soil material is 250 mg.

Microbial genomic DNA purified using the MagAttract PowerSoil Pro DNA Kit and MagAttract PowerSoil Pro DNA EP Kit are ready for use in enzymatic reactions, such as PCR or NGS, or it can be stored at -30 to -15°C .

Bead beating

These kits require bead beating for efficient lysis of microbial cells. The MagAttract PowerSoil Pro DNA Kit and MagAttract PowerSoil Pro DNA EP Kit are designed to be used in combination with either PowerBead Pro Plates (cat. no. 19311) or PowerBead Pro Tubes (cat. no. 19301). The use of other disruption media can lead to reduced yields of microbial genomic DNA.

For convenient high-throughput 96-well homogenization, we offer the TissueLyser II (cat. no. 85300) and Plate Adapter Set (cat. no. 11990). In conjunction with PowerBead Pro Plates, the TissueLyser II provides high-throughput processing for simultaneous, rapid, and effective disruption of up to 2 x 96 samples in only a few minutes.

For disruption using 2 ml PowerBead Pro Tubes, the TissueLyser II provides simultaneous disruption of up to 48 samples in combination with the TissueLyser Adapter Set 2 x 24 (cat. no. 69982) or up to 96 samples in combination with the 2 ml Tube Holder Set (cat. no. 11993) and Plate Adapter Set.

Alternately, the PowerLyzer® 24 Homogenizer (cat. no. 13155) allows the simultaneous disruption of up to 24 PowerBead Pro Tubes.

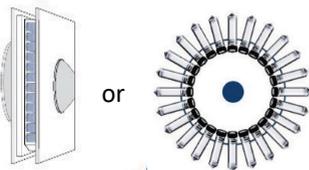
Homogenization using Vortex-Genie® 2 with Vortex Adapter for 1.5–2 ml tubes (cat. no. 13000-V1-24) can also be used for disruption in conjunction with PowerBead Pro Tubes.

The order of placement of components and reagents for the platform portion of the protocol will be described in the software specific to the KingFisher and epMotion platform being used. Please contact the QIAGEN Technical Services for questions regarding the software and the MagAttract PowerSoil Pro DNA/MagAttract PowerSoil Pro DNA EP scripts.

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



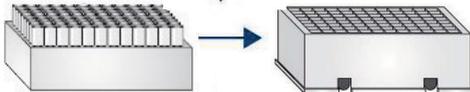
Add soil or stool sample and Solution CD1 to PowerBead Pro Plate or PowerBead Pro Tube



TissueLyser II or Vortex-Genie 2



Transfer supernatant to CMTRs
Add Solution CD2 and mix



Transfer up to 450 μ l of the supernatant to the KingFisher or Sarstedt deep well block depending on the platform

Figure 1. MagAttract PowerSoil Pro DNA/MagAttract PowerSoil Pro 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.

- PowerBead Pro Plates (cat. no. 19311) or PowerBead Pro Tubes (cat. no. 19301) are purchased separately
- Centrifuge capable of handling two 96-well blocks (13 cm x 8.5 cm x 6.0 cm) at 4500 x *g* or a microcentrifuge (up to 15,000 x *g*) if using bead tubes
Note: If you have a centrifuge with a maximum speed less than 4500 x *g*, see the “Troubleshooting Guide”.
- High-velocity bead beater such as the TissueLyser II with corresponding adapter sets for use with PowerBead Pro Plates (Plate Adapter Set) or PowerBead Pro Tubes (TissueLyser Adapter Set 2 x 24, 2 ml Tube Holder Set, and Plate Adapter Set)
- Alternately, for use with PowerBead Pro Tubes, a Vortex-Genie 2 with Vortex Adapter for 24 (1.5–2 ml) tubes or the PowerLyzer 24 Homogenizer
- Multichannel pipettor (50–1000 µl)
- Extra-long pipette tips (1000–1250 µl) for collection microtubes, racked (CMTRs)
- Isopropanol, 100% ethanol, and 80% ethanol
- Please contact your Thermo Fisher Scientific representative for specific KingFisher Flex consumables

ThermoFisher Flex description

Cat. no.

