Quick-Start Protocol August 2016

AllPrep® PowerFecal® DNA/RNA Kit

Store the kit components dry at room temperature (15–25°C) if not otherwise stated on the label.

Further information

- *AllPrep PowerFecal DNA/RNA Handbook:* **www.qiagen.com/HB-2190**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Add ethanol to buffers AW1, AW2 and RPE according to the labels on the bottles.
- Before use, warm buffer PM1 to 55°C for 10 min. and use while still warm.
- Prepare 80% ethanol in water.

Procedure

1. Place 100–200 mg of stool into the Microbial Lysis Tube and add 650 µl Buffer PM1 and 25 µl DTT. Tightly cap the lid.
2. Lyse the bacterial cells using one of the verified options from the handbook.
3. Centrifuge at ≥ 18,000 x g for 1 min. Transfer the supernatant to a clean 1.5 ml collection tube, add 150 µl of Solution IRS, and vortex briefly. Incubate at 4°C for 5 min.
4. Centrifuge at ≥ 13,000 x g for 1 min. Transfer 300 µl of the supernatant to a clean 2 ml collection tube. Shake to mix Buffer C4 before use. Add 400 µl of Buffer C4 to the supernatant and mix well by pipetting.
5. Transfer 700 µl of the mix to an AllPrep DNA MinElute® spin column (white) placed in a 2 ml collection tube. Centrifuge at ≥ 8,000 x g for 30 sec., collecting the flow-through for RNA purification, then place the spin column in a new 2 ml collection tube. Store at 4°C for later DNA purification.

RNA purification

6. Add 1 volume of 80% ethanol to the flow-through from step 5 and mix well by pipetting.
7. Transfer up to 700 µl of the mix to an RNeasy® Mini spin column (pink) placed in a 2 ml collection tube. Centrifuge at ≥ 8,000 x g for 30 sec. Discard the flow-through. Centrifuge successive aliquots in the same spin column, discarding the flow-through.
8. Add 700 µl Buffer RW1 to the spin column. Centrifuge at ≥ 8,000 x g for 30 sec. Discard the flow-through. Repeat with 500 µl Buffer RPE.
9. Add 500 µl Buffer RPE, and centrifuge at ≥ 18,000 x g for 2 min. Discard the flow-through and place the spin column in a clean 2 ml collection tube. Centrifuge at ≥ 18,000 x g for 1 min.
10. Place the spin column in a clean 1.5 ml elution tube. Add 30 µl RNase-free water directly to the membrane. Centrifuge at ≥ 8,000 x g for 1 min. Repeat for increased RNA yield.

DNA purification

11. Add 500 µl AW1 to the spin column from step 5. Centrifuge at ≥ 8,000 x g for 30 sec. Discard the flow-through.
12. Add 500 µl Buffer AW2 to the spin column. Centrifuge at ≥ 18,000 x g for 2 min. Discard the collection tube and the flow-through
13. Place the spin column in a clean 2 ml collection tube. Centrifuge at ≥ 18,000 x g for 1 min.
14. Place the spin column in a clean 1.5 ml elution tube. Add 30 µl Buffer EB directly to the membrane, incubate at room temperature for 1 min, and centrifuge at ≥ 8,000 x g for 1 min. Repeat for increased DNA yield.

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

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