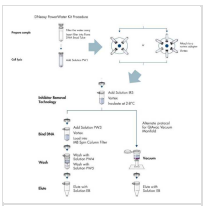

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QIAGEN DNeasy Power Water SOP

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QIAGEN DNeasy PowerWater SOP

Purpose: For the isolation of genomic DNA from filter water samples, inculding turbid water

Introduction: The DNeasy PowerWater Kit can isolate genomic DNA from a variety of filtered water samples. Utilizing Inhibitor Removal Technology® (IRT), even water containing heavy amounts of contaminants can be processed to provide DNA of high quality and yield. The DNeasy PowerWater Kit can isolate DNA equally well from any commonly used type of filter membrane. Purified DNA is ready to use in a final elution volume of 100 µl.

Principle & Procedure: The DNeasy PowerWater Kit starts with the filtration of a water sample onto a filter membrane. Filter membranes may be user supplied. The membrane is then added to our special 5 ml bead beating tube containing a unique bead mix. Rapid and thorough lysis occurs through vortexing in a specially formulated lysis buffer that enhances the isolation of microorganisms from filter membranes. After the protein and inhibitor removal steps, total genomic DNA is captured on an MB Spin Column. High-quality DNA is then washed and eluted from the MB Spin Column membrane for use in downstream applications including PCR and qPCR.

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- 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.
- Perform all centrifugation steps at room temperature (15–25°C).

Procedure 23m

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. See below for types of water samples.

1.1 **Clear water samples:** Larger volumes of clear water can be processed because there is less chance of filter clogging.
Potable drinking water will generally allow for very high volumes depending on the quality and particulate count. In most cases, **100 mL** to **10 L** can be processed, although some users report processing even higher volumes.

1.2 **Turbid Water Samples:** Turbid samples with high levels of suspended solids or sediments will tend to clog filters with
smaller pore sizes (0.22 µm). Use of 0.45 µm filters is recommended for these types of samples. Prior to filtering, samples can be stored in a container to allow suspended solids to settle out. For samples where settling does not occur or is not desired, a method involving stacking filters with larger pore sizes on top of the filter membrane of the desired pore size is recommended. A common set-up is to stack a sterile 1 µm filter. This layering will filter out large debris and allow the smaller micron filter to trap microorganisms. The layered filter system can be washed with sterile water or sterile phosphate buffer to knock down some of the trapped microorganisms on the larger pore size filters. Although this is not completely efficient, it will increase the overall yield of microbial DNA.

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.

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.

5.1 For samples containing organisms that are difficult to lyse (e.g. fungi, algae) 10m an additional heating step can be included. Heating can aid the lysis of some organisms (fungi, algae). After adding Solution PW1 (Step 5 of the protocol), heat the sample at **65 °C** for **00:10:00** . Resume protocol from step 6.

Secure the tube horizontally to a Vortex Adapter (cat. no. 13000-V1-5/13000-V1-15).

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7 Vortex at maximum speed for **00:05:00** . Centrifuge the tubes at **4000 x g, Room temperature, 00:01:00** (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). 6m

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 **13000 x g, Room temperature, 00:01:00** 1m

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 **00:05:00** . 5m

12 Centrifuge the tubes at **13000 x g, Room temperature, 00:01:00** . 1m

13 Avoiding the pellet, transfer the supernatant to a clean 2 ml **2 mL** Collection Tube (provided).

14 Add **650 µL** of Solution PW3 and vortex briefly to mix.

15 Load 650 µl of supernatant onto an MB Spin Column. Centrifuge at 1m

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13000 x g, Room temperature, 00:01:00 . 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 **13000 x g, Room temperature, 00:01:00** . 1m

18 Discard the flow-through and add **650 µL** of ethanol (provided) and centrifuge at **13000 x g, Room temperature, 00:01:00** . 1m

19 Discard the flow-through and centrifuge again at **13000 x g, Room temperature, 00:02:00** . 2m

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 **13000 x g, Room temperature, 00:01:00** . 1m

23 Discard the MB Spin Column. The DNA is now ready for downstream applications.

23.1 QIAgen recommend storing DNA frozen (**-90 °C** to **-15 °C**) as Solution EB does not contain EDTA

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