February 2020

QIAamp® PowerFecal® Pro DNA Kit Handbook

For the isolation of microbial genomic DNA from stool and gut samples

Sample to Insight

QIAGEN

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Kit Contents

QIAamp PowerFecal Pro DNA Kit	**(50)**
Catalog no.	**51804**
Number of preps	**50**
PowerBead Pro Tubes	50
MB Spin Columns	50
Solution CD1	40 ml
Solution CD2	15 ml
Solution CD3	35 ml
Solution EA	36 ml
Solution C5	30 ml
Solution C6	9 ml
Microcentrifuge Tubes (2 ml)	100
Elution Tubes (1.5 ml)	50
Collection Tubes (2 ml)	100
Quick-Start Protocol	1

Storage

Solution CD2 should be stored at 2–8°C upon arrival. All other components and reagents of the QIAamp PowerFecal Pro DNA Kit can be stored at room temperature (15–25°C) until the expiration date printed on the box label.

Intended Use

All QIAamp products are intended for molecular biology applications. These products are not intended for the diagnosis, prevention or treatment of a disease.

QIAcube® Connect is designed to perform fully automated purification of nucleic acids and proteins in molecular biology applications. The system is intended for use by professional users trained in molecular biological techniques and the operation of QIAcube Connect.

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.

WARNING	**Solution EA and Solution C5 are flammable.**

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

Solution CD1 and Solution CD3 contain chaotropic 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 QIAamp PowerFecal Pro DNA Kits is tested against predetermined specifications to ensure consistent product quality.

Introduction

The QIAamp PowerFecal Pro DNA Kit comprises a novel and proprietary method for isolating both microbial and host genomic DNA from stool and gut samples. The kit uses QIAGEN's second-generation Inhibitor Removal Technology® (IRT), and is intended for use with samples containing inhibitory substances commonly found in stool, such as polysaccharides, heme compounds and bile salts. Improved IRT combined with more efficient bead beating and lysis chemistry yields high-quality DNA that can be used immediately in downstream applications, including PCR, qPCR and next-generation sequencing (16S and whole-genome).

Principle and procedure

The QIAamp PowerFecal Pro DNA Kit is effective at removing PCR inhibitors from even the most difficult stool types. Samples are added to a bead beating tube for rapid and thorough homogenization. Cell lysis occurs by mechanical and chemical methods. Total genomic DNA is captured on a silica membrane in a spin-column format. DNA is then washed and eluted from the membrane and ready for NGS, PCR and other downstream applications.

Bead beating options

The QIAamp PowerFecal Pro DNA Kit does not require homogenization using a high-velocity bead beater. However, if the microorganism of interest requires stronger homogenization than provided by a vortex, or if using a bead beater is desired, the QIAamp PowerFecal Pro DNA Kit contains bead tubes suitable for high-powered bead beating and may be used in conjunction with the PowerLyzer® 24 Homogenizer (110/220V) (cat. no. 13155) or the TissueLyser II (cat. no. 85300) using a 2 ml Tube Holder Set (cat no. 11993).

The PowerLyzer 24 Homogenizer: Optimized for complete homogenization of any sample

The PowerLyzer 24 Homogenizer is a highly efficient bead beating system that allows for optimal DNA extraction from a variety of biological samples. The instrument's velocity and proprietary motion combine to provide the fastest homogenization time possible, minimizing the time spent processing samples. The programmable display enables hands-free, walk-away homogenization with up to ten cycles of bead beating for as long as 5 minutes per cycle. Even the toughest and most difficult samples, such as pine needles, seeds, spores and fungal mats are easily and effectively lysed. For more information and protocols, please contact QIAGEN Technical Service at **support.qiagen.com**.