KingFisher 96 deep-well plate, v-bottom, polypropylene 50 pieces	95040450
KingFisher 96 tip comb for deep-well magnets, 10 x 10 pcs/box	97002434
KingFisher 96 microplate (200 µl), 48 pieces	97002540

- Please contact your Eppendorf representative for additional epMotion consumables not included in EP accessories such as pipet tips or epMotion components such as the magnet or the thermomixer unit (TMX).

Eppendorf epMotion 5075 description	Cat. no.
epT.I.P.S. [®] Motion pipette tips, 1000 µl, filtered	30014499
epMotion reservoir, 100 ml	30126513
epMotion reservoir, 400 ml	5075751364
Reservoir rack	5075754002
Eight-channel dispensing tool, 8-channel, 40–1000 µl	5280000258
Gripper	5282000018
Magnum FLX magnetic adapter 96-well plate	5075751836
TMX module to shake, heat, or cool samples and reagents	contact Eppendorf

Protocol: MagAttract PowerSoil Pro DNA Kit with KingFisher

This protocol describes the use of the MagAttract PowerSoil Pro DNA Kit (cat. no. 47109) with the KingFisher Flex instrument.

Solution CD2 should be stored at 2–8°C upon arrival. All other reagents and kit components should be stored at room temperature (15–25°C).

Notes before starting

- Add 400 µl RNase A solution to 80 ml Solution CD1 for each 96-well plate to be processed.
- Prepare Buffer QSB1 and Buffer MW1 according to the instructions on the bottles (addition of 100% ethanol and isopropanol, respectively).
- In this protocol, 80% ethanol is required and needs to be supplied by the user.
- KingFisher consumables: 16 KingFisher 96 deep-well plates, 4 KingFisher 96-tip combs for deep-well magnets, and 4 KingFisher 96 microplates (200 µl) will be needed for 384 samples.

Procedure

1. Spin the PowerBead Pro Plate or the PowerBead Pro Tube briefly to ensure that the beads have settled at the bottom of the wells or tube.
2. Add up to 250 mg of soil or 100 mg of stool into the plate/tube and 800 µl Solution CD1/RNase A solution. Seal the plate with sealing film or recap the tube.
3. Homogenize samples thoroughly using one of the following methods:

3a. If using a PowerBead Pro Plate with the TissueLyser II, seal the plate carefully using the sealing film (watch [link](#) for detailed instructions), place a silicone compression mat on top of the sealed plate and then place the entire assembly between 2 Plate Adapter Sets. Shake for 5 min at 25 Hz.

Reorient the plates so that the sides that were closest to the machine body are now furthest from it. Shake again for 5 min at 25 Hz.

Important: When using this assembly, do not exceed the recommended disruption time and setting of 2 x 5 min at 25 Hz, because extended processing might lead to leakage

3b. If using PowerBead Pro Tubes with the TissueLyser II, place the tubes into a TissueLyser Adapter Set 2 x 24 or into a 2 ml Tube Holder and Plate Adapter Set. Fasten the adapter into the TissueLyser II. Shake for 5 min at 25 Hz. Reorient the adapter so that the side that was closest to the machine body becomes furthest from it. Shake again for 5 min at 25 Hz.

3c. If using the PowerBead Pro Tubes with Vortex Adapters, secure the PowerBead Pro Tube horizontally on a Vortex Adapter for 1.5–2 ml tubes. Vortex at maximum speed for 10 min.

Note: If using Vortex Adapter for more than 12 preps simultaneously, increase the vortexing time by 5–10 min.

3d. If using the PowerBead Pro Tubes with the PowerLyzer 24 Homogenizer, PowerBead Pro Tubes must be properly balanced in the tube holder of the PowerLyzer 24 Homogenizer. We recommend homogenizing the sample at 2000 rpm for 30 s, pausing for 30 s, then homogenizing again at 2000 rpm for 30 s.

Note: Homogenizing samples at higher speeds (up to 4000 rpm) may increase yields but may result in more fragmented DNA.

