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QIAamp® DNA Microbiome Handbook

For isolation of bacterial microbiome DNA from mixed samples

Sample & Assay Technologies

QIAGEN Sample and Assay Technologies

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QIAGEN sets standards in:

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- Nucleic acid and protein assays
- microRNA research and RNAi
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Contents

Kit Contents

QIAamp DNA Microbiome Kit	**(50)**
Catalog no.	**51704**
Number of preps	**50**
QIAamp UCP Mini Columns	50
Collection tubes (2 ml)	150
Pathogen Lysis tubes L	50
Elution tubes (1.5 ml)	100
Buffer AHL	5 bottles
Buffer RDD	35 ml
Benzonase®	2 vials
Buffer ATL	50 ml
Reagent DX (clear cap)	1 ml
Buffer APL2*	14 ml
Buffer AW1* (concentrate)	19 ml
Buffer AW2† (concentrate)	13 ml
Proteinase K (green cap)	2 vials
Buffer AVE†	3 vials
Quick-Start Protocol	1

* Contains chaotropic salt. Not compatible with disinfecting agents containing bleach.
† Contains sodium azide as a preservative.

Shipping and Storage

The components of the QIAamp DNA Microbiome Kit are shipped on dry ice. Upon receipt, Buffer AHL and Benzonase should be stored at –20°C.

Ready-to-use Proteinase K solution is dissolved in a specially formulated storage buffer and stable for up to 1 year after delivery when stored at room temperature (15–25°C). To prolong the lifetime of Proteinase K, storage at 2–8°C is recommended.

QIAamp UCP Mini Columns should be stored at 2–8°C upon arrival, but storage for up to 4 weeks at room temperature (15–25°C) does not affect their performance. All of the buffers of the QIAamp DNA Microbiome Kit except Buffer AHL can be stored at room temperature (15–25°C).

Intended Use

The QIAamp DNA Microbiome Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

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 the QIAamp DNA 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 the excess of host DNA. In the case of whole metagenome sequencing from certain sample types, microbial data output is severely limited due to a significant decrease in sequencing capacity.

The QIAamp DNA 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 bodily fluids or swabs. The method is specific for the identification of intact bacteria so it prevents false results due to nucleic acids from dead bacteria. The QIAamp DNA 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 Microbiome Kit efficiently depletes human and animal host DNA and yields enriched bacterial DNA. An optimized combination of mechanical and chemical 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 remove host nucleic acids from samples, the contaminating nucleic acids are degraded before the isolation of bacterial DNA. This is achieved thanks to a differential lysis of the human or animal host cells based on differences in the physiology of the host and bacterial cells. During this lysis step, the bacterial cells are kept intact so that only exposed nucleic acids are degraded during the incubation with Benzonase.

Lysing bacterial cells

To ensure efficient lysis of both Gram-negative and Gram-positive bacteria, the QIAamp DNA Microbiome Kit uses an optimized cellular disruption method.

Samples are disrupted using Pathogen Lysis Tubes and a lysis buffer that contains detergent. The Pathogen Lysis Tubes L included in the kit contain large beads. Pathogen Lysis Tubes S are available as an accessory product (see page 22).

Adsorption to the QIAamp UCP Mini membrane

The buffer conditions of the sample are adjusted by the addition of ethanol 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 Buffer AVE. The elution buffer 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.