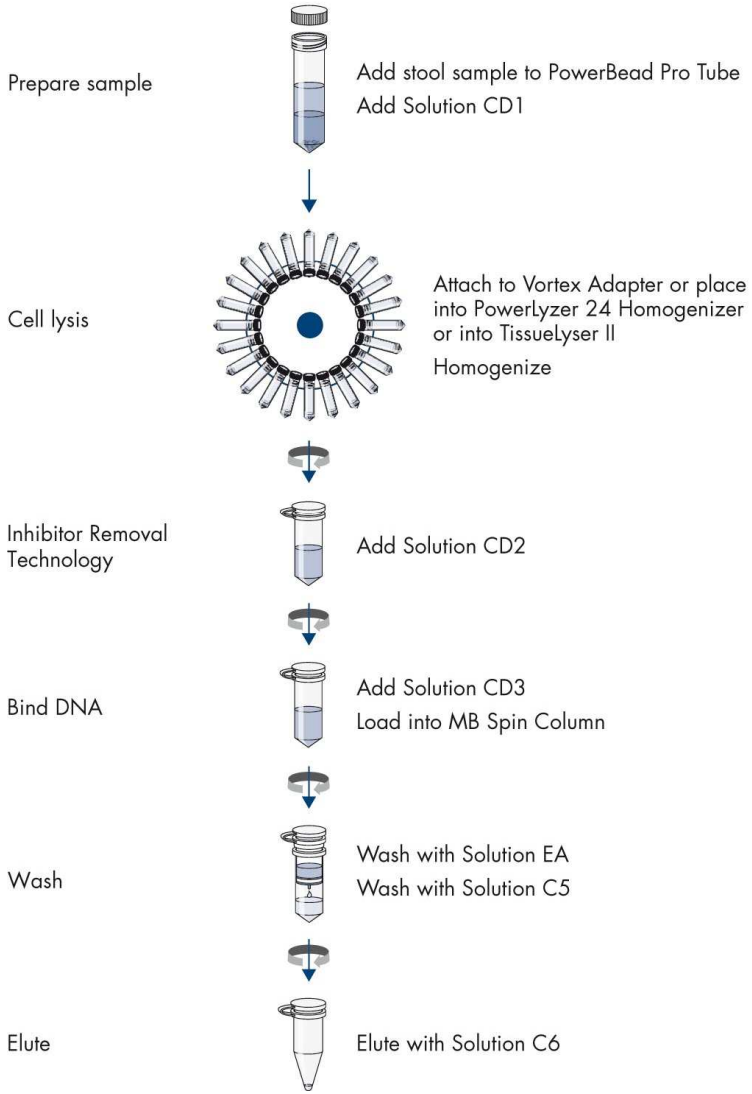
The TissueLyser II: Optimized for medium- to high-throughput sample disruption

The TissueLyser II simultaneously disrupts multiple biological samples through high-speed shaking in plastic tubes with stainless steel, tungsten carbide or glass beads. Using the appropriate adapter set, up to 48 or 192 samples can be processed at the same time. Alternatively, a grinding jar set can be used to process large samples. A range of beads, bead dispensers and collection microtubes and caps are also available.

High-throughput options

For high-throughput options, we offer the DNeasy 96 PowerSoil Pro Kit (384) (cat. no. 47017) for processing up to 2 x 96 samples using a centrifuge capable of spinning two stacked 96-well blocks (13 cm x 8 cm x 5.5 cm) at 4500 x *g*. For 96-well homogenization of stool, we offer the TissueLyser II and Plate Adapter Set (cat. no. 85300 and 11990, respectively). We also offer the DNeasy 96 PowerSoil Pro QIAcube HT Kit (5) (cat. no. 47021) and the QIAsymphony PowerFecal Pro Kit (192) (cat. no. 938036) for automatic processing.

QIAamp PowerFecal Pro DNA Kit Procedure



Automated purification of DNA on QIAcube instruments

Purification of DNA can be fully automated on QIAcube Connect or the classic QIAcube. The innovative QIAcube instruments use advanced technology to process QIAGEN spin columns, enabling seamless integration of automated, low-throughput sample prep into your laboratory workflow. Sample preparation using QIAcube instruments follows the same steps as the manual procedure (i.e., lyse, bind, wash and elute), enabling you to continue using the QIAamp PowerFecal Pro DNA Kit for purification of high-quality DNA.

QIAcube instruments are preinstalled with protocols for purification of plasmid DNA, genomic DNA, RNA, viral nucleic acids and proteins, plus DNA and RNA cleanup. The range of protocols available is continually expanding, and additional QIAGEN protocols can be downloaded free of charge at **www.qiagen.com/qiacubeprotocols**.

Note: The lyse and remove inhibitors steps is not automated in QIAcube Connect and must be performed manually.

QIAcube Connect.

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.

- Microcentrifuge (up to16,000 x *g*)
- Pipettor (50–1000 µl)
- Vortex-Genie® 2
- Vortex Adapter for 24 (1.5 or 2 ml) tubes (cat. no. 13000-V1-24)

Protocol: Experienced User

Important notes before starting

- Ensure that the PowerBead Pro Tubes rotate freely in the centrifuge without rubbing.
- If Solution CD3 has precipitated, heat at 60°C until precipitate dissolves.
- Perform all centrifugation steps at room temperature (15–25°C).

Procedure

1. Spin the PowerBead Pro Tube briefly to ensure that the beads have settled at the bottom. Add up to 250 mg of stool and 800 µl of Solution CD1. Vortex briefly to mix.
2. Secure the PowerBead Pro Tube horizontally on a Vortex Adapter for 1.5– 2 ml tubes (cat. no. 13000-V1-24). Vortex at maximum speed for 10 min.

