

DNeasy[®] PowerWater[®] Sterivex[™] Kit

The DNeasy PowerWater Sterivex Kit should be stored at room temperature (15–25°C). After Solution ST1A is added to make Solution ST1B, store at 2–8°C.

Further information

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

Notes before starting

- We recommend you use Sterivex filter units (Millipore cat. no. SVGPL10RC). If you have non-Luer style Sterivex filters, please refer to the Troubleshooting Guide or contact technical services for recommendations.
 - Solution ST1A must be added to the bottle labeled Solution ST1B and mixed well.
 - Solutions MBL and MR **must be** warmed at 65°C for 5–10 minutes to dissolve precipitates prior to use. Solutions MBL and MR must be used while still warm.
 - Shake to mix Solution PW before use.
1. Filter water sample through a Sterivex filter unit. Remove as much of the remaining liquid as possible using a syringe containing air. Cap both ends with the Inlet and Outlet Caps.
 2. Remove the Inlet Cap and add 0.9 ml of Solution ST1B using a pipet tip. Insert pipet completely into inlet so that pipet tip is visible inside the unit just above the membrane.
 3. Re-cap the inlet and secure the Sterivex filter unit horizontally, with the inlet facing out, to a Vortex Adapter (cat. no. 13000-V1-15 or 13000-V1-5).
 4. Vortex at **minimum** speed for 5 min.
 5. While still attached to the vortex adapter, rotate the Sterivex filter unit 180 degrees from the original position. Vortex at **minimum** speed for an additional 5 min.
 6. Set the Sterivex filter unit with the inlet facing up and remove the Inlet Cap. Add 0.9 ml of Solution MBL using a pipet tip. Insert pipet completely into the inlet so that the pipet tip is visible inside the unit just above the membrane. Re-cap the inlet.
 7. Incubate the Sterivex filter unit at 90°C for 5 min. Ensure heat is evenly distributed.
Note: Do **not** heat at higher temperatures or for longer than 5 min.
 8. Cool the unit at room temperature for 2 min. Ensure that the caps are on tightly.
 9. Secure the Sterivex filter unit horizontally, with the inlet facing out, to a Vortex Adapter.
 10. Vortex at maximum speed for 5 min. Set the Sterivex filter unit with the inlet facing up and remove the Inlet Cap.

11. Pull back the plunger of a 3 ml Syringe to fill the barrel with 1 ml of air and attach it to the inlet of Sterivex filter unit. Push air into the unit until there is resistance and release the plunger. Continue to pull back on the plunger to remove as much of the lysate as possible. Detach the syringe from the Sterivex filter unit.
12. Add the lysate to 5 ml glass PowerBead Tubes. Secure the PowerBead Tubes horizontally to a Vortex Adapter (cat. no. 13000-V1-15 or 13000-V1-5).
13. Vortex at **maximum** speed for 5 min. Centrifuge at 4,000 x g for 1 min.
14. Transfer all the supernatant to a clean 2.2 ml collection tube.
15. Add 300 µl of Solution IRS and vortex briefly to mix. Incubate at 4°C for 5 min.
16. Centrifuge the tube at 13,000 x g for 1 min. Avoiding the pellet, transfer the supernatant to a clean 5 ml collection tube.
17. Place a Tube Extender firmly into an MB Spin Column.
18. Attach the Tube Extender/MB Spin Column unit to a VacConnector and VacValve on the QIAVac 24 Plus Manifold (cat. no. 19413).
19. Add 3 ml of Solution MR to the collection tube containing supernatant. Vortex to mix.
20. Load the entire 4.5 ml of supernatant into the Tube Extender/MB Spin Column.
21. Turn on the vacuum source and open the stopcock of the port. Allow the lysate to pass through. After the lysate has passed through completely, close the stopcock of that port.
22. While keeping the MB Spin Column attached to the Luer-Lok stopcock, remove the Tube Extender from the MB Spin Column and discard.
23. Add 0.8 ml of ethanol to the MB Spin Column. Open the stopcock. Allow the ethanol to pass through the column completely. Close the stopcock.
24. Add 0.8 ml of Solution PW to the MB Spin Column. Open the stopcock and allow Solution PW to pass through the column completely. Continue to pull a vacuum for another minute to dry the membrane. Close the port.
25. Add 0.8 ml of ethanol to the MB Spin Column. Open the stopcock and apply a vacuum until the ethanol has passed through the column completely. Continue to pull a vacuum for another minute to dry the membrane. Close the port.
26. Turn off the vacuum source and open an unused port to vent the manifold. If all 20 ports are in use, break the vacuum at the source.
27. Remove the MB Spin Column and place in a 2.2 ml collection tube. Centrifuge the tube at 13,000 x g for 2 min to completely dry the membrane.
28. Transfer the MB Spin Column to a new 2.2 ml collection tube. Add 100 µl of Solution EB or sterile DNA-free PCR-grade water to the center of the white filter membrane.
29. Centrifuge at 13,000 x g for 1 min at room temperature. Discard the MB Spin Column. The DNA is now ready for any downstream application.

For up-to-date licensing information and productspecific disclaimers, see the respective QIAGEN kit handbook or user manual. Trademarks: QIAGEN®, Sample to Insight®, DNeasy®, PowerWater® (QIAGEN Group), Sterivex™ (Millipore). 1104502 03/2017 HB-2224002 © 2017 QIAGEN, all rights reserved.