4. Centrifuge the PowerBead Pro Plate at 4500 x *g* for 6 min or the PowerBead Pro Tubes at 15,000 x *g* for 1 min.

5. Transfer the supernatant to the CMTRs.

Note: Expect 500–600 μ l. The supernatant may still contain some soil/stool particles.

6. Add 300 µl Solution CD2. Seal the CMTRs with the caps provided, and then vortex.
7. Centrifuge the CMTRs at 4500 x *g* for 6 min at room temperature (15–25°C).
8. Taking care to avoid any residual pellet, transfer no more than 450 µl supernatant from each well to a clean KingFisher Microtiter® 96 deep-well plate.
9. Resuspend the MagAttract Suspension G beads by vortexing. For each 96-well plate to be processed, add 3 ml of the resuspended MagAttract Suspension G beads to 44 ml Buffer QSB1 and mix well. Immediately transfer to a multichannel pipette reservoir.

Note: Maintain the MagAttract Suspension G beads in suspension to ensure uniform distribution.

KingFisher Flex

Step	Buffer/Mixture	Volume (µl)	Plate
Bind	MagAttract Suspension G beads/Buffer QSB1	470	KingFisher 96 deep-well plate
Wash 1	Buffer MW1	500	KingFisher 96 deep-well plate
Wash 2	80% ethanol (user provided)	500	KingFisher 96 deep-well plate
Wash 3	80% ethanol (user provided)	500	KingFisher 96 deep-well plate
Elute	Solution C6	100	KingFisher 96 microplate

10. Add 470 µl of the MagAttract Suspension G beads/Buffer QSB1 mix to each well containing lysate in a KingFisher 96 deep-well plate.
11. Place the plate on the robotic deck at the specified location indicated in the program.
12. Add 500 µl Buffer MW1 to each well of 1 clean KingFisher 96 deep-well plate. Add 80% ethanol (provided by the user) to each well of 2 clean KingFisher 96 deep-well plates. Place the plates on the robotic deck at the specified locations indicated in the program.
13. Add 100 µl Solution C6 to each well of a clean KingFisher 96 microplate and place on the robotic deck at the specified location. Initiate the robotic program.
14. Upon completion of the robotic program, cover the wells of the KingFisher 96 microplate with an appropriate storage seal (not provided). DNA is now ready for downstream applications.

Protocol for Low Biomass Samples: MagAttract PowerSoil Pro DNA Kit with KingFisher – 2 Binding Steps

This protocol describes the use of the MagAttract PowerSoil Pro DNA Kit with the KingFisher Flex instrument for low biomass samples.

Please note that when using the low biomass sample protocol, less than 384 samples can be processed.

Solution CD2 should be stored at 2–8°C upon arrival. All other reagents and kit components should be stored at room temperature (15–25°C).

Important points before starting

- Use extra-long pipette tips (1000–1250 µl) for collection microtube racks (CMTRs).
- Add 400 µl RNase A solution to 80 ml Solution CD1 for each 96-well plate to be processed.
- Prepare Buffer QSB1 and Buffer MW1 according to the instructions on the bottles (addition of 100% ethanol or isopropanol, respectively).
- In this protocol, 80% ethanol is required and needs to be supplied by the user.
- KingFisher consumables: 20 KingFisher 96 deep-well plate, 4 KingFisher 96 tip comb for deep-well magnets, and 4 KingFisher 96 microplate (200 µl) will be needed for 384 samples.

