Quick-Start Protocol March 2016

QIAamp® DNA Microbiome Kit

Components of the QIAamp DNA Microbiome Kit are shipped on dry ice. Upon receipt, Buffer AHL and Benzonase® should be stored at –30 to –15°C and QIAamp UCP Mini columns should be stored at 2–8°C. All other buffers (except Buffer AHL) can be stored at room temperature (15–25°C).

Further information

- *QIAamp DNA Microbiome Handbook*: **www.qiagen.com/HB-1792**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- This protocol is for the depletion of host DNA.
- Remove all components from the refrigerator or freezer.
- Preheat different heating blocks/water baths to 37°C, 56°C and 70°C.
- Add ethanol (96–100%) to Buffers AW1 and AW2 as indicated on the bottles.
- If a precipitate has formed in Buffer ATL or Buffer APL2, heat at 56°C to dissolve.
- Before use, add 100 µl Reagent DX to 15 ml Buffer ATL and mix well. After preparation, the mixture is stable for 6 months at room temperature (15–25°C).
- For swab samples, swirl swab in 1 ml transport media or PBS for at least 20 s.

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.
2. Centrifuge the tube at 10,000 x *g* for 10 min and remove the supernatant.
3. Add 190 µl of Buffer RDD and 2.5 µl of 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. Then, briefly spin the tube at slow speed.

5. Add 200 µl Buffer ATL (containing Reagent DX). Mix well and transfer into Pathogen Lysis Tube L. Place Pathogen Lysis Tube L into a FastPrep®-24 instrument, applying a velocity of 6.5 m/s twice for 45 s with a 5 min intermission, while samples are stored on ice. For alternative lysis options, refer to the kit handbook. **Optional**: After lysis, heat to 95°C for 5 min.
6. Centrifuge Pathogen Lysis Tube L at 10,000 x *g* for 1 min and transfer the supernatant into a fresh microcentrifuge tube. Add 40 µl Proteinase K, vortex to mix and incubate at 56°C for 30 min at 600 rpm in a heating block or water bath.
7. Add 200 µl Buffer APL2. Mix by pulse-vortexing for 30 s. Incubate at 70°C for 10 min and briefly spin the tube.
8. Add 200 µl ethanol to the lysate and mix by pulse-vortexing for 15–30 s. Apply up to 700 µl of this mixture to the QIAamp UCP Mini spin column without wetting the rim. Close the cap and centrifuge at 6,000 x *g* for 1 min. Discard the flow-through* and put the column back into the same collection tube. Repeat with any remaining ethanol-lysate mixture.
9. Transfer the QIAamp UCP Mini spin column to a fresh collection tube. Open the cap and add 500 µl Buffer AW1 without wetting the rim. Close the cap and centrifuge at 6000 x *g* for 1 min. Place the QIAamp UCP Mini spin column into a fresh 2 ml collection tube and discard the filtrate.*
10. Open the QIAamp UCP Mini spin column and add 500 µl Buffer AW2† without wetting the rim. Centrifuge at 20,000 x *g* for 3 min.
11. Place the QIAamp UCP Mini spin column into a fresh 2 ml collection tube and discard the filtrate. Centrifuge at 20,000 x *g* for 1 min.
12. Place the QIAamp UCP Mini spin 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. Centrifuge at 6000 x *g* for 1 min to elute the DNA.

* Flow-through containing Buffer APL2 or Buffer AW1 is not compatible with bleach.

† Buffers AW2 and AVE contain sodium azide as a preservative.

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