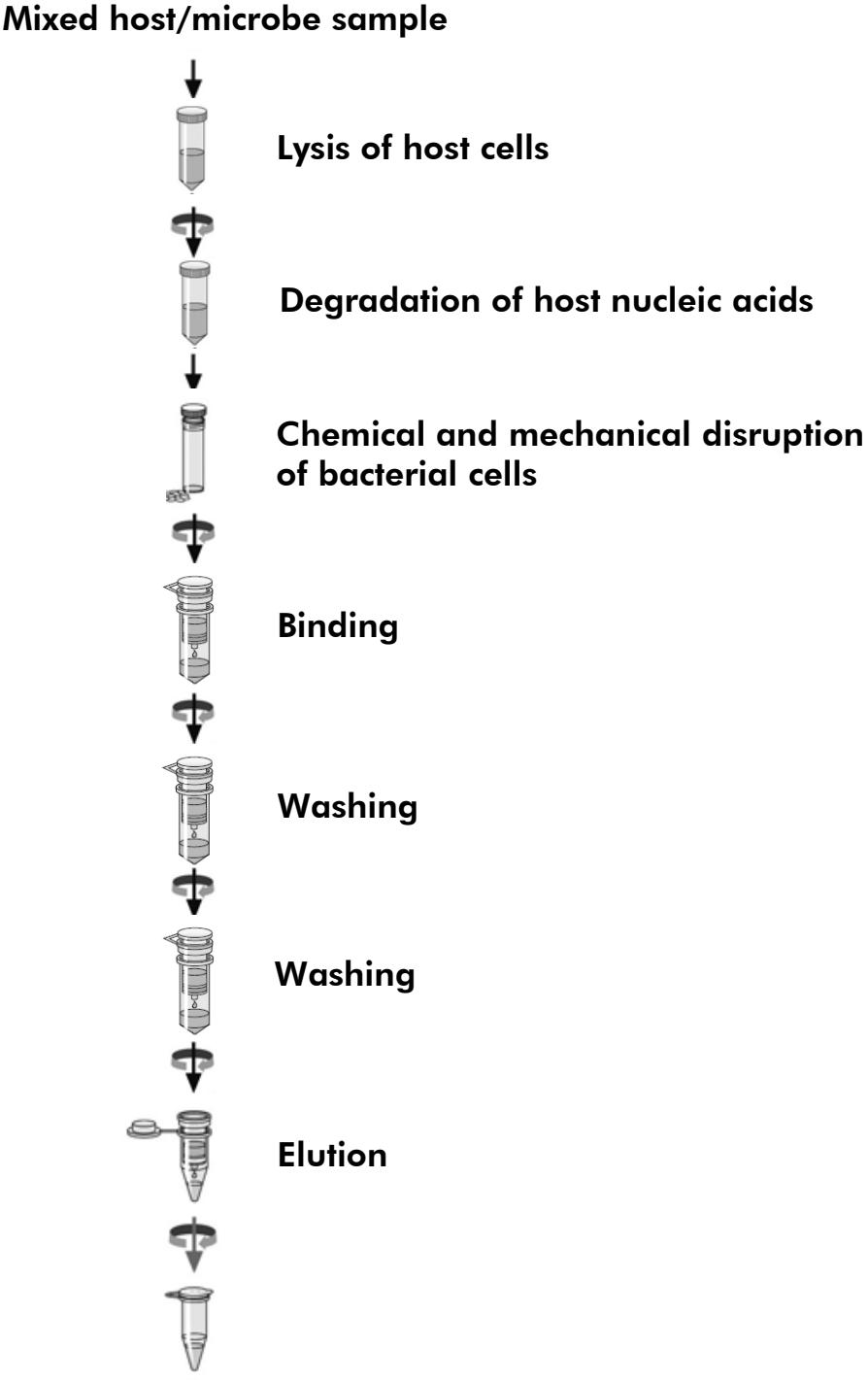
An elution volume of 50 µl is recommended to obtain a concentrated sample. For downstream applications that require a larger starting volume, the elution volume can be increased to up to 200 µl. However, the increase in elution volume will decrease the concentration of nucleic acids in the eluate. The 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. If you are using water for elution, ensure that its pH is at least 7.0 (deionized water from certain sources can be acidic). Nucleic acids stored in water are subject to degradation by acid hydrolysis.

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 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 pipet tips with aerosol barriers to prevent cross contamination
- Ethanol (96-100%)†
- Microcentrifuge tubes (2 ml)
- Phosphate-buffered saline (PBS; only required for some samples)
- Overhead shaker, e.g., available from Heidolph®
  (cat. no. 541-21001-00)*
- 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)*
- TissueLyser® LT (cat. no. 85600), TissueLyser II (cat. no. 85300), Scientific Industries® Vortexer with Microtube insert (cat. no. 504-0234-00), or MP Biomedicals® FastPrep® 24 (cat. no. 116004500)*

* This is not a complete list of suppliers and does not include many important vendors of biological supplies.

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

Important notes

Sample collection and handling

The QIAamp DNA 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, make sure that the components do not compromise microbial cells. Keep in mind that sample storage and handling might impact microbial composition.

To avoid false results due to contamination, keep your work area clean and wear protective clothing. 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

Buffer AHL

Thaw Buffer AHL at room temperature or at 2–8°C and mix well prior to use. To ensure optimal performance, do not freeze and thaw more than 3 times. In case less than a full bottle of Buffer AHL is needed, make sure to use sterile technique and/or work under laminar flow when removing the required amount of buffer to avoid contamination and microbial growth in the remaining buffer.

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 pipet tips between all liquid transfers.
- Avoid touching the QIAamp membrane with the pipet 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 (15–25°C).

Centrifugation of the QIAamp UCP Mini Column is performed at 6,000 x g in order to reduce noise. Centrifugation at full speed (20,000 x g) will not affect DNA yield. 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. Note that 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.
- For efficient parallel processing of multiple samples, fill a rack with collection tubes to which the QIAamp UCP Mini Columns can be transferred after centrifugation. Used collection tubes containing filtrate can be discarded and the new collection tubes containing the QIAamp UCP Mini Columns can be placed into the microcentrifuge directly.

