QIAGEN Supplementary Protocol

Purification of MAP DNA from tissue using the QIAamp® *cador*® Pathogen Mini Kit

This protocol is designed for purification of *Mycobacterium avium* spp. *paratuberculosis* (MAP) DNA from up to 25 mg of tissue.

In this protocol, tissue is homogenized using the TissueLyser II or an equivalent electric homogenizer. Samples are then processed according to the standard QIAamp protocols.

IMPORTANT: Please read the *QIAamp* cador *Pathogen Mini Kit Handbook*, paying careful attention to the "Safety Information" and "Important Notes" sections, before beginning this procedure.

Equipment and reagents

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.

- QIAamp *cador* Pathogen Mini Kit [cat. no. 54104 (50) or 54106 (250)]
- Equipment for sample disruption and homogenization
- PBS or 0.9% NaCl solution
- Pipets and pipet tips (pipet tips with aerosol barriers for preventing cross-contamination are recommended)
- Microcentrifuge with rotor for 1.5 ml and 2 ml tubes
- Thermomixer or rocking platform for shaking and heating at 99°C
- Ethanol (96–100%)
- Vortexer
- *bactotype*® MAP PCR Kit (96)* (cat. no. 285905), *bactotype* MAP PCR Reagent (96) (cat. no. 285915), or *intype* IC-DNA (cat no. 289980)

* Not available in the USA.

Suppliers of equipment for disruption and homogenization

QIAGEN

- TissueLyser II (Cat. no. 85300)
- Pathogen Lysis Tubes S (Cat. no. 19091)

MP Biomedicals*

- FastPrep®-24 Instrument (Catalog no. 116004500)
- Lysing Matrix E (Catalog no. 116914050, 116914100 or 116914500)

PEQLAB Biotechnologie GMBH*

- Precellys® 24 (cat. no. 91-PCS24)
- Precellys Glas/Keramik-Kit SK38 (Cat. no. 91-PCS-SK38)

Important points before starting

- If using the QIAamp *cador* Pathogen Mini Kit for the first time, read "Important Notes" in the *QIAamp* cador *Pathogen Mini Kit Handbook*.
- All centrifugation steps are carried out at room temperature (15–25°C) in a microcentrifuge.

Things to do before starting

- Before beginning the procedure, read "Important Notes" (page 14) in the *QIAamp* cador *Pathogen Mini Kit Handbook*.
- Add 1µl IC-DNA to 100µll Buffer VXL

  Note: Buffer VXL + IC-DNA should be prepared fresh.

Procedure

1. **Place up to 25 mg tissue in a 2 ml lysis tube (not provided).**

   For tissues with a very high number of cells for a given mass of tissue (e.g., spleen), a reduced amount of starting material (5–10 mg) should be used.

   If working with fibrous tissue, cutting the tissue into smaller pieces before starting disruption will improve disruption efficiency.

* Non-QIAGEN equipment needs to be validated.

2. **Add 300 µl PBS or 0.9% NaCl solution to each tube.**

3. **Homogenize the sample until the sample is thoroughly homogenized.**

   Homogenize the sample using a conventional homogenizer until it is uniformly homogeneous.

   Disruption and homogenization using the TissueLyser II

   1. Place the Pathogen Lysis Tubes S in the TissueLyser Adapter Set 2 x 24.
   2. Operate the TissueLyser II for 3 min at 30 Hz.
   3. Rearrange the tubes so that the outermost tubes are innermost, and the innermost tubes are outermost.
   4. Operate the TissueLyser II for another 3 min at 30 Hz.

   Disruption and homogenization using the FastPrep-24 Instrument

   1. Place the Lysing Matrix E tubes in the QuickPrep (24 x 2 ml) or HiPrep (48 x 2 ml) Adapter.
   2. Operate the FastPrep-24 Instrument for 1 min at 6.5 m/s.

