

QIAamp® 96 PowerFecal QIAcube® HT Kit

This protocol is for use with QIAcube HT Prep Manager software.

Further information

- *QIAamp 96 PowerFecal QIAcube HT Handbook*: www.qiagen.com/HB-2162
- Safety Data Sheets: www.qiagen.com/safety
- Technical assistance: support.qiagen.com

Notes before starting

- This protocol is for the purification of nucleic acids from stool samples using the QIAamp 96 PowerFecal QIAcube HT Kit with the QIAcube HT Prep Manager software.
- Do not overload the QIAamp membrane as this can impair nucleic acid extraction and/or performance in downstream assays. See the kit handbook for more information.
- Avoid repeated freezing and thawing of samples, as this may reduce nucleic acid yield and quality.
- Prepare Buffers AW1 and AW2 according to the instructions in the kit handbook.
- Warm Buffer PW1 at 60° C for 10 min before use. The solution should still be warm when used. Make sure there are no precipitates in the solution.

Preparing the samples

1. Place 200 mg of each stool sample into a Pathogen Lysis Tube L (cat. no. 19092) or a PowerBead DNA Plate, Garnet (cat. no. 12955-4-BP). If using tubes, close each tube and place it on ice.

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2. Add 650 μ l prewarmed Buffer PW1 to each sample. After addition of Buffer PW1, all of the following steps can be carried out at room temperature (15–25°C).

Note: If working with dehydrated stool samples, it might be necessary to increase this volume. Resuspend the stool sample in enough Buffer PW1 (cat. no. 2600-50-1) to obtain 400 μ l of supernatant after centrifugation in step 4.

3. Homogenize each sample thoroughly by one of these methods:
 - Vortex continuously for 10 min or until the stool sample is thoroughly homogenized.
 - Homogenize the samples using the TissueLyser II for 5 min at 30 Hz. If using the PowerBead DNA Plate, Garnet and the TissueLyser II for disruption, please also use two TissueLyser Adapter Bottom Plates (cat. no. 69983). For more information see the kit handbook.

Note: Vortexing is recommended when isolating human DNA. Homogenization using the TissueLyser II is recommended when isolating pathogen DNA to ensure complete disruption of microorganisms.

Note: It is important to homogenize the samples thoroughly. This helps ensure maximum DNA concentration in the final eluate.

4. Centrifuge at full speed for 1 min (tubes) or 5 min (plate) to pellet stool particles.
5. Pipet approximately 400 μ l supernatant from step 4 into a new tube or S-Block (cat. no. 19585) (not provided).

IMPORTANT: Do not transfer any solid material. If particles are still visible in the supernatant, centrifuge the sample again

6. Add 150 μ l of Buffer C3 to each supernatant and mix thoroughly by carefully vortexing or pipetting.
 7. Incubate for 5 min at 4 °C.
 8. Centrifuge at full speed for 1 min (tubes) or 5 min (S-Block).
 9. For each sample, add 20 μ l Proteinase K to a new S-Block well. Add 300 μ l of each supernatant from step 8 to these wells and mix. Incubate for 10 min at room temperature (15–25° C).
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Note: If you only want to isolate human DNA from the stool samples, leave out the Proteinase K and incubation step, and only transfer 300 µl of supernatant to the S-Block.

QIAamp 96 PowerFecal Protocol on the QIAcube HT

1. Start the QIAcube HT Prep Manager software. Click on the **Home** icon in the main toolbar to access the Home screen.
 2. Select QIAamp 96 PowerFecal from the **Create Experiment** list. Follow the instructions in the wizard and fill in all required fields.
 3. In the **Setup** step, select **Sample type** and **Pre-treatment** for documentation.
 4. Select the protocol: QIAamp 96 PowerFecal protocol. For information about optional steps and advanced options see the kit handbook.
 5. Define samples in the **Labware selection** step.
 6. Arrange samples to the output plate in the **Assignment** step. **Note:** The instrument must be switched on and connected to the software before entering the **Worktable** step.
 7. Follow the instructions for loading the worktable.
 8. Add the volume of sample indicated in the **Worktable** step to the selected S-Block wells.
 9. Save the experiment by clicking the **Save** button in the button bar.
 10. Click the **Start** run button to start the run.

Important: The protocol includes a vacuum performance check after the first vacuum step. Therefore, the software will show a dialog that needs to be confirmed after the vacuum has finished.
 11. When the protocol is complete, cover the elution plate (EMTR) with the lid and remove it from the elution chamber.

Note: If using Top Elute fluid, there may be 2 liquid phases in the elution microtubes. Top Elute fluid will be the top layer over the elution buffer.
 12. Create a report (if required).
 13. Follow the cleaning procedure.
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Scan QR code for handbook.

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

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