Protocol: Depletion of host DNA

Things to do before starting

- Remove all components from refrigerator or freezer.
- If not using a complete bottle of Buffer AHL, make sure to use sterile technique to remove the needed amount of buffer. For this step, work under laminar flow is recommended.
- If a precipitate has formed in Buffer ATL or Buffer APL2, dissolve by incubation at 56°C.
- 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 (15–25°C).
- 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.

Procedure for 1 ml sample volume:

1. **Add 500 µl Buffer AHL to 1 ml of sample in a 2 ml tube (not provided) and incubate for 30 min at room temperature with end-over-end rotation.**

   For smaller sample volumes adjust the volume of Buffer AHL accordingly.

   Optional: Instead of using end-over-end rotation, you can incubate non-viscous samples in a thermomixer at 600 rpm. Periodically check that the sample and Buffer AHL are mixing correctly.
2. **Centrifuge the tube at 10,000 x g for 10 min and carefully remove the supernatant.**

   Do not disturb the pellet as this will result in loss of bacterial material.
3. **Add 190 µl Buffer RDD and 2.5 µl Benzonase. Mix well and incubate at 37°C for 30 min at 600 rpm in a heating block or water bath.**
4. **Add 20 µl Proteinase K and incubate at 56°C for 30 min at 600 rpm in a heating block or water bath.**

5. **Briefly spin the tube at slow speed to remove condensation. Add 200 µl Buffer ATL (containing Reagent DX). Mix well to avoid loss of sample material and transfer into a Pathogen Lysis Tube L.**

   Note: Careful mixing after the centrifugation step ensures efficient transfer of intact bacteria.

6. **Lyse bacterial cells with Pathogen Lysis Tube L. The following three options have been verified as compatible with the QIAamp DNA Microbiome Kit.**

   A. Place the Pathogen Lysis Tube L in a TissueLyser LT for 10 min at 50 Hz or a TissueLyser II for 10 min at 30 Hz.

   B. Place the Pathogen Lysis Tube L into a FastPrep-24 instrument. Apply a velocity of 6.5 m/s twice for 45 s each with a 5-min interval during which the samples should be stored on ice.

   C. Place the Pathogen Lysis Tube L on a vortexer with a microtube foam insert and vortex for 10 min at maximum speed.

   Note: Avoid heating of samples during lysis.

7. **Centrifuge the Pathogen Lysis Tube L at 10,000 x g for 1 min to reduce the amount of foam after lysis. Mix carefully and transfer the supernatant to a fresh microcentrifuge tube.**

   Note: Careful mixing after the centrifugation step will transfer possibly intact bacteria that are at the bottom of the tube. Do not transfer beads from the Pathogen Lysis Tube to subsequent reactions.

8. **Add 40 µl Proteinase K, mix by vortexing, and incubate at 56°C for 30 min at 600 rpm in a heating block or water bath.**
9. **Add 200 µl Buffer APL2. Mix by pulse vortexing for 30 s.**
10. **Incubate at 70°C for 10 min and briefly spin the tube.**
11. **Add 200 µl ethanol to the lysate. Mix thoroughly by pulse vortexing for 15–30 s.**
12. **Carefully apply up to 700 µl of the mixture from step 11 to the QIAamp UCP Mini Column without wetting the rim. Close the cap and centrifuge at 6,000 x g for 1 min.**
13. **Discard the flow-through. Put the column back into the collection tube to repeat step 12 with any remaining mixture from step 11.**

    Note: Flow-through containing Buffer APL2 or Buffer AW1 is not compatible with bleach. See "Safety Information" on page 5.

14. **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. Place the QIAamp UCP Mini Column into a fresh 2 ml collection tube and discard the filtrate.**

    Note: Flow-through containing Buffer APL2 or Buffer AW1 is not compatible with bleach. See "Safety Information" on page 5.

15. **Carefully open the QIAamp UCP Mini Column and add 500 µl Buffer AW2 without wetting the rim. Centrifuge at full speed (20,000 x g) for 3 min.**
16. **Place the QIAamp UCP Mini Column into a fresh 2 ml collection tube. Discard the filtrate. Centrifuge at full speed (20,000 x g) for 1 min.**
17. **Place the QIAamp UCP Mini Column into a fresh 1.5 ml tube and apply 50 µl Buffer AVE directly onto the center of the membrane. Close the lid and incubate at room temperature for 5 min.**

    Optional: Depending on the downstream application, water or Buffer EB may be used instead.

18. **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 at our Technical Support Center: www.qiagen.com/FAQ/FAQList.aspx. The scientists of QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or sample and assay technologies (for contact information, see back cover or visit www.qiagen.com).

