Quick-Start Protocol December 2017

QIAamp® Fast DNA Stool Mini Kit

The QIAamp Fast DNA Stool Mini Kit (cat. no. 51604) can be stored at room temperature (15–25°C) for up to 12 months.

Further information

- *QIAamp Fast DNA Stool Mini Kit Handbook*: **www.qiagen.com/HB-1764**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Prepare a thermomixer with 2 ml inlays or a water bath at 70°C for use in steps 3 and 8.
- Perform all centrifugation steps at room temperature (15–25°C) at 20,000 x *g* (~14,000 rpm).
- Redissolve any precipitates in Buffer AL and InhibitEX® Buffer by heating and mixing.
- Add ethanol to Buffer AW1 and Buffer AW2 concentrates.
- Mix all buffers before use.
- Symbols: ● pathogen detection; ▲ human DNA analysis

1. Weigh 180–220 mg stool in a 2 ml microcentrifuge tube (not provided) and place tube on ice.
2. Add 1 ml InhibitEX Buffer to each stool sample. Vortex continuously for 1 min or until the stool sample is thoroughly homogenized.
3. ▲ Skip this step and continue with step 4. ● Heat the suspension for 5 min at 70°C. The lysis temperature can be increased to 95°C for cells that are difficult to lyse. Vortex for 15 s.
4. Centrifuge sample for 1 min to pellet stool particles.

5. Pipet ● 15 µl or ▲ 25 µl Proteinase K into a new ● 1.5 ml or ▲ 2 ml microcentrifuge tube (not provided).
6. Pipet ● 200 µl or ▲ 600 µl supernatant from step 4 into the ● 1.5 ml or ▲ 2 ml microcentrifuge tube containing Proteinase K.
7. Add ● 200 µl or ▲ 600 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.
8. Incubate at 70°C for 10 min.
9. Add ● 200 µl or ▲ 600 µl of ethanol (96–100%) to the lysate, and mix by vortexing.
10. Carefully apply 600 µl lysate from step 9 to the QIAamp spin column. Close the cap and centrifuge for 1 min. Place the QIAamp spin column in a new 2 ml collection tube, and discard the tube containing the filtrate. ▲ Repeat step 10 until all lysate is loaded.
11. Carefully open the QIAamp spin column and add 500 µl Buffer AW1. Centrifuge for 1 min. Place the QIAamp spin column in a new 2 ml collection tube, and discard the collection tube containing the filtrate.
12. Carefully open the QIAamp spin column and add 500 µl Buffer AW2. Centrifuge for 3 min. Discard the collection tube containing the filtrate.
13. Place the QIAamp spin column in a new 2 ml collection tube (not provided) and discard the old collection tube with the filtrate. Centrifuge for 3 min.
14. Transfer the QIAamp spin column into a new, labeled 1.5 ml microcentrifuge tube (not provided) and pipet 200 µl Buffer ATE directly onto the QIAamp membrane. Incubate for 1 min at room temperature, then centrifuge for 1 min to elute DNA. If yield will be quantified by UV absorbance, blank the measuring device using Buffer ATE to avoid false results.

Changes between Revision 2 and Revision 3

Step 5. Addition of 2 ml microcentrifuge tube for the human DNA analysis protocol.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual.