March 2025

Quick-Start Protocol

QIAamp® DNA Host-Free Microbiome Kit

The QIAamp DNA Host-Free Microbiome Kit is shipped at room temperature (15–25°C). Upon receipt, store the Host Depletion Solution and the QIAamp UCP Mini Spin Columns at 2–8°C, Benzonase at –20°C, and all other kit components dry at room temperature.

Further information

- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Dissolve lyophilized Host Depletion Solution in 1.8 mL PBS. Mix gently by inverting. Do not spin down. For long-term storage, store single-use aliquots at –15°C to –25°C. Thawed aliquots can be stored at 2–8°C for 6 weeks. Note that precipitate may form during storage but do not compromise the activity of the product.
- Tissue samples: mince or grind sample before starting the protocol (e.g., using TissueRuptor II, cat. no. 990890).
- 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 down (10 min, 10,000 × *g*) and remove the supernatant.

- If a precipitate has formed in Buffer ATL or Buffer APL2, dissolve by incubating at 56°C.
- Ensure that Buffers AW1 and AW2 have been prepared according to the instructions on the bottle.
- 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 preparation, the mixture is stable for 6 months at room temperature (15–25°C).
- Spin the PowerBead Pro Tube briefly to ensure that the beads have settled at the bottom.

Equipment and reagents to be supplied by user

- Ethanol (96–100 %)
- Phosphate-buffered saline (PBS)
- Microcentrifuge (with rotor for 2 mL tubes)
- Shaker–incubator
- Equipment for sample disruption and homogenization, one of the following:
  - 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)

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 (cat. no. 13000-V1-24). Orient the tube caps to point toward the center of the vortex adapter. Vortex at maximum speed for 10 min.
    b. Use TissueLyser III. 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 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 × *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. Close the cap and centrifuge at 6000 × *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 6000 × *g* for 1 min. Discard the filtrate.

11. Add 500 µL Buffer AW2 to the QIAamp UCP Mini Column without wetting the rim. Centrifuge at full speed (20,000 × *g*) for 3 min. Discard the filtrate.
12. Centrifuge at full speed (20,000 × *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.
14. Centrifuge at 6000 × *g* for 1 min to elute the DNA.

Document Revision History

Date	Description
03/2025	Initial release