April 2025

QIAamp® DNA Host-Free Microbiome Handbook

For host DNA removal and isolation of bacterial microbiome DNA from mixed samples

Sample to Insight

Table of Contents

Kit Contents

QIAamp DNA Host-Free Microbiome Kit Catalog no. Number of preps	(50) 51904 50
QIAamp UCP Mini Columns	50
Collection tubes (2 mL)	50
PowerBead Pro tubes	50
Elution tubes (1.5 mL)	50
Host Depletion Solution (15 mg)	1 bottle
Buffer RDD	35 mL
Benzonase®	2 vials
Buffer ATL	14 mL
Reagent DX (clear cap)	1 mL
Buffer APL2*	14 mL
Buffer AW1* (concentrate)	19 mL
Buffer AW2 (concentrate)	17 mL
Proteinase K (green cap)	1 vial
RNase-free Water	6 vials
Quick-Start Protocol	1

* Contains chaotropic salt. Not compatible with disinfecting agents containing bleach.

Shipping and Storage

The components of the QIAamp DNA Host-Free Microbiome Kit are shipped in two boxes: Box 1 is shipped at room temperature (15–25°C); and Box 2 is shipped on wet ice at 4°C. Upon receipt, the Benzonase should be stored at –20°C.

QIAamp UCP Mini Columns and lyophilized Host Depletion Solution should be stored at 2–8°C upon arrival, but storage for up to 4 weeks at room temperature does not affect their performance. All of the buffers of the QIAamp DNA Host-Free Microbiome Kit can be stored at room temperature.

Intended Use

The QIAamp DNA Host-Free Microbiome Kit is intended for molecular biology applications. This product is 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 waste containing Buffer APL2 or Buffer AW1.

Buffer APL2 and Buffer AW1 contain guanidine salts, which can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilt, clean with 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 DNA Host-Free Microbiome Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

Analyzing microbial DNA content in mixed samples derived from host material can pose a major challenge due to an excess of host DNA. Consequently, microbial data output from whole genome sequencing can be severely limited due to a significant decrease in relative sequencing depth of the bacterial DNA fraction in the sample.

The QIAamp DNA Host-Free Microbiome Kit provides an easy-to-use workflow for selective isolation of bacterial DNA from samples that are intrinsically rich in host DNA; such as tissues, bodily fluids, or swabs. The method specifically targets eukaryotic cells and removes nucleic acids from these host cells as well as free nucleic acids derived from dead bacteria. The QIAamp DNA Host-Free Microbiome Kit allows isolation of enriched bacterial DNA suitable for a variety of applications, including qPCR and whole metagenome, or 16S rRNA gene sequencing.

Principle and procedure

The QIAamp DNA Host-Free Microbiome Kit efficiently depletes human and animal host DNA, and yields enriched bacterial DNA. An optimized combination of mechanical and chemical microbial lysis allows efficient disruption of bacterial cells. Target DNA is purified through adsorption to the silica membrane of QIAamp UCP Mini Columns, which have undergone proprietary DNA decontamination processes.

Depletion of host nucleic acid

In order to reduce the host DNA background in the sample, host nucleic acids are removed before isolating bacterial DNA. This is achieved by breaking down complex matrices in tissues samples and degradation of the host cells. Host-specific selectivity is based on differences in the physiology of eukaryotic and bacterial cells. During the host DNA depletion

step, the bacterial cells remain intact, ensuring that only released host nucleic acids are degraded during the incubation with Benzonase.

Lysing bacterial cells

The QIAamp DNA Host-Free Microbiome Kit uses an optimized cellular disruption method to ensure efficient lysis of both Gram-negative and Gram-positive bacteria.

Samples are disrupted using PowerBead Pro Tubes and a lysis buffer that contains detergent. The PowerBead Pro Tubes included in the kit contain a mix of differently sized beads, which strongly improves bacterial cell lysis.

Adsorption to the QIAamp UCP Mini membrane

The buffer conditions of the sample are adjusted to allow optimal binding of DNA to the membrane of the QIAamp UCP Mini Column. Lysates are then transferred to the QIAamp UCP Mini Column, and microbial nucleic acids are adsorbed onto the silica membrane.

Removal of contaminants

Nucleic acids bound to the silica membrane are washed twice with wash buffers AW1 and AW2 combined with centrifugation steps. These conditions ensure complete removal of residual contaminants without affecting DNA binding.

