Supplementary Protocol April 2015

# Purification of MAP DNA from feces using the QIAamp® DNA Stool Mini Kit

This protocol is designed for purification of *Mycobacterium avium* spp. *paratuberculosis* (MAP) DNA from up to 220 mg of feces.

In this protocol, feces are homogenized using the FastPrep®-24 Instrument or an equivalent electric homogenizer. Samples are then processed according to the standard QIAamp protocols.

**IMPORTANT:** Please read the *QIAamp DNA Stool 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 DNA Stool Mini Kit (cat. no. 51504)
- Equipment for sample disruption and homogenization
- Pipets and pipet tips (pipet tips with aerosol barriers for preventing cross-contamination are recommended)
- 1.5 ml and 2 ml microcentrifuge tubes (the 2 ml tubes should be wide enough to accommodate an InhibitEX® tablet, for example, Eppendorf® Safe-Lock, cat. no. 0030120.094 or Sarstedt Safe-Seal, cat. no. 72.695)
- 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. † Not available in Germany.

- **Optional**: Feces container with conical bottom and support skirt, screw cap with spoon (e.g., Greiner cat. no. 443102)

## Suppliers of equipment for disruption and homogenization

### MP Biomedicals

- FastPrep®-24 Instrument (cat. no. 116004500)
- Lysing Matrix E (cat. 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 DNA Stool Mini Kit for the first time, read "Important Notes" in the *QIAamp DNA Stool Mini Kit Handbook*
- All centrifugation steps are carried out at room temperature (15–25°C) in a microcentrifuge

## Things to do before starting

- Ensure that Buffer AW1 and Buffer AW2 have been prepared according to the instructions on the labels
- Mix all buffers before use
- Add 1 µl IC-DNA to 1 ml Buffer ASL

  **Note**: Buffer ASL + IC-DNA should be prepared fresh

## Procedure

1. Mix the sample thoroughly by stirring with a clean spoon or spatula (not provided).
2. Weigh 180–220 mg mixed stool in a 2 ml lysis tube (not provided) and place tube on ice.

   For a liquid sample, pipet 200 µl into the lysis tube. Cut the end of the pipet tip to make pipetting easier.

If the sample is frozen, use a scalpel or spatula to scrape bits of stool into a 2 ml lysis tube on ice.

**Note**: When using frozen stool samples, take care that the samples do not thaw until Buffer ASL is added in step 2 to lyse the sample; otherwise the DNA in the sample may degrade. After addition of Buffer ASL, all following steps can be performed at room temperature (15–25°C).

3. Add 1 ml Buffer ASL + IC-DNA mix to each stool sample.
4. Homogenize the sample until the stool sample is thoroughly homogenized.

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

**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.

5. Incubate at 99°C for 5 min at 1400 rpm.
6. Place the sample on ice for 5 min.
7. Centrifuge sample at full speed for 1 min to pellet stool particles.
8. Pipet all the supernatant into a new 2 ml microcentrifuge tube (not provided) and discard the pellet.

At this point, the protocol is the same as the protocol entitled "Isolation of DNA from Stool for Pathogen Detection" (from step 6 onwards), in the *QIAamp DNA Stool Mini Kit Handbook*

9. Add 1 InhibitEX Tablet to each sample and vortex immediately and continuously at room temperature to allow inhibitors to adsorb to the InhibitEX matrix.
10. Centrifuge sample at full speed for 3 min to pellet inhibitors bound to InhibitEX matrix.
11. Pipet all the supernatant into a new 1.5 ml microcentrifuge tube (not provided) and discard the pellet. Centrifuge the sample at full speed for 3 min.

    Transfer of small quantities of pelleted material from step 10 will not affect the procedure.
12. Pipet 15 µl proteinase K into a new 1.5 ml microcentrifuge tube (not provided).
13. Pipet 200 µl supernatant from step 11 into the 1.5 ml microcentrifuge tube containing proteinase K.
14. Add 200 µl Buffer AL and vortex for 15 s.

**Note**: Do not add proteinase K directly to Buffer AL. It is essential that the sample and Buffer AL are thoroughly mixed to form a homogeneous solution.

15. Incubate at 70°C for 10 min.

    Centrifuge briefly to remove drops from the inside of the tube lid (optional).

16. Add 200 µl of ethanol (96–100%) to the lysate, and mix by vortexing.

    Centrifuge briefly to remove drops from the inside of the tube lid (optional).

17. Label the lid of a new QIAamp spin column placed in a 2 ml collection tube. Carefully apply the complete lysate from step 16 to the QIAamp spin column without moistening the rim. Close the cap and centrifuge at full speed for 1 min. Place the QIAamp spin column in a new 2 ml collection tube, and discard the tube containing the filtrate.

    Close each spin column to avoid aerosol formation during centrifugation. If the lysate has not completely passed through the column after centrifugation, centrifuge again until the QIAamp spin column is empty.

18. Carefully open the QIAamp spin column and add 500 µl Buffer AW1. Close the cap and centrifuge at full speed for 1 min. Place the QIAamp spin column in a new 2 ml collection tube, and discard the collection tube containing the filtrate.

19. Carefully open the QIAamp spin column and add 500 µl Buffer AW2. Close the cap and centrifuge at full speed for 3 min. Discard the collection tube containing the filtrate.

    **Note:** Residual Buffer AW2 in the eluate may cause problems in downstream applications. Some centrifuge rotors may vibrate upon deceleration, resulting in the flow-through, which contains Buffer AW2, contacting the QIAamp spin column. Removing the QIAamp spin column and collection tube from the rotor may also cause flow-through to come into contact with the QIAamp spin column.

20. **Recommended**: Place the QIAamp spin column in a new 2 ml collection tube (not provided) and discard the old collection tube with the filtrate. Centrifuge at full speed for 1 min.

    This step helps to eliminate the chance of possible Buffer AW2 carryover.

21. Transfer the QIAamp spin column into a new, labeled 1.5 ml microcentrifuge tube (not provided). Carefully open the QIAamp spin column and pipet 200 µl Buffer AE directly onto the QIAamp membrane. Close the cap and incubate for 1 min at room temperature, then centrifuge at full speed for 1 min to elute DNA.

## Automated DNA purification on the QIAcube®

Purification of DNA from stool samples using the QIAamp DNA Stool 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 DNA Stool Mini Kit for purification of high-quality DNA.

Treatment of the sample with an InhibitEX Tablet cannot be performed on the QIAcube and must be carried out manually. Use the QIAcube Protocol "Isolation of DNA from stool for pathogen detection"

After treatment with an InhibitEX Tablet in step 11, transfer at least 350 µl of the supernatant obtained after the first manual centrifugation step to position 2 of the rotor adapter. The QIAcube will then purify DNA from 200 µl of each supernatant.

## Troubleshooting

For general troubleshooting, please consult the "Troubleshooting Guide" in the *QIAamp DNA Stool 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.