4. **Disassemble the adapter set. Centrifuge the samples at 14,000 x *g* for 2 min at room temperature (15–25°C).**

At this point, use 200 µl of the supernatant from step 4 as starting material for the protocol "Purification of Pathogen Nucleic acids from Fluid Samples" in the *QIAamp* cador *Pathogen Mini Kit Handbook*.

5. **Pipet 20 µl proteinase K into a 2 ml microcentrifuge tube (not provided).**

6. **Add 200 µl supernatant (step 4) to the proteinase K.**

   Note: If processing lower sample volumes, adjust the volume to 200 µl with PBS or 0.9% NaCl.

7. **Add 100 µl Buffer VXL+ IC-DNA mix. Close the cap and mix by pulse vortexing.**

   To ensure sufficient lysis, thoroughly mix the sample and Buffer VXL to yield a homogenous solution. If using sample fluid containing Buffer ATL, for example, after enzymatic digestion of tissue, precipitates may form. Precipitates can be dissolved by brief incubation at 56°C. However, they have no influence on subsequent protocol steps.

   Note: If processing cell-free samples, ensure that 1 µg Carrier RNA is added per 100 µl of Buffer VXL before use.

8. **Incubate at 20–25°C for 15 min.**

9. **Briefly centrifuge the 2 ml tube to remove drops from the inside of the lid.**

10. **Add 350 µl Buffer ACB to the sample, close the cap, and mix thoroughly by pulse-vortexing. Ensure that isopropanol was added to the Buffer ACB concentrate before use.**
11. **Briefly centrifuge the 2 ml tube to remove drops from inside the lid.**
12. **Transfer the lysate from step 10 to the QIAamp Mini column placed in a 2 ml collection tube without wetting the rim. Close the cap, and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp Mini column into a clean 2 ml collection tube, and discard the collection tube containing the filtrate.**

    If the lysate has not completely passed through the column after centrifugation, centrifuge again at a higher speed (up to 20,000 x *g*; 14,000 rpm) until the QIAamp Mini column is empty.
13. **Open the QIAamp Mini column, and add 600 µl Buffer AW1 without wetting the rim. Close the cap, and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp Mini column in a clean 2 ml collection tube, and discard the tube containing the filtrate.**
14. **Open the QIAamp Mini column, and add 600 µl Buffer AW2 without wetting the rim. Close the cap, and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp Mini column in a clean 2 ml collection tube, and discard the tube containing the filtrate.**
15. **Centrifuge at full speed (20,000 x *g*; 14,000 rpm) for 2 min to dry the membrane.**
16. **Place the QIAamp Mini column in a clean 1.5 ml microcentrifuge tube (not provided), and discard the collection tube containing the filtrate. Open the QIAamp Mini column and add 150 µl Buffer AVE to the center of the membrane. Close the cap, and incubate at room temperature (15–25°C) for 1 min. Centrifuge at full speed (20,000 x *g*; 14,000 rpm) for 1 min.**

    Important: Ensure that the elution buffer is equilibrated to room temperature. To reduce noise, the centrifugation speed for elution can be set to 6000 x *g*. If this is done, the recovered eluate volume will be approximately 5 µl less than elution buffer volume applied onto the column.

Automated DNA purification on the QIAcube®

Purification of DNA from stool samples using the QIAamp *cador* Pathogen Mini Kit can be automated on the QIAcube.

The innovative QIAcube uses advanced technology to process QIAGEN spin columns, enabling seamless integration of automated, low-throughput sample prep into your laboratory workflow.

Sample preparation using the QIAcube follows the same steps as the manual procedure (i.e., lyse, bind, wash, and elute), enabling you to continue using the QIAamp *cador* Pathogen Mini Kit for purification of high-quality DNA.

Use the QIAcube Protocol "Purification of pathogen nucleic acids from animal samples — standard" to continue processing at step 8 or use the QIAcube Protocol "Purification of pathogen nucleic acids from animal samples – Manual lysis" to continue processing at step 12.

Troubleshooting

For general troubleshooting, please consult the Troubleshooting Guide in the *QIAamp* cador *Pathogen Mini Kit Handbook*.

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.

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