Procedure

1. Spin the PowerBead Pro Plate or the PowerBead Pro Tube briefly to ensure that the beads have settled at the bottom of the wells or tube.
2. Add up to 250 mg of soil or 100 mg of stool into the plate/tube and 800 µl Solution CD1/RNase A solution. Seal the plate with sealing film or recap the tube.
3. Homogenize samples thoroughly using one of the following methods:
 - 3a. If using a PowerBead Pro Plate with the TissueLyser II, seal the plate carefully using the sealing film (watch [link](#) for detailed instructions), place a silicone compression mat on top of the sealed plate, and then place the entire assembly between 2 Plate Adapter Sets. Shake for 5 min at 25 Hz.
Reorient the plates so that the sides that were closest to the machine body are now furthest from it. Shake again for 5 min at 25 Hz.
Important: When using this assembly, do not exceed the recommended disruption time and setting of 2 x 5 min at 25 Hz, because extended processing might lead to leakage.
 - 3b. If using the PowerBead Pro Tubes with the TissueLyser II, place the tubes into a TissueLyser Adapter Set 2 x 24 or into a 2 ml Tube Holder and Plate Adapter Set. Fasten the adapter into the TissueLyser II. Shake for 5 min at 25 Hz. Reorient the adapter so that the side that was closest to the machine body becomes furthest from it. Shake again for 5 min at 25 Hz.
 - 3c. If using the PowerBead Pro Tubes with Vortex Adapters, secure the PowerBead Pro Tube horizontally on a Vortex Adapter for 1.5–2 ml tubes. Vortex at maximum speed for 10 min.
Note: If using Vortex Adapter for more than 12 preps simultaneously, increase the vortexing time by 5–10 min.
 - 3d. If using the PowerBead Pro Tubes with the PowerLyzer 24 Homogenizer, the PowerBead Pro Tubes must be properly balanced in the tube holder of the PowerLyzer 24 Homogenizer. We recommend homogenizing the sample at

2000 rpm for 30 s, pausing for 30 s, then homogenizing again at 2000 rpm for 30 s.

Note: Homogenizing samples at higher speeds (up to 4000 rpm) may increase yields but may result in more fragmented DNA.

4. Centrifuge the PowerBead Pro Plate at 4500 x *g* for 6 min or the PowerBead Pro Tubes at 15,000 x *g* for 1 min.

5. Transfer the supernatant to the CMTRs.

Note: Expect 500–600 μ l. The supernatant may still contain some soil/stool particles.

6. Add 300 μ l Solution CD2. Seal the CMTRs with the caps provided, and then vortex.

7. Centrifuge the CMTRs at 4500 x *g* for 6 min at room temperature (15–25°C).

8. Taking care to avoid any residual pellet, transfer the supernatant from each well to a clean KingFisher 96 deep-well plate.

9. Resuspend the MagAttract Suspension G beads by vortexing. For each 96-well plate to be processed, add 3 ml of the resuspended MagAttract Suspension G beads to 60 ml of Buffer QSB1 and mix well. Immediately transfer to a multichannel pipette reservoir.

Note: Maintain the MagAttract Suspension G beads in suspension to ensure uniform distribution.

10. Add 630 μ l of the MagAttract Suspension G beads/Buffer QSB1 to each well containing lysate in a KingFisher 96 deep-well plate and mix by pipetting.

ThermoFisher Flex: 2 binding steps

Step	Buffer/Mixture	Volume	Plate
Bind 1	MagAttract Suspension G beads/Buffer QSB1/lysate	920 μ l	KingFisher 96 deep-well plate
Bind 2	MagAttract Suspension G beads/Buffer QSB1/lysate	remaining mix	KingFisher 96 deep-well plate
Wash 1	Buffer MW1	500 μ l	KingFisher 96 deep-well plate
Wash 2	80% ethanol (user provided)	500 μ l	KingFisher 96 deep-well plate
Wash 3	80% ethanol (user provided)	500 μ l	KingFisher 96 deep-well plate
Elute	Solution C6	100 μ l	KingFisher 96 microplate

11. Transfer no more than 920 μ l of the MagAttract Suspension G beads/Buffer QSB1/lysate mix from each sample to another KingFisher 96 deep-well plate (this will be used in the first binding step). The remaining MagAttract Suspension G beads/Buffer QSB1/lysate mix will be processed in the second binding step.

Note: The volume of the MagAttract Suspension G beads/Buffer QSB1/lysate mix used for the second binding step will vary depending on the sample. The script is set to total volume of 920 μ l but works with less.