Elution of pure bacterial nucleic acids

Purified bacterial DNA is eluted from the QIAamp UCP Mini Column with RNAse-free water. The water should be equilibrated at room temperature before it is applied to the membrane. The yield is increased with a 5-minute incubation step at room temperature.

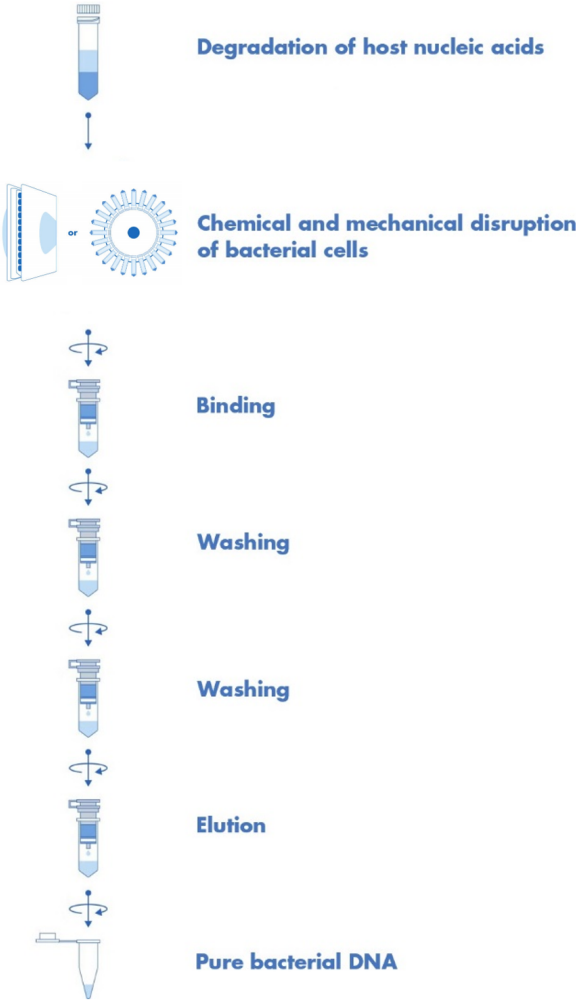
An elution volume of 50 µL is recommended to obtain a concentrated sample. For downstream applications that require a larger starting volume, the elution volume can be increased to up to 200 µL. However, the increase in elution volume will decrease the concentration of nucleic acids in the eluate. The recovered eluate can be up to 5 µL less than the volume of elution buffer applied to this column. The volume of eluate recovered depends on the nature of the sample.

Eluted nucleic acids can be stored at 2–8°C for 24 hours. For periods longer than 24 hours, storage at –15 to –30°C is recommended.

Yield and analysis

Depending on the sample type, the removal of host nucleic acids can result in a severe decrease in the overall yield. Using a spectrophotometer to determine DNA concentration might not be sufficient. Fluorescence-based methods may be used as an alternative. Variation in yield is possible depending on the sample donor.

QIAamp DNA Host-Free Microbiome 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

- Sterile pipette tips with aerosol barriers to prevent cross contamination
- Ethanol (96-100%)*
- Microcentrifuge tubes (2 mL)
- Phosphate-buffered saline (PBS)
- Microcentrifuge (with rotor for 2 mL tubes)
- Shaker-incubator, such as the Eppendorf® Thermomixer® Comfort (cat. no. 5355 000.011) and an Eppendorf Thermoblock for 24 x 2 mL tubes (cat. no. 5362 000.019)
- Equipment for sample disruption and homogenization, one of:
    - Vortex Genie 2 and Vortex Adapter for 24 (1.5–2 mL) tubes (cat. no. 13000-V1-24)
    - TissueLyser III (cat. no. 9003240) with adapter sets for use with the PowerBead Pro Tubes (TissueLyser Adapter Set 2 x 24, cat. no. 69982), or 2 mL Tube Holder, cat. no. 11993, in conjunction with Plate Adapter Set, cat. no. 11990)

*Do not use denatured alcohol that contains other substances, such as methanol or methylethylketone.

