Quick-Start Protocol July 2022

RNeasy® PowerWater® Kit

Lyophilized DNase I should be stored at 2–8°C upon arrival. All other reagents and components of the RNeasy PowerWater Kit should be stored at room temperature (15–30°C).

Further information

- *RNeasy PowerWater Kit*: **www.qiagen.com/HB-2268**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Solution PM1 must be warmed at 55°C for 5–10 min prior to use.
- Shake to mix Solution PM5 before use.
- Prepare Solution PM1 by adding 10 µl β-mercaptoethanol (β-ME) for every 990 µl of Solution PM1 (a total of 1 ml for each prep).
- Prepare DNase I stock enzyme by adding 550 µl of RNase-free water to the DNase I (RNase-free) lyophilized powder and mixing gently. Aliquot the DNase I stock enzyme in 50 µl portions and store at –20°C for long term storage (but do not freeze/thaw more than three times). To prepare DNase I Solution, thaw and combine 5 µl of DNase I stock enzyme with 45 µl of DNase Digestion Solution per prep.

1. Filter water samples using a reusable or disposable filter funnel (0.22 or 0.45 µm filter membranes) attached to a vacuum source. The volume of water filtered will depend on the microbial load and turbidity of the water sample.
2. If using a reusable filter funnel, remove the upper portion of the apparatus.
3. Using two sets of sterile forceps, pick up the white filter membrane at opposite edges and roll the filter into a cylinder with the top side facing inward.

   Note: Do not tightly roll or fold the filter membrane.
4. Insert the filter into a 5 ml PowerWater Bead Pro Tube.
5. Add 1 ml of Solution PM1/βME to the PowerWater Bead Pro Tube. Alternatively, you can add 990 µl of PM1 and 10 µl of βME directly to the tube.
6. Make sure the PowerWater Bead Pro Tube cap is securely tightened.

   Note: For samples containing difficult-to-lyse organisms (e.g., fungi, and algae) an additional heating step can be included. Refer to Troubleshooting Guide.
7. Secure the PowerWater Bead Pro Tube horizontally to a Vortex Adapter (cat. no. 13000-V1-5). Tube caps should point toward the center of the Vortex Adapter.
8. Vortex at maximum speed for 5 min. Centrifuge the tubes ≤ 4000 x *g* for 1 min.

   Note: This step is optional if a centrifuge with a 15 ml tube rotor is not available.
9. Transfer all the supernatant to a clean 2 ml collection tube (provided). Draw up the supernatant using a 1 ml pipette tip by placing it down into the beads.

Note: Placing the pipette tip down into the beads is required. Pipette more than once to ensure removal of all supernatant. Expect to recover between 600 and 650 µl of supernatant.

10. Centrifuge at 13,000 x *g* for 1 min. Avoiding the pellet, transfer the supernatant to a clean 2 ml collection tube (provided).
11. Add 200 µl of Solution IRS and vortex briefly to mix. Incubate at 4°C for 5 min.
    Note: This step can be omitted for non-turbid water samples that are known to be free of PCR inhibitors. Continue the protocol at step 13.
12. Repeat step 10. Then proceed to step 13.
13. Add 650 µl each of Solution PM3 and Solution PM4. Vortex briefly to mix.
14. Load 650 µl of supernatant onto an MB RNA Spin Column. Centrifuge at 13,000 x *g* for 1 min. Discard the flow-through and repeat until all the supernatant has been loaded.
15. Add 650 µl of Solution PM5. Centrifuge at 13,000 x *g* for 1 min. Discard the flow-through.
    Note: Skip steps 16–18 if you want to isolate both RNA and DNA.
16. Centrifuge again at 13,000 x *g* for 1 min and place the MB RNA Spin Column into a clean 2 ml collection tube (provided).
17. Add 50 µl of DNase I Solution to the center of the column membrane and incubate at room temperature for 15 min.
18. Add 400 µl Solution PM7 and centrifuge the column at 13,000 x *g* for 1 min.
19. Discard the flow-through. Add 650 µl of Solution PM5. Centrifuge at 13,000 x *g* for 1 min.
20. Discard the flow-through. Add 650 µl of Solution PM4. Centrifuge at 13,000 x *g* for 1 min.
21. Discard the flow-through and centrifuge again at 13,000 x *g* for 2 min.
22. Place the MB Spin Column into a clean 2 ml collection tube (provided).
23. Add 100 µl of RNase-free water (provided) to the center of the white filter membrane.
24. Centrifuge at 13,000 x *g* for 1 min. Discard the MB Spin Column. The RNA is now ready for downstream applications and can be stored at –90 to –65°C.

Date	Changes
07/2022	Replaced “PowerWater DNA Bead Tubes” with “PowerWater Bead Pro Tubes”. Deleted reference to a video.

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