Issue	Comments and suggestions
Host DNA depletion:	
Poor removal of host DNA	
a. Insufficient incubation time with Buffer AHL	Incubation for 30 min is required for sufficient lysis of host cells.
b. Insufficient mixing during incubation with Buffer AHL	Ensure proper mixing of sample with Buffer AHL. End-over-end rotation is recommended, especially for samples with high numbers of host cells.
c. Insufficient activity of Benzonase	Make sure incubation was performed at 37°C for at least 30 min in Buffer RDD to achieve sufficient degradation of host nucleic acids.
Bacterial DNA:	
No recovery of bacterial DNA	
a. Bacterial cells in sample material were compromised	Preferably use fresh sample material and avoid freezing if possible. Make sure transport media does not contain components that will result in premature microbial lysis.
b. Pellet containing bacterial cells disturbed	Make sure not to disturb the pellet containing bacterial cells when removing the supernatant after host cell lysis. Note that the pellet might not be visible.
c. Decreased activity of Proteinase K	Decreased activity might result in inefficient degradation of Benzonase. Store Proteinase K at the appropriate temperature, preferably 2–8°C.

Issue	Comments and suggestions
d. Inefficient mechanical lysis of bacteria	Make sure that the Pathogen Lysis 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 LT at 50 Hz, TissueLyser II at 30 Hz, or FastPrep-24 instrument as described in the protocol.
e. No ethanol added to the lysate before applying it to the QIAamp UCP Mini Column	Repeat the purification procedure with a new sample.
f. Low-percentage ethanol used instead of 96–100%	Repeat the purification procedure with new samples and 96–100% ethanol. Do not use denatured alcohol, which contains other substances, such as methanol or methylethylketone.
g. 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.
h. 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.
i. 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.
j. QIAamp UCP Mini Column not incubated at room temperature (15–25°C) for 5 min	After addition of Buffer AVE the QIAamp UCP Mini Column should be incubated at room temperature for 5 min.
k. If eluting with water, pH of water incorrect (acidic)	Low pH may reduce DNA yield. Ensure that the pH of the water is at least 7.0 or use Buffer AVE or Buffer EB for elution.

Issue	Comments and suggestions
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.
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.
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.

Issue	Comments and suggestions
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 the section "Handling of QIAamp UCP Mini Columns" on page 11. Repeat the procedure

Appendix: General Remarks

General handling

Proper microbiological, aseptic technique should always be used when purifying bacterial DNA with the QIAamp DNA Microbiome Kit. Hands and dust particles may carry bacteria and molds 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 Microbiome Kit (50)	For 50 samples: 50 QIAamp UCP Mini Columns, Collection Tubes, Pathogen Lysis Tubes, Elution Tubes and Buffers	51704
Accessories		
Pathogen Lysis Tubes S	50 Pathogen Lysis Tubes and 1 vial Reagent DX	19091
Pathogen Lysis Tubes L	50 Pathogen Lysis Tubes and 1 vial Reagent DX	19092
TissueLyser II	Universal laboratory mixer-mill disruptor, 100–120/220–240 V, 50/60 Hz; requires the TissueLyser Adapter Set 2 x 24 or TissueLyser Adapter Set 2 x 96 (available separately)	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
TissueLyser Adapter Set 2 x 96	2 sets of adapter plates for use with Collection Microtubes (racked) on the TissueLyser II	69984
TissueLyser LT	Bead mill for low- to medium throughput sample disruption	85600
TissueLyser LT Adapter, 12-Tube	Adapter for disruption of up to 12 samples in 2 ml microcentrifuge tubes on the TissueLyser LT	69980
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

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

Product	Contents	Cat. no.
GeneRead Library I Core Kit (12) *	For 12 reactions: Buffers and reagents for end-repair, A-Addition, and ligation, for use with Illumina instruments	180432
GeneRead DNA I Amp Kit (100)	For 100 reactions: Buffers and reagents for library amplification, for use with Illumina instruments	180455
GeneRead Adapter I Set 12-Plex (72)	For 72 reactions: 12 barcoded adapters for ligation to DNA library, for use with Illumina instruments	180984
GeneRead Adapter I Set 1-Plex (12)	For 12 reactions: Adapters for DNA ligation, for use with Illumina instruments	180912
GeneRead DNA Library L Core Kit (12)	For 12 reactions: Buffers and reagents for end-repair, ligation, and nick-repair, for use with Life Technology instruments	180462
GeneRead DNA L Amp Kit (100)	For 100 reactions: Buffers and reagents for library amplification, for use with Life Technology instruments	180485
GeneRead Adapter L Set 12-plex (72)	For 72 reactions: 12 barcoded adapters for ligation to DNA library, for use with instruments from Life Technologies	180994
GeneRead Adapter L Set 1-plex (12)	For 12 reactions: Adapters for DNA ligation, for use with Life Technologies instruments	180922

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

Notes

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