PowerWater® Sterivex™ DNA Isolation Kit

(For isolation of genomic DNA from Sterivex™ filter units, Millipore catalog# SVGPL10RC)

Catalog No.	Quantity	Filters
14600-50-NF	50 Preps	No filters

Instruction Manual

Inhibitor Removal Technology® (IRT) is a registered trademark of MO BIO Laboratories, Inc. and is covered by the following patents USA US 7,459,548 B2, Australia 2005323451, Japan 5112064 and India 246946.

Please recycle

Version: 01162013

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Introduction

The PowerWater® Sterivex™ DNA Isolation Kit is designed to isolate genomic DNA from Sterivex™ filter units (Millipore Catalog #SVGPL10RC) without the need for enzymes or hazardous organic chemicals. Utilizing a novel filter membrane treatment, microbes are released from the Sterivex™ filter units without extensive incubation times or having to cut open the plastic casing holding the membrane. *Patented Inhibitor Removal Technology® (IRT) is included to provide high quality DNA from all types of water samples even those containing heavy amounts of contaminants. In addition, a special designed silica binding column and column extender allows the one-step addition of all sample lysate (4.5 ml) with elution in a 50-100 µl volume.

Protocol Overview

The PowerWater® Sterivex™ DNA Isolation Kit starts with the addition and incubation of the Sterivex™ units with a novel filter membrane treatment. The lysis buffer is added to the units which are then mixed and the lysate removed for additional mechanical lysis in a 5 ml bead beating tube. After the protein and inhibitor removal steps, total genomic DNA is captured on a silica binding column under vacuum*. The DNA is washed then high quality DNA is eluted from the binding column membrane for use in downstream applications including PCR and qPCR.

***** A vacuum manifold is highly recommended for this protocol.

This kit is for research purposes only. Not for diagnostic use.

Other Related Products	Catalog No.	Quantity
Vortex Adapter for Vortex Genie® 2	13000-V1-15 13000-V1-5	Holds 4 (5 ml or 15 ml) Tubes Holds 6 (5 ml) Tubes
Vortex Genie® 2 Vortex	13111-V 13111-V-220	1 unit (120V) 1 unit (220V)
PowerVac™ Manifold	11991	1 manifold
PCR Water (Certified DNA-free)	17000-1 17000-5 17000-10 17000-11	1 ml 5 x 1 ml 10 x 1 ml 10 ml bottle

PowerWater® Sterivex™ DNA Isolation Kit

Prepare Sample

- Filter the water sample
- Cap filter ends
- Add Solution ST1B

Cell Lysis

- Attach to vortex adapter
- Vortex
- Add Solution ST2
- Vortex

- Heat at 90°C
- Vortex
- Attach 3 ml syringe
- Transfer lysate to 5 ml bead tube
- Bead beat

Centrifuge

Inhibitor Removal Technology®

- Add Solution ST3
- Incubate at 4°C

Centrifuge

Vacuum

- Add Solution ST4
- Load supernatant into syringe/binding column
- Wash with Solution ST5
- Wash with Solution ST6
- Wash with Solution ST5

Centrifuge

Elute

- Elute with Solution ST7

Equipment Required

Centrifuge for 15 ml tubes (≤4,000 x *g*)
Microcentrifuge (13,000 x *g*)
Pipettors
Water bath or heat block at 90°C
Vortex-Genie® 2 Vortex (MO BIO Catalog# 13111-V or 13111-V-220)
Vortex Adapter for 5 ml tubes (MO BIO Catalog# 13000-V1-5 or 13000-V1-15)
PowerVac™ Manifold (MO BIO Catalog# 11991)

Kit Contents

	Kit Catalog #14600-50	
Components	**Catalog #**	**Amount**
Solution ST1A	14600-50-NF-1A	44 ml
Solution ST1B	14600-50-NF-1B	5.86 g
Solution ST2	14600-50-NF-2	50 ml
Solution ST3	14600-50-NF-3	22 ml
Solution ST4	14600-50-