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MagAttract® PowerSoil® Pro DNA Kit Handbook

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

Sample to Insight

QIAGEN

Contents

Kit Contents

MagAttract PowerSoil Pro DNA Kit	**(384)**
Catalog no.	**47109**
No. of preps	**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	200 ml
MW1 concentrate	250 ml
Solution C6	66 ml
Collection microtubes, racked (CMTRs)	4
Caps for collection microtubes (55 x 8)	1
Quick-Start Protocol	1

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 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 	QSB1, MW1, and ethanol are flammable.

CAUTION 	DO NOT add bleach or acidic solutions directly to the sample preparation waste.

Solution CD1, QSB1, and 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 allows automated high-throughput isolation of DNA from up to 384 soil samples in less than 1 day and is optimized for use with the Thermo Scientific® KingFisher® Flex platform. Protocols for other instruments are being developed; please contact QIAGEN Technical Services if you are interested in using a different instrument with this kit.

The MagAttract PowerSoil Pro DNA Kit comprises a novel and proprietary method for isolating microbial genomic DNA from environmental and stool samples. The kit uses QIAGEN's second-generation Inhibitor Removal Technology® (IRT) and is 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 is 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 platform 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.

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 is 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, though 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 is ready for use in enzymatic reactions, such as PCR or NGS, or it can be stored at –30 to –15°C.

Bead beating

This kit requires bead beating for efficient lysis of microbial cells. The MagAttract PowerSoil Pro DNA Kit is 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.

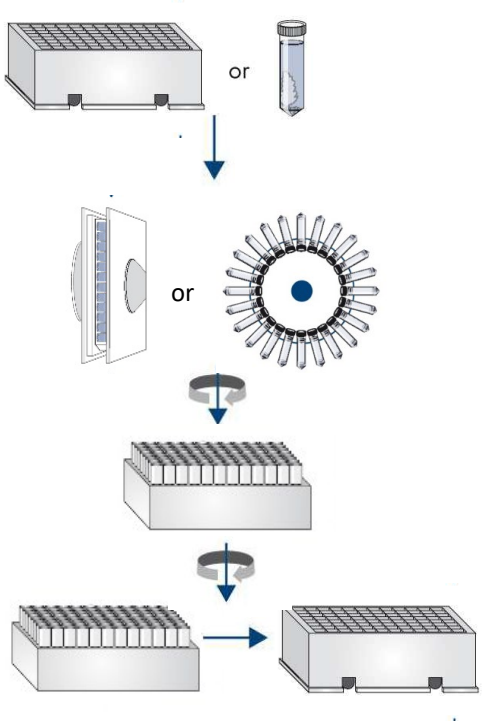
For disruption using 2 ml PowerBead Pro Tubes, the TissueLyser II provides simultaneous disruption of up to 48 samples in combination with the TissueLyser Adapter Set 2 x 24 (cat. no. 69982) or up to 96 samples in combination with the 2 ml Tube Holder Set (cat. no. 11993) and Plate Adapter Set.

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 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 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 µl of the supernatant to the KingFisher Microtiter® 96 deep-well plate

Figure 1. MagAttract PowerSoil Pro 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 60 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 (0.1–1000 µl) for CMTRs (optional)
- 80% ethanol
- Multichannel pipettor reagent reservoirs for 10–150 ml
- Please contact your Thermo Fisher Scientific representative for specific KingFisher Flex consumables

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).

Important points before starting

- Use extra-long pipette tips (1000–1250 µl) for 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.
- 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, place a silicone compression mat on top of the sealing film and then place the sealed plate and the mat 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.

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 µl. 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.

10. Add 470 µl of the MagAttract Suspension G beads/Buffer QSB1 mix to each well containing lysate in a KingFisher Microtiter 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 Microtiter 96 deep-well plate. Add 80% ethanol (provided by the user) to each well of 2 clean KingFisher Microtiter 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.

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 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.
- 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, place a silicone compression mat on top of the sealing film and then place the sealed plate and the mat 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.

   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 µl. 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 the 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 60 ml of QSB1 binding solution 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 µl of the MagAttract Suspension G beads/QSB1 binding solution to each well containing lysate in a KingFisher Microtiter 96 deep-well plate and mix by pipetting.
11. Transfer no more than 920 µl of the mix from each sample to another KingFisher Microtiter 96 deep-well plate (this will be used in the first binding step). The remaining mix of lysate, MagAttract Suspension G beads, and QSB1 binding solution will be processed in the second binding step.

    Note: The volume of the lysate/MagAttract Suspension G beads/QSB1 binding solution mix used for the second binding step will vary depending on the sample. The script is set to total volume of 920 µl but works with less.
12. Place the plate on the robotic deck at the specified location indicated in the program.
13. Add 500 µl of MW1 wash solution to each well of 1 clean KingFisher Microtiter 96 deep-well plate. Add 500 µl of 80% ethanol (provided by the user) to each well of 2 clean KingFisher Microtiter 96 deep-well plates. Place on the robotic deck at the specified locations indicated in the program.
14. Add 100 µl 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 microplate 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 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)	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 x *g*	Multiply the protocol time and speed to determine the total x *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 x *g* = 45,000 If centrifuge has a maximum speed of 2500 x *g*: 45,000 ÷ 2500 = 18 minutes of centrifugation.

		Comments and suggestions
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.
c)	Concentrating the eluted DNA	The final volume of the eluted DNA will be 50–100 µl. The DNA may be concentrated by adding 5–10 µl of 3 M NaCl and inverting 3–5 times to mix. Next, add 100 µl 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 x *g* for 5 minutes 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).
d)	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 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).
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 minutes. Resume protocol from step 2.
b)	Reduction of shearing of DNA	After adding Solution CD1, vortex 3–4 seconds, then heat to 70°C for 5 minutes. 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
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
DNeasy 96 PowerSoil Pro Kit (384)	For 384: manual high-throughput isolation of microbial genomic DNA from all soil and stool types	47017
DNeasy PowerMax® Soil Kit (10)	For 10 preps: isolation of microbial DNA from large quantities of stool; great for samples with low microbial load	12988-10

Product	Contents	Cat. no.
TissueLyser II	For medium- to high-throughput sample disruption for molecular analysis	85300
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	19301
Plate Adapter Set	Set of four adapters required to assemble two 96-well plates onto the 96-well plate shaker.	11990
2 ml Tube Holder Set	For sample homogenization in 2 ml bead tubes on a TissueLyser II	11993
TissueLyser Adapter Set 2 x 24	Two sets of adapter plates and 2 racks for use with 2 ml microcentrifuge tubes on the TissueLyser II	69982
PowerLyzer 24 Homogenizer (110/220 V)	For the most efficient and complete lysis and homogenization of any biological sample	13155
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

Product	Contents	Cat. no.
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 variable regions and fungal ITS regions using phased primer; sufficient for 96 samples	333815
QIAseq 16S/ITS Region Panel (24)	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	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 96 samples	333845

Product	Contents	Cat. no.
QIAseq 16S/ITS 24-Index I (96)	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)	333822
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

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.

Document Revision History

Date	Changes
11/2020	Initial release.

Notes

Limited License Agreement for MagAttract® PowerSoil® Pro DNA Kit

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

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