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

   Note: For alternative ways to homogenize samples, see the detailed protocol on page 13–14
3. Centrifuge the PowerBead Pro Tube at 15,000 x *g* for 1 min.
4. Transfer the supernatant to a clean 2 ml Microcentrifuge Tube (provided).

   Note: Expect 500–600 µl. The supernatant may still contain some stool particles.
5. Add 200 µl of Solution CD2 and vortex for 5 s.
6. Centrifuge at 15,000 x *g* for 1 min. Avoiding the pellet, transfer up to 700 µl of supernatant to a clean 2 ml Microcentrifuge Tube (provided).

   Note: Expect 500–600 µl.
7. Add 600 µl of Solution CD3 and vortex for 5 s.
8. Load 650 µl of the lysate onto an MB Spin Column and centrifuge at 15,000 x *g* for 1 min.

9. Discard the flow-through and repeat step 8 to ensure that all of the lysate has passed through the MB Spin Column.
10. Carefully place the MB Spin Column into a clean 2 ml Collection Tube (provided). Avoid splashing any flow-through onto the MB Spin Column.
11. Add 500 µl of Solution EA to the MB Spin Column. Centrifuge at 15,000 x *g* for 1 min.
12. Discard the flow-through and place the MB Spin Column back into the same 2 ml Collection Tube.
13. Add 500 µl of Solution C5 to the MB Spin Column. Centrifuge at 15,000 x *g* for 1 min.
14. Discard the flow-through and place the MB Spin Column into a new 2 ml Collection Tube (provided).
15. Centrifuge at up to 16,000 x *g* for 2 min. Carefully place the MB Spin Column into a new 1.5 ml Elution Tube (provided).
16. Add 50–100 µl of Solution C6 to the center of the white filter membrane.
17. Centrifuge at 15,000 x *g* for 1 min. Discard the MB Spin Column. The DNA is now ready for downstream applications.

    Note: We recommend storing the DNA frozen (–30°C to –15°C or –90°C to –65°C) as Solution C6 does not contain EDTA. To concentrate DNA, refer to the Troubleshooting Guide.

Protocol: Detailed

Important notes before starting

- Ensure that the PowerBead Pro Tubes rotate freely in the centrifuge without rubbing.
- If Solution CD3 has precipitated, heat at 60°C until precipitate dissolves.
- Perform all centrifugation steps at room temperature (15–25°C).

Procedure

1. Spin the PowerBead Pro Tube briefly to ensure that the beads have settled at the bottom. Add up to 250 mg of stool and 800 µl of Solution CD1. Vortex briefly to mix.

   Note: After the sample has been loaded into the PowerBead Pro Tube, the next step is a homogenization and lysis procedure. The PowerBead Pro Tube contains a buffer that will (a) help disperse the stool particles, (b) begin to dissolve inhibitors and (c) protect nucleic acids from degradation. Gentle vortexing mixes the components in the PowerBead Pro Tube and begins to disperse the sample in the buffer.

2. Homogenize samples using one of the following methods:

   A. Secure the PowerBead Pro Tube horizontally on a Vortex Adapter for 1.5–2 ml tubes (cat. no. 13000-V1-24). Vortex at maximum speed for 10 min.

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

   Note: Using the Vortex Adapter will maximize homogenization, which can lead to higher DNA yields. Avoid using tape, which can become loose and result in reduced homogenization efficiency, inconsistent results and reduced yields.

B. Use a PowerLyzer 24 Homogenizer. PowerBead Pro Tubes must be properly balanced in the tube holder of the PowerLyzer 24 Homogenizer. We recommend homogenizing the tissue 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 result in more fragmented DNA.

C. Use a TissueLyser II. Place the PowerBead Pro Tube into the TissueLyser Adapter Set 2 x 24 (cat. no. 69982) or 2 ml Tube Holder (cat. No. 11993) and Plate Adapter Set (cat. no.1190). Fasten the adapter into the instrument and shake for 5 min at speed 25 Hz. Re-orient the adapter so that the side that was closest to the machine body is now furthest from it. Shake again for 5 min at speed 25 Hz.

Note: Vortexing/shaking is critical for complete homogenization and cell lysis. Cells are lysed by a combination of chemical agents from step 1 and mechanical shaking introduced at this step. Randomly shaking the beads in the presence of disruption agents will cause the beads to collide with microbial cells and lead to the cells breaking open.