12. Place the plate on the robotic deck at the specified location indicated in the program.
13. Add 500 μ l of Buffer MW1 wash solution to each well of 1 clean KingFisher 96 deep-well plate. Add 500 μ l of 80% ethanol (provided by the user) to each well of 2 clean KingFisher 96 deep-well plates. Place on the robotic deck at the specified locations indicated in the program.
14. Add 100 μ l of Solution C6 to each well of a clean KingFisher 96 microplate and place on the robotic deck at the specified location. Initiate the robotic program.
15. 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: MagAttract PowerSoil Pro DNA EP Kit with epMotion

This protocol describes the use of the MagAttract PowerSoil Pro DNA EP Kit (cat. no. 47119) with the Eppendorf epMotion 5975 TMX instrument.

Solution CD2 should be stored at 2–8°C upon arrival. All other reagents and kit components should be stored at room temperature (15–25°C).

Important points before starting

- Use extra-long pipette tips (1000–1250 µl) for collection microtube racks (CMTRs).
- Add 400 µl RNase A solution to 80 ml Solution CD1 for each 96-well plate to be processed.
- Prepare Buffer QSB1 and Buffer MW1 according to the instructions on the bottles (addition of 100% ethanol and isopropanol, respectively).
- In this protocol, 80% ethanol is required and needs to be supplied by the user.

Procedure

1. Spin the PowerBead Pro Plate or the PowerBead Pro Tube briefly to ensure that the beads have settled at the bottom of the wells or tube.
2. Add up to 250 mg of soil or 100 mg of stool into the plate/tube and 800 µl Solution CD1/RNase A solution. Seal the plate with sealing film or recap the tube.
3. Homogenize samples thoroughly using one of the following methods:
 - 3a. If using a PowerBead Pro Plate with the TissueLyser II, seal the plate carefully using the sealing film (watch <https://youtu.be/gWu-x7AT75g> for detailed instructions),

place a silicone compression mat on top of the sealed plate and then place the entire assembly between 2 Plate Adapter Sets. Shake for 5 min at 25 Hz. Reorient the plates so that the sides that were closest to the machine body are now furthest from it. Shake again for 5 min at 25 Hz.

Important: When using this assembly, do not exceed the recommended disruption time and setting of 2 x 5 min at 25 Hz, because extended processing might lead to leakage.

3b. If using PowerBead Pro Tubes with the TissueLyser II, place the tubes into a TissueLyser Adapter Set 2 x 24 or into a 2 ml Tube Holder and Plate Adapter Set. Fasten the adapter into the TissueLyser II. Shake for 5 min at 25 Hz. Reorient the adapter so that the side that was closest to the machine body becomes furthest from it. Shake again for 5 min at 25 Hz.

3c. If using the PowerBead Pro Tubes with Vortex Adapters, secure the PowerBead Pro Tube horizontally on a Vortex Adapter for 1.5–2 ml tubes. Vortex at maximum speed for 10 min.

Note: If using Vortex Adapter for more than 12 preps simultaneously, increase the vortexing time by 5–10 min.

3d. If using the PowerBead Pro Tubes with the PowerLyzer 24 Homogenizer, PowerBead Pro Tubes must be properly balanced in the tube holder of the PowerLyzer 24 Homogenizer. We recommend homogenizing the sample at 2000 rpm for 30 s, pausing for 30 s, then homogenizing again at 2000 rpm for 30 s.

Note: Homogenizing samples at higher speeds (up to 4000 rpm) may increase yields but may result in more fragmented DNA.

4. Centrifuge the PowerBead Pro Plate at 4500 x *g* for 6 min or the PowerBead Pro Tubes at 15,000 x *g* for 1 min.

5. Transfer the supernatant to the CMTRs.

Note: Expect 500–600 μ l. The supernatant may still contain some soil/stool particles.

6. Add 300 μ l Solution CD2. Seal the CMTRs with the caps provided, and then vortex.

7. Centrifuge the CMTRs at 4500 x g for 6 min at room temperature (15–25°C).
8. Taking care to avoid any residual pellet, transfer no more than 450 µl supernatant from each well to a clean Sarstedt deep-well block (provided).

Note: If you wish to use the remaining supernatant in each well, transfer to another Sarstedt deep-well block and store at 2–8°C until they can be processed.