Important Notes

Sample collection and handling

- The QIAamp DNA Host-Free Microbiome Kit protocol is designed to isolate DNA from intact bacterial cells. To achieve optimal recovery of bacterial DNA and avoid biased results regarding community composition, the samples should be fresh. If storage is necessary, 2–8°C is preferable to freezing.
- Freeze–thaw cycles can compromise bacterial integrity, so the Benzonase treatment for the degradation of host DNA might lead to loss of exposed bacterial DNA.
- When using samples in transport media, ensure that the components do not compromise microbial cells. Keep in mind that sample storage and handling might impact microbial composition.
- Keep your work area clean and wear protective clothing to avoid false results due to contamination. Minimize the risk for cross-contamination by proper handling of sample material and always close vials and bottles directly after use.
- Use DNA-free pipette tips and consumables.

For preparation of buffers

- **Host Depletion Solution**:
  - Dissolve in 1.8 mL PBS. Mix gently by inverting, do not spin down.
  - For long-term storage store single-use aliquots at –15 to –25°C. Thawed aliquots can be stored at 2–8°C for 6 weeks.
  - Precipitates may form during storage but do not compromise the activity of the product.

- **Buffer AW1**: Add the appropriate amount of ethanol (96–100%) as indicated on the bottle and mix well.
- **Buffer AW2**: Add the appropriate amount of ethanol (96–100%) as indicated on the bottle and mix well.

Handling of QIAamp UCP Mini Columns

To avoid cross-contamination during sample preparation, adhere to the following guidelines:

- Pipet the sample into the column without wetting the rim.
- Use aerosol barrier tips and change pipette tips between all liquid transfers.
- Avoid touching the QIAamp membrane with the pipette tip
- Wear gloves throughout the procedure. Change your gloves if you come into contact with a sample.

Centrifugation

- All of the centrifugation steps are performed at room temperature.
- It is recommended to centrifuge always at the recommended speed. If this is not possible or in order to reduce noise, centrifugation at lower speed is possible as long as each solution passes through the QIAamp UCP Mini membrane.
- Centrifugation at lower speeds is possible as long as each solution passes through the QIAamp UCP Mini membrane.

Processing of QIAamp UCP Mini Columns

- Close the QIAamp UCP Mini Column before placing it in the microcentrifuge and spin as described in the protocol.
- Remove the QIAamp UCP Mini Column and collection tube from the microcentrifuge. Place the QIAamp UCP Mini Column into a new collection tube. Discard the filtrate and the collection tube. The filtrate may contain hazardous waste and should be disposed of appropriately.
- Open only one QIAamp UCP Mini Column at a time, taking care to avoid generating aerosols.

Protocol: Depletion of host DNA

Things to do before starting

- Remove all components from refrigerator or freezer.
- If a precipitate has formed in Buffer ATL or Buffer APL2, dissolve by incubation at 56°C.
- Vortex the (thawed) Host DNA Depletion solution. Do not spin down.
- Preheat heating blocks or water baths to 37°C, 56°C, and 70°C.
- Ensure that Buffers AW1 and AW2 have been prepared according to the instructions on the bottle.
- Before use, add 100 µL Reagent DX to 15 mL Buffer ATL. If smaller amounts are needed, transfer 1.5 mL of Buffer ATL into a sterile 2 mL vial and add 10 µL Reagent DX. Mix well after adding Reagent DX. After preparation, the mixture is stable for 6 months at room temperature.
- For tissue samples, mince or grind sample before starting the protocol, e.g. using TissueRuptor II (cat.no. 9002756 with Disposable Probes (25), cat.no. 990890).
- For swab samples, swirl the swab in 1 mL transport media or PBS for at least 20 s and dry off by pressing against the wall of the tube multiple times.
- Spin the PowerBead Pro Tube briefly to ensure that the beads have settled at the bottom.

Procedure

1. Add 220 µL of Buffer RDD, 3 µL Benzonase, and 30 µL Host Depletion Solution to up to 100 mg of tissue or the pre-processed swab sample in a 2 mL microcentrifuge tube (in that order). Mix well and incubate at 37°C for 45 min at 600 rpm in a heating block or water bath.