3. Centrifuge the PowerBead Pro Tube at 15,000 x *g* for 1 min.
4. Transfer the supernatant to a clean 2 ml Microcentrifuge Tube (provided).

   Note: Expect 500–600 µl. The supernatant may still contain some stool particles.
5. Add 200 µl of Solution CD2 and vortex for 5 s.

   Note: Solution CD2 contains Inhibitor Removal Technology (IRT), which is a reagent that can precipitate non-DNA organic and inorganic material including polysaccharides, cell debris and proteins. It is important to remove contaminating organic and inorganic matter that may reduce DNA purity and inhibit downstream DNA applications.
6. Centrifuge at 15,000 x *g* for 1 min. Avoiding the pellet, transfer up to 700 µl of supernatant to a clean 2 ml Microcentrifuge Tube (provided).

   Note: Expect 500–600 µl.

   Note: The pellet at this point contains non-DNA organic and inorganic material including polysaccharides, cell debris and proteins. For best DNA yields and quality, avoid transferring any of the pellet.

7. Add 600 µl of Solution CD3 and vortex for 5 s.

   Note: Solution CD3 is a high-concentration salt solution. Since DNA binds tightly to silica at high salt concentrations, Solution CD3 will adjust the DNA solution salt concentration to allow binding of DNA, but not non-DNA organic and inorganic material that may still be present at low levels, to the MB Spin Column filter membrane.

8. Load 650 µl of the lysate onto an MB Spin Column and centrifuge at 15,000 x *g* for 1 min.

   Note: DNA is selectively bound to the silica membrane in the MB Spin Column in the presence of high salt solution. Contaminants pass through the filter membrane, leaving only DNA bound to the membrane.

9. Discard the flow-through and repeat step 8 to ensure that all of the lysate has passed through the MB Spin Column.

10. Carefully place the MB Spin Column into a clean 2 ml Collection Tube (provided). Avoid splashing any flow-through onto the MB Spin Column.

11. Add 500 µl of Solution EA to the MB Spin Column. Centrifuge at 15,000 x *g* for 1 min.

    Note: Solution EA is a wash buffer that removes protein and other non-aqueous contaminants from the MB Spin Column filter membrane.

12. Discard the flow-through and place the MB Spin Column back into the same 2 ml Collection Tube.

13. Add 500 µl of Solution C5 to the MB Spin Column. Centrifuge at 15,000 x *g* for 1 min.

    Note: Solution C5 is an ethanol-based wash solution used to further clean the DNA that is bound to the silica filter membrane in the MB Spin Column. This wash solution removes residual salt, inhibitors and other contaminants while allowing the DNA to stay bound to the silica membrane.

14. Discard the flow-through and place the MB Spin Column into a new 2 ml Collection Tube (provided).

15. Centrifuge at up to 16,000 x *g* for 2 min. Carefully place the MB Spin Column into a new 1.5 ml Elution Tube (provided).

    Note: This spin removes residual Solution C5. It is critical to remove all traces of Solution C5 because the ethanol in it can interfere with downstream DNA applications, such as PCR, restriction digests and gel electrophoresis.

16. Add 50–100 µl of Solution C6 to the center of the white filter membrane.

    Note: Placing Solution C6 in the center of the small white membrane will make sure the entire membrane is wet. This will result in a more efficient and complete release of the DNA from the MB Spin Column filter membrane. As Solution C6 passes through the silica membrane, DNA that was bound in the presence of high salt is selectively released by Solution C6 (10 mM Tris), which lacks salt.

17. Centrifuge at 15,000 x *g* for 1 min. Discard the MB Spin Column. The DNA is now ready for downstream applications.

    Note: We recommend storing the DNA frozen (–30°C to –15°C or –90°C to –65°C) as Solution C6 does not contain EDTA. To concentrate DNA, refer to the Troubleshooting Guide.

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
Stool Processing		
a)	Amount of stool to process	The QIAGEN QIAamp PowerFecal Pro Kit is designed to process 0.25 grams of stool. For inquiries regarding the use of larger sample amounts, please contact Technical Support for suggestions.
b)	Stool sample is high in water content	Remove contents from the PowerBead Pro Tube (beads) and transfer into another sterile microcentrifuge tube (not provided). Add stool sample to PowerBead Pro Tube and centrifuge at room temperature (15–25°C) for 30 seconds at 10,000 x *g*. Remove as much liquid as possible with a pipette tip. Add beads back to PowerBead Pro Tube and resume protocol from step 2.
DNA		
a)	DNA does not amplify	Ensure that you 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 QIAamp PowerFecal Pro DNA Kit. However, it should still be attempted. If DNA will still not amplify after trying the steps above, then PCR optimization may be needed.

		Comments and suggestions
b)	Eluted DNA is brown	If you observe coloration in your samples, please contact Technical Support for suggestions.
c)	Concentrating eluted DNA	The final volume of 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°C to –15°C for 30 minutes and centrifuge at 10,000 x *g* for 5 minutes at room temperature. Decant all liquid. Briefly dry residual ethanol in a speed vac or ambient air. Avoid over-drying the pellet or resuspension may be difficult. Resuspend precipitated DNA in desired volume of 10 mM Tris (Solution C6).
d)	DNA floats out of a well when loading a gel	This usually occurs because residual Solution C5 remains in the final sample. Prevent this by being careful in step 15 and not transferring liquid onto the bottom of the spin filter basket. Ethanol precipitation (described in "Concentrating eluted DNA") is the best way to remove residual Solution C5.
e)	Storing DNA	DNA is eluted in Solution C6 (10 mM Tris) and must be stored at –30°C to –15°C or –90°C to –65°C to prevent degradation. DNA can be eluted in TE without loss, but the EDTA may inhibit downstream reactions such as PCR and automated sequencing. DNA may also be eluted in sterile, DNA-Free PCR-Grade water (cat. no. 17000-10).
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.
QIAamp PowerFecal Pro DNA Kit (50)	For the isolation of microbial DNA from stool and gut samples	51804
QIAcube Connect — for fully automated nucleic acid extraction with QIAGEN spin-column kits		
QIAcube Connect*	Instrument, connectivity package, 1-year warranty on parts and labor	Inquire
Starter Pack, QIAcube	Filter-tips, 200 µl (1024), 1000 µl filter-tips (1024), 30 ml reagent bottles (12), rotor adapters (240), elution tubes (240), rotor adapter holder	990395
Related products		
DNeasy PowerSoil Pro Kit (250)	For the isolation of microbial genomic DNA from all soil types	47016
DNeasy 96 PowerSoil Pro Kit (384)	For 384 preps: Isolate microbial genomic DNA from all soil types in a 96-well format	47017
DNeasy 96 PowerSoil Pro QIAcube HT Kit (480)	For 480 preps: Automated high-throughput purification of microbial genomic DNA from all stool types	47021
QIAsymphony PowerFecal Pro DNA Kit (192)	For the isolation of microbial genomic DNA from stool and soil on the QIAsymphony	938036
DNeasy PowerMax Soil Kit (10)	For the isolation of microbial DNA from large quantities of soil with low microbial load	12988-10

Product	Contents	Cat. no.
MagAttract PowerSoil DNA KF Kit	For the automated, hands-free isolation of DNA from soil	27000-4-KF
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
Vortex Adapter	For vortexing 1.5 ml or 2 ml tubes using the Vortex-Genie 2	13000-V1-24
PowerLyzer 24 Homogenizer	For complete lysis and homogenization of any biological sample	13155
TissueLyser II	For medium- to high-throughput sample disruption for molecular analysis	85300
TissueLyser Adapter Set (2 x 24)	2 sets of adapter plates and 2 racks for use with 2 ml microcentrifuge tubes on the TissueLyser II	69982
2 ml Tube Holder Set	For sample homogenization in 2 ml bead tubes on a TissueLyser II	11993
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

* All QIAcube Connect instruments are provided with a region-specific connectivity package, including tablet and equipment necessary to connect to the local network. Further, QIAGEN offers comprehensive instrument service products, including service agreements, installation, introductory training and preventive subscription. Contact your local sales representative to learn about your options.

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
May 2019	Added storage condition for Solution CD2 in the Storage section; Deleted photo of QIAcube; Added QIAcube Connect references; Updated reference link from www.qiagen.com/MyQIAcube to www.qiagen.com/MyQIAcubeConnect; Updated Ordering Information section for QIAcube Connect; Layout updates
January 2020	Updated text, ordering information and intended use for QIAcube Connect. Updated cross-references to homogenization sections. Removed statement about sterility of Solution C6. Updated storage information.

Limited License Agreement for QIAamp PowerFecal Pro DNA Kit

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

1. The product may be used solely in accordance with the protocols provided with the product and this handbook and for use with components contained in the kit only. QIAGEN grants no license under any of its intellectual property to use or incorporate the enclosed components of this kit with any components not included within this kit except as described in the protocols provided with the product, this handbook, and additional protocols available at www.qiagen.com. Some of these additional protocols have been provided by QIAGEN users for QIAGEN users. These protocols have not been thoroughly tested or optimized by QIAGEN. QIAGEN neither guarantees them nor warrants that they do not infringe the rights of third-parties.
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