9. Place the Sarstedt deep-well block containing the supernatant on the epMotion robotic deck as indicated in the epMotion program worktable.

Eppendorf epMotion 5075 TMX

Step	Buffer/Mixture	Volume for 96 samples (ml)	Reservoir (ml)
Bind	MagAttract Suspension G beads/Buffer QSB1	51.3	100
Wash 1	Buffer MW1	53	100
Wash 2	80% ethanol (user provided)	53	100
Wash 3	80% ethanol (user provided)	53	100
Elute	Solution C6	14	100

10. For each 96-well block to be processed, add 53 ml Buffer MW1 into an 100 ml Eppendorf reservoir and add 2 x 53 ml 80% ethanol into two other 100 ml Eppendorf reservoirs. Place all three reservoirs into the Eppendorf tub holder at the appropriate location on the deck as indicated in the epMotion program worktable.
11. For each 96-well plate to be processed, add 14 ml Solution C6 into a 100 ml Eppendorf reservoir placed at the appropriate location on the deck as indicated in the epMotion program worktable.
12. Resuspend MagAttract Suspension G Beads by vortexing. For each 96-well plate to be processed, add 3.3 ml of the resuspended MagAttract Suspension G Beads to 48 ml Buffer QSB1 and mix well. Immediately transfer the entire volume of MagAttract Suspension G Beads/Buffer QSB1 into a 100 ml Eppendorf reservoir placed at the appropriate location on the deck as indicated on the epMotion program worktable.

Note: Maintain the MagAttract Suspension G Beads in suspension to ensure uniform distribution.

13. 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.

14. Upon completion, cover the wells of the microtiter 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 in 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 or protocols in this handbook (for contact information, visit support.qiagen.com).

Comments and suggestions

Sample Processing

- | | | |
|----|--|---|
| a) | Amount of soil and stool to process | The QIAGEN MagAttract PowerSoil Pro DNA Kit is designed to process 0.25 g of soil and up to 0.1 g of stool. |
| b) | Soil or stool sample is high in water content | Weigh the slurry and dispense into the wells. We suggest restricting the starting amount to 0.25 g soil and 0.1 g stool. Increasing the amount used will increase the amount of volume in the subsequent steps. |
| c) | Centrifuge available has maximum speed less than $4500 \times g$ | Multiply the protocol time and speed to determine the total $\times g$. Divide the total by the maximum speed of the centrifuge (round up if necessary). This will be the number of minutes that the centrifuge will need to run to achieve the appropriate overall force.
Example: 10 minutes at $4500 \times g = 45,000$
If centrifuge has a maximum speed of $2500 \times g$: $45,000 \div 2500 = 18$ min of centrifugation. |

DNA

- | | | |
|----|--------------------------|---|
| a) | The 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 the DNA isolated using the MagAttract PowerSoil Pro DNA Kit. However, it should still be attempted.
If the DNA will still not amplify after trying the steps above, then PCR optimization may be needed. |
| b) | The eluted DNA is brown | If you observe coloration in your samples, please contact Technical Support for suggestions. |

Comments and suggestions

- | | |
|--|--|
| c) Inefficient removal of inhibitory DNA | If you observe inhibition, please contact Technical Support for suggestions. |
| d) Concentrating the eluted DNA | The final volume of the eluted DNA will be 50–100 μ l. The DNA may be concentrated by adding 5–10 μ l of 3 M NaCl and inverting 3–5 times to mix. Next, add 100 μ l of 100% cold ethanol and invert 3–5 times to mix. Incubate at –30 to –15°C for 30 minutes and centrifuge at 10,000 $\times g$ for 5 min at room temperature (15–25°C). 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 the precipitated DNA in desired volume of 10 mM Tris (Solution C6). |
| e) Storing the DNA | The DNA is eluted in Solution C6 (10 mM Tris) and must be stored at –30 to –15°C or at –90 to –65°C to prevent degradation. The DNA can be eluted in Buffer 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 Water (cat. no. 17000-10). |
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Alternative lysis methods