2. Add 20 µL Proteinase K, mix well, and incubate at 56°C for 20 min at 600 rpm in a heating block or water bath..
3. Add 200 µL Buffer ATL (containing Reagent DX). Mix well to avoid loss of sample material, and transfer into a PowerBead Pro Tube.
4. Mechanically disrupt samples using one of the following methods:
    a. Secure the PowerBead Pro Tube horizontally on a Vortex Adapter for 24 (1.5–2 mL) tubes. Orient the tube caps to point toward the center of the vortex adapter. Vortex at maximum speed for 10 min.
    b. Use a TissueLyser III. Place the PowerBead Pro Tube into the TissueLyser Adapter Set 2 x 24 or 2 mL Tube Holder, and Plate Adapter Set. Fasten the adapter into the instrument and shake for 5 min at 30 Hz. Reorient the adapter so that the side that was closest to the machine body is now furthest from it. Shake again for 5 min at 30 Hz.
5. Centrifuge the PowerBead Pro Tube at 10,000 x *g* for 1 min. Transfer the supernatant to a fresh microcentrifuge tube.
6. Add 200 µL APL2. Mix by pulse vortexing for 30 s.
7. Incubate at 70°C for 10 min and briefly spin the tube to remove condensation.
8. Carefully apply up to 700 µL of the mixture from step 7 to the QIAamp UCP Mini Column without wetting the rim. Close the cap and centrifuge at 6,000 x *g* for 1 min.
9. Discard the flow-through. Put the column back into the collection tube to repeat step 8 with any remaining mixture from step 7.
10. Transfer the QIAamp UCP Mini Column to a fresh collection tube. Carefully open the cap and add 500 µL Buffer AW1 without wetting the rim. Close the cap and centrifuge at 6,000 x *g* for 1 min. Discard the filtrate and place the column back in the same collection tube.

11. Add 500 µL Buffer AW2 to the QIAamp UCP Mini Column without wetting the rim. Centrifuge at full speed (20,000 x *g*) for 3 min. Discard the filtrate and place the column back in the same collection tube.
12. Centrifuge at full speed (20,000 x *g*) for 1 min.
13. Place the QIAamp UCP Mini Column into a fresh 1.5 mL tube and apply 50 µL RNase-free Water directly onto the center of the membrane. Close the lid and incubate at room temperature for 5 min.
13. Centrifuge at 6,000 x *g* for 1 min to elute the DNA.

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**).

Issue	Comments and suggestions
Host DNA depletion and tissue homogenisation:	
Poor removal of host DNA	
Insufficient incubation time with Host Depletion Solution	Incubation with Host DNA Depletion Solution and Benzonase for 45 min is required for sufficient degradation of host cells. Can be extended to 60 min for DNA-rich or tough tissue types.
Insufficient tissue homogenisation	
Tissue pieces visible after incubation with proteinase K	Incubation time with proteinase K can be extended to 45 min to improve homogenization of fibrous or especially tough tissues. Incubation should not exceed 45 min to avoid compromising bacterial cell envelopes.
Bacterial DNA:	
No recovery of bacterial DNA	
a) Bacterial cells in sample material were compromised	Preferably use fresh sample material and avoid (repeated) freezing if possible. Make sure transport media does not contain components that will result in premature microbial lysis or might inhibit enzyme activity.
b) Low bacterial biomass in starting material	Bacterial cell numbers in some sample types can be extremely low. Make sure to process sufficient material.
c) Inefficient mechanical lysis of bacteria	Make sure that the PowerBead Pro Tube vortexed for 10 min at maximum speed using a Microtube foam insert of a Vortex Genie®; or that sufficient disruption was carried out in a TissueLyser III at 30 Hz, or FastPrep-24 instrument as described in the protocol.

Issue	Comments and suggestions
d) Buffer AW1 or Buffer AW2 prepared incorrectly	Check that Buffer AW1 and Buffer AW2 concentrates were diluted with the correct volume of ethanol as indicated on the respective bottles. Repeat the purification procedure with new samples.
e) Buffer AW1 or Buffer AW2 prepared with 70% ethanol	Check that Buffer AW1 and Buffer AW2 concentrates were diluted with 96–100% ethanol as indicated on the bottles. Repeat the purification procedure with new samples.
f) Buffers AW1 and AW2 used in the wrong order	Repeat the purification procedure and ensure that Buffers AW1 and AW2 are used in the correct order.
g) QIAamp UCP Mini Column not incubated at room temperature (15–25°C) for 5 min	After addition of RNAse-free water the QIAamp UCP Mini Column should be incubated at room temperature for 5 min.
Eluted bacterial nucleic acids do not perform well in downstream reactions	
a) Little or no DNA in the eluate	See "No recovery of bacterial DNA" above for possible reasons. If possible, increase the amount of eluate added to the reaction. The eluate may be re-applied to the QIAamp UCP Mini Column to maximise yields. Incubate again for 5 min at room temperature and elute at 6 000 x *g* for 1 min.
b) Inappropriate elution volume used	Determine the maximum volume of eluate suitable for your downstream reaction. Reduce or increase the volume of eluate added to the downstream reaction accordingly. The elution volume can be adapted proportionally. Elution volume of lower than 50 µL will reduce overall yield.
c) Buffers not mixed thoroughly	Salt and ethanol components of wash Buffer AW2 may have separated out after being left for a long period between runs. Always mix buffers thoroughly before each run.
d) Residual ethanol in the eluate	Use the recommended drying step and make sure that the QIAamp UCP Mini Column does not come into contact with the filtrate prior to elution.
White precipitate after addition of Buffer APL2	
White precipitate after addition of Buffer APL2	In most cases, the precipitate formed after addition of Buffer APL2 will dissolve during incubation at 70°C. The precipitates do not interfere with the procedure or with any subsequent application.

