Quick-Start Protocol June 2016

DNeasy® PowerWater® Kit

The DNeasy PowerWater Kit can be stored at room temperature (15–25°C) until the expiry date printed on the box label.

Further information

- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Solution PW1 must be warmed at 55°C for 5–10 minutes to dissolve precipitates prior to use. Solution PW1 should be used while still warm.
- If Solution PW3 has precipitated, heat at 55°C for 5–10 minutes to dissolve precipitate.
- Shake to mix Solution PW4 before use.

1. Filter water samples using a filter funnel attached to a vacuum source. The volume of water filtered will depend on the microbial load and turbidity of the water sample.
   Note: Please see Types of Water Samples in the Hints and Troubleshooting Guide.
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, please visit the DNeasy® PowerWater® Kit product page at www.mobio.com.
4. Insert the filter into a 5 ml PowerWater DNA Bead Tube.
5. Add 1 ml of Solution PW1 to the PowerWater DNA Bead Tube.
   Note: For samples containing organisms that are difficult to lyse (e.g. fungi, algae) an additional heating step can be included. See Alternate Lysis Method in the Hints and Troubleshooting Guide.
6. Secure the tube horizontally to a vortex adapter (Cat. # 13000–V1–15/13000–V1–5).
7. Vortex at maximum speed for 5 min. Centrifuge the tubes ≤ 4000 x *g* for 1 min at room temperature. (This centrifugation step is optional if a centrifuge with a 15 ml tube rotor is not available, but will result in minor loss of supernatant).
8. Transfer 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 until you have removed all the supernatant. Expect to recover 600–650 µl of supernatant.
9. Centrifuge at 13,000 x *g* for 1 min at room temperature.
10. 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 2–8°C for 5 min.
12. Centrifuge the tubes at 13,000 x *g* for 1 min.
13. Avoiding the pellet, transfer the supernatant to a clean 2 ml collection tube (provided).
14. Add 650 µl of Solution PW3 and vortex briefly to mix.
15. Load 650 µl of supernatant onto a MB Spin Column. Centrifuge at 13,000 x *g* for 1 min. Discard the flow-through. Repeat until all the supernatant has been processed.
16. Place the MB Spin Column Filter into a clean 2 ml collection tube (provided).
17. Add 650 µl of Solution PW4 (shake before use). Centrifuge at 13,000 x *g* for 1 min.
18. Discard the flow-through and add 650 µl of ethanol (provided) and centrifuge at 13,000 x *g* for 1 min.
19. Discard the flow through and centrifuge again at 13,000 x *g* for 2 min.
20. Place the MB Spin Column into a clean 2 ml collection tube (provided).
21. Add 100 µl of Solution EB to the center of the white filter membrane.
22. Centrifuge at 13,000 x *g* for 1 min.
23. Discard the MB Spin Column. The DNA is now ready for downstream applications.

Protocol 4: DNA Isolation from Water Samples

Pre-College Comp Bio Protocol 4 (start on Step 5)

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Notes before starting

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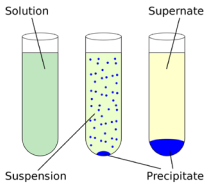
- Shake to mix Solution PW4 before use.

1. Filter water samples using a filter funnel attached to a vacuum source. The volume of water filtered will depend on the microbial load and turbidity of the water sample.
   Note: Please see Types of Water Samples in the Hints and Troubleshooting Guide.
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, please visit the DNeasy® PowerWater® Kit product page at www.mobio.com.
4. Insert the filter into a 5 ml PowerWater DNA Bead Tube.
5. Add 1 ml of Solution PW1 to the PowerWater DNA Bead Tube.
   Note: For samples containing organisms that are difficult to lyse (e.g. fungi, algae) an additional heating step can be included. See Alternate Lysis Method in the Hints and Troubleshooting Guide.



Supernatant: liquid above pellet/precipitate after centrifugation.

6. Secure the tube horizontally to a vortex adapter (Cat. # 13000–V1–15/13000–V1–5).
7. Vortex at maximum speed for 5 min. Centrifuge the tubes ≤ 4000 x *g* for 1 min at room temperature. (This centrifugation step is optional if a centrifuge with a 15 ml tube rotor is not available, but will result in minor loss of supernatant).
8. Transfer 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.
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13. Avoiding the pellet, transfer the supernatant to a clean 2 ml collection tube (provided).
14. Add 650 µl of Solution PW3 and vortex briefly to mix.
15. Load 650 µl of supernatant onto a MB Spin Column. Centrifuge at 13,000 x *g* for 1 min. Discard the flow-through. Repeat until all the supernatant has been processed.
16. Place the MB Spin Column Filter into a clean 2 ml collection tube (provided).
17. Add 650 µl of Solution PW4 (shake before use). Centrifuge at 13,000 x *g* for 1 min.
18. Discard the flow-through and add 650 µl of ethanol (provided) and centrifuge at 13,000 x *g* for 1 min.
19. Discard the flow through and centrifuge again at 13,000 x *g* for 2 min.
20. Place the MB Spin Column into a clean 2 ml collection tube (provided).
21. Add 100 µl of Solution EB to the center of the white filter membrane.
22. Centrifuge at 13,000 x *g* for 1 min.
23. Discard the MB Spin Column. The DNA is now ready for downstream applications.

Most important step: Keep flow-through after #23. That's your DNA!

No need for this. Just vortex for 5-10 minutes using our a 3D printed vortex adaptor.

It is OK to accidentally pick up beads here.

It is **NOT OK** to accidentally pick up beads here.

Use refrigerator or ice bath.

2 mL Eppendorf tube

Flow-through: liquid that went through your spin column after centrifugation.

Centrifuge

Store your labelled tube of DNA in a ice bath or refrigerator. It will be moved to a freezer when the whole class is done.

Waste: Leave liquid waste on your bench. Put everything else in the biohazard bin.