September 2023

Quick-Start Protocol

RNeasy® PowerMax® Soil Pro Kit

Solution CD2, RDD Buffer and Lyophilized DNase I should be stored at 2–8°C upon arrival. All other reagents and kit components should be stored at room temperature (15–25°C).

RNeasy PowerMax Soil Pro Kit is for the isolation of microbial RNA from soil samples.

- *RNeasy PowerMax Soil Pro Kit Handbook*: **www.qiagen.com/HB-3451**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Prepare DNase I stock enzyme by adding 550 µL RNase-Free Water (provided) to the DNase I (RNase-free) lyophilized powder and mixing gently. Aliquot the DNase I stock enzyme in 100 µL portions and store at –30°C to –15°C for long-term storage (but do not freeze—thaw more than 3 times). To prepare DNase I Solution, thaw and combine 100 µL DNase I stock enzyme with 900 µL RDD Buffer per prep. DNase I is sensitive to physical denaturation; do not vortex resuspended DNase I.
- Perform all centrifugation steps at room temperature.
- Prepare fresh 80% ethanol use RNase-Free Water (not provided).

Procedure

1. Add 5–15 g of soil, 16 mL of Solution CD1, 2 mL of Solution CD2 and 2 mL phenol–chloroform–isoamyl alcohol (25:24:1, pH 6.5–8.0) to the PowerMax Bead Pro Tube and vortex briefly to mix.
2. Place the PowerMax Bead Pro Tube into the 50 mL TissueLyser Adapter (cat. no. 11960). Fasten the adapter into the TissueLyser III (cat. no. 9003240) instrument and shake for 10 min at speed 25 Hz.
3. Centrifuge the PowerMax Bead Pro Tube at 2500 x *g* for 10 min.
4. Transfer the supernatant to a clean 50 mL Collection Tube (provided).

   Note: Expect a volume of 15 mL. The supernatant may still contain some soil particles.
5. Add 5 mL of Solution CD2 and vortex for 5 s. Centrifuge at 5500 x *g* for 10 min.
6. Avoiding the pellet, transfer the supernatant to a clean 50 mL Collection Tube (provided).

   Note: The supernatant can be decanted when the pellet is solid. Expect a volume of 20 mL.
7. Add equal volume of Solution EA to the supernatant. Vortex briefly to mix.
8. Load 15 mL binding mix (supernatant + Solution EA) into an MB Maxi Spin Column and centrifuge at 2500 x *g* for 2 min. Discard the flow-through.
9. Repeat step 8 until the whole binding mix is processed.
10. Add 15 mL Solution EA to the spin column and centrifuge at 2500 x *g* for 2 min. Discard the flow-through.
11. Add 1 mL DNase I Solution to the center of the spin column, (prepared by mixing 900 µL RDD Buffer and 100 µL DNase I stock enzyme; see “Notes before starting”).
12. Incubate at room temperature for 15 min. Add 10 mL Solution EA and centrifuge at 2500 x *g* for 2 min.
13. Discard flow-through. Add 10 mL Solution C5. Centrifuge at 2500 x *g* for 2 min.
14. Discard flow-through. Add 15 mL 80% EtOH. Centrifuge at 2500 x *g* for 3 min.

15. Discard flow-through and place the MB Maxi Spin Column into a clean 50 mL Collection Tube (provided). Centrifuge at 5500 x *g* for 5 min to dry.
16. Place the MB Maxi Spin Column into a clean 50 mL Collection Tube (provided). Add 1 mL RNase-Free Water to the center of the white filter membrane. Incubate at room temperature for at least 1 min.
17. Centrifuge at 5500 x *g* for 5 min. Discard the MB Maxi Spin Column. Transfer the Eluate into a clean 1.5 mL LoBind Tube. The RNA is now ready for any downstream application.

Document Revision History

Date	Changes
09/2023	Initial release

Scan QR code for handbook.

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