Quick-Start Protocol March 2016

QIAamp® *cador*® Pathogen Mini Kit

The QIAamp *cador* Pathogen Mini Kit (cat. nos. 54104 and 54106) can be stored at room temperature (15–25°C) until the expiration date on the kit box.

Further information

- *QIAamp* cador *Pathogen Mini Handbook*: **www.qiagen.com/HB-0934**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- This Quick-Start Protocol is for viruses and easy-to-lyse bacteria from fluid samples. For difficult-to-lyse bacteria or tissue samples please refer to the pretreatments described in the *QIAamp* cador *Pathogen Mini Handbook* (see Table 1).
- Add isopropanol (100%) to Buffer ACB and ethanol (96–100%) to Buffers AW1 and AW2 before use. See the respective bottle labels for volumes.
- Dissolve carrier RNA using Buffer AVE and add it to Buffer VXL for processing cell-free samples.
- Carry out all centrifugation steps at room temperature in a conventional table-top microcentrifuge.

1. Pipet 20 µl proteinase K into a 2 ml microcentrifuge tube (not provided).
2. Add 200 µl fluid sample to the proteinase K. For lower sample volumes, adjust the volume to 200 µl with PBS or 0.9% NaCl.
3. Add 100 µl Buffer VXL. Close the cap and mix by pulse vortexing. For cell-free samples, ensure that 1 µg carrier RNA is added per 100 µl Buffer VXL.
4. Incubate at 20–25°C for 15 min.
5. Briefly centrifuge the tube to remove drops from the inside of the lid.
6. Add 350 µl Buffer ACB to the sample, and mix thoroughly by pulse-vortexing.

7. Briefly centrifuge the tube to remove drops from the inside of the lid.
8. Transfer the lysate to a QIAamp Mini column placed in a 2 ml collection tube. Close the cap, and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Transfer the QIAamp Mini column to a clean 2 ml collection tube, and discard the collection tube containing the filtrate.
9. Add 600 µl Buffer AW1 and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Transfer the QIAamp Mini column to a clean 2 ml collection tube, and discard the collection tube containing the filtrate.
10. Add 600 µl Buffer AW2 and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Transfer the QIAamp Mini column to a clean 2 ml collection tube, and discard the collection tube containing the filtrate.
11. Centrifuge the QIAamp Mini column at 20,000 x *g* (14,000 rpm) for 2 min.
12. Place the QIAamp Mini column in a clean 1.5 ml microcentrifuge tube (not provided), and discard the collection tube containing the filtrate. Add 50–150 µl Buffer AVE to the center of the membrane, close the cap and incubate at room temperature for 1 min.
13. Centrifuge at 20,000 x *g* (14,000 rpm) for 1 min.

Table 1. Pretreatments (see *QIAamp* cador *Pathogen Mini Handbook*)

Name	Application
Pretreatment B1	For difficult-to-lyse bacteria in whole blood or tissue homogenates
Pretreatment B2	For difficult-to-lyse bacteria in cell-free fluids
Pretreatment B3	For easy-to-lyse bacteria in high-volume cell-free fluids
Pretreatment T1	Mechanical disruption of tissue
Pretreatment T2	Enzymatic digestion of tissue
Pretreatment T3	Rapid partial disruption of tissue
Pretreatment T4	Organic extraction for difficult tissue

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