- | | |
|---------------------------------|---|
| a) Cells are difficult to lyse | After adding Solution CD1 and prior to the bead beating step, incubate at 65°C for 10 min. Resume protocol from step 2. |
| b) Reduction of shearing of DNA | After adding Solution CD1, vortex 3–4 s, then heat to 70°C for 5 min. Repeat once. This alternative procedure will reduce shearing but may also reduce yield. |

Ordering Information

Product	Contents	Cat. no.
MagAttract PowerSoil Pro DNA Kit	For 4 x 96 preps: automated high-throughput isolation of DNA from stool or soil samples in less than 1 day	47109
MagAttract PowerSoil Pro DNA EP Kit	For 4 x 96 preps: automated high-throughput isolation of DNA from stool or soil samples in less than 1 day on the epMotion	47117
PowerBead Pro Plates (4)	Bead plates ready for rapid and reliable biological sample lysis from a wide variety of starting materials	19311
PowerBead Pro Tubes (2 ml) (50)	Bead tubes ready for rapid and reliable biological sample lysis from a wide variety of starting materials, 2 ml	19301
Related products		
DNeasy® PowerSoil Pro Kit (50)	For 50 preps: isolate microbial genomic DNA from all soil types	47014
DNeasy PowerSoil Pro Kit (250)	For 250 preps: isolate microbial genomic DNA from all soil types	47016
QIAamp PowerFecal Pro DNA Kit (50)	For 50 preps: isolation of microbial genomic DNA from stool and gut samples	51804
DNeasy 96 PowerSoil Pro QIAcube® HT Kit (480)	For 480 preps: automated high-throughput purification of microbial genomic DNA from all soil and stool types	47021

Product	Contents	Cat. no.
DNeasy 96 PowerSoil Pro Kit (384)	For 384: manual high-throughput isolation of microbial genomic DNA from all soil and stool types	47017
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
Vortex Adapter for 24 (1.5–2.0 ml) tubes	For vortexing 1.5 and 2 ml tubes using the Vortex-Genie 2 Vortex	13000-V1-24
PCR Water (10 x 1 ml)	Water certified to be free of DNA, DNase, and RNase contamination	17000-10
UCP Multiplex PCR Kit (100)	For 100 reactions: for highly specific and sensitive multiplex PCR with minimized background using nucleic acid-depleted reagents	206742
UCP Multiplex PCR Kit (500)	For 500 reactions: for highly specific and sensitive multiplex PCR with minimized background using nucleic acid-depleted reagents	206744
QIAseq® 16S/ITS Screening Panel (24)	Profiling of bacterial and fungal communities by constructing a library of all bacterial 16S rRNA gene variable regions and fungal ITS regions using phased primer; sufficient for 24 samples	333812
QIAseq 16S/ITS Screening Panel (96)	Profiling of bacterial and fungal communities by constructing a library of all bacterial 16S rRNA gene	333815

Product	Contents	Cat. no.
QIAseq 16S/ITS Region Panel (24)	variable regions and fungal ITS regions using phased primer; sufficient for 96 samples	333842
QIAseq 16S/ITS Region Panel (96)	Profiling of bacterial and fungal communities by constructing a library of specific bacterial 16S rRNA gene variable regions and fungal ITS regions using phased primer; sufficient for 24 samples	333845
QIAseq 16S/ITS 24-Index I (96)	Profiling of bacterial and fungal communities by constructing a library of specific bacterial 16S rRNA gene variable regions and fungal ITS regions using phased primer; sufficient for 96 samples	333822
QIAseq 16S/ITS 96-Index I (384)	Adapters and sample indexes for use in conjunction with QIAseq 16S/ITS panels to generate Illumina-compatible libraries; sufficient adapters for indexing 96 samples (4 x 24 samples)	333825
QIAseq 16S/ITS 96-Index I (384)	Adapters and sample indexes (Set A) for use in conjunction with QIAseq 16S/ITS panels to generate Illumina-compatible libraries; sufficient adapters for indexing 384 samples (4 x 96 samples)	333825

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Document Revision History

Date	Changes
11/2020	Initial release
09/2022	Updated to include epMotion.

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