Issue	Comments and suggestions
White precipitate in Buffer ATL or Buffer APL2	
White precipitate may form after storage at low temperature or prolonged storage	Any precipitate in Buffer ATL or Buffer APL2 must be dissolved by incubation of the buffer at 56°C. The precipitate has no effect on function. Dissolving the precipitate at high temperature will not compromise the yield or quality of the purified nucleic acids.
General handling	
a) Lysate not completely passed through the membrane	Centrifuge for 1 min at full speed or until all the lysate has passed through the membrane
b) Cross-contamination between samples	To avoid cross-contamination when handling QIAamp UCP Mini Columns, please read "Handling of QIAamp UCP Mini Columns" on page 13 . Repeat the procedure

Appendix A: General Remarks

General handling

Proper microbiological, aseptic technique should always be used when purifying bacterial DNA with the QIAamp DNA Host-Free Microbiome Kit. Hands and dust particles may carry microorganisms and are the most common sources of contamination. Always wear latex or vinyl gloves while handling reagents and consumables to prevent contamination from the surface of the skin or from dusty laboratory equipment. Change gloves frequently and keep tubes closed whenever possible. Keep purified nucleic acids on ice when aliquots are pipetted for downstream applications.

Disposable plastic ware

The use of sterile, disposable polypropylene tubes is recommended throughout the procedure. These tubes are generally free of contaminating nucleic acids and do not require pretreatment.

Ordering Information

Product	Contents	Cat. no.
QIAamp DNA Host-Free Microbiome Kit (50)	For 50 samples: 50 QIAamp UCP Mini Columns, Collection Tubes, Pathogen Lysis Tubes, Elution Tubes and Buffers	51904
Accessories		
PowerBead Pro Tubes (2 mL) (50)	50 PowerBead Pro Tubes	19301
TissueLyser III	Bead mill (100–120/220–240 V, 50/60 Hz) for medium-to-high throughput sample disruption for molecular analysis; requires the TissueLyser Adapter Set 2 x 4 for 50 mL Tubes	9003240
TissueLyser Adapter Set 2 x 24	Two sets of adapter plates and 2 racks for use with 2 mL microcentrifuge tubes on the TissueLyser III	69982
TissueLyser Adapter Set 2 x 96	Two sets of adapter plates for use with Collection Microtubes (racked) on the TissueLyser III	69984
TissueRuptor® II	Handheld rotor–stator homogenizer	Variable*
TissueRuptor, Disposable probes	25 nonsterile plastic disposable probes for use with the TissueRuptor II	990890
Related products		
REPLI-g® Single Cell Kit (24)†	REPLI-g sc Polymerase, Buffers, and Reagents for 24 whole genome amplification reactions (yields up to 40 µg/reaction)	150343
GeneRead DNA Library I Core Kit (48)†	For 48 reactions: Buffers and reagents for end-repair, A-Addition, and ligation, for use with Illumina instruments	180434
GeneRead DNA I Amp Kit (100)	For 100 reactions: Buffers and reagents for library amplification, for use with Illumina instruments	180455
QIAseq FX DNA Library UDI-A Kit (96) ‡	Buffers and reagents for DNA fragmentation (including end repair and A-addition), ligation and library amplification; for use with Illumina instruments	180479

* Contact your QIAGEN sales team or local distributor for the instrument available in your country.

† Other kit sizes and/or formats available; see **www.qiagen.com**

‡ For more information on QIAseq Library Kits and accessories please visit **www.qiagen.com**

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
04/2025	Initial revision.

Limited License Agreement for QIAamp® DNA Host-Free Microbiome Kit

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

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