

Quick-Start Protocol

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 minutes prior to use.
 - Shake to mix Solution PM5 before use.
 - Prepare Solution PM1 by adding 10 µl β-mercaptoethanol (βME) 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 3 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 µm 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. To see a video of this technique, please visit the RNeasy PowerWater Kit product page at www.mobio.com.

4. Insert the filter into a 5 ml PowerWater Bead Tube.

5. Add 1 ml of Solution PM1/ β ME to the PowerWater Bead Tube. Alternatively, you can add 990 μ l of PM1 and 10 μ l of β ME directly to the tube.

6. Make sure the PowerWater Bead Tube cap is securely tightened.

Note: For samples containing difficult-to-lyse organisms (fungi, algae) an additional heating step can be included. Refer to Troubleshooting Guide.

7. Secure the PowerWater Bead Tube horizontally to a Vortex Adapter (cat. nos. 13000-V1-15 or 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 $\leq 4000 \times g$ 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 – 650 μ l of supernatant.

10. Centrifuge at 13,000 $\times g$ for 1 min. Avoiding the pellet, transfer the supernatant to a clean 2 ml Collection Tube (provided).

11. Add 200 μ l of Solution IRS and vortex briefly to mix. Incubate at 4°C for 5 minutes.

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 μ l each of Solution PM3 and Solution PM4. Vortex briefly to mix.

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14. Load 650 μ l of supernatant onto an MB RNA Spin Column. Centrifuge at 13,000 $\times g$ for 1 min. Discard flow through and repeat until all the supernatant has been loaded.
 15. Add 650 μ l of Solution PM5. Centrifuge at 13,000 $\times g$ for 1 min. Discard flow through.
Note: Skip steps 16 - 18 if you want to isolate both RNA and DNA.
 16. Centrifuge again at 13,000 $\times g$ for 1 min and place the MB RNA Spin Column into a clean 2 ml collection tube (provided).
 17. Add 50 μ l of DNase I Solution to the center of the column membrane and incubate at room temperature for 15 min.
 18. Add 400 μ l Solution PM7 and centrifuge the column at 13,000 $\times g$ for 1 min.
 19. Discard flow through. Add 650 μ l of Solution PM5. Centrifuge at 13,000 $\times g$ for 1 min.
 20. Discard flow through. Add 650 μ l of Solution PM4. Centrifuge at 13,000 $\times g$ for 1 min.
 21. Discard flow through and centrifuge again at 13,000 $\times g$ for 2 min.
 22. Place the MB Spin Column into a clean 2 ml collection tube (provided).
 23. Add 100 μ l of RNase-free water (provided) to the center of the white filter membrane.
 24. Centrifuge at 13,000 $\times g$ for 1 min. Discard the MB Spin Column. The RNA is now ready for downstream applications and can be stored at -90°C to -65° .
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Revision History

Revision no.	Description of change
R2 12/2018	Revised the "Notes before starting" section to update the preparation of DNase I stock enzyme using 550 µl instead of 300 µl. Added link to RNeasy® PowerWater® Kit Handbook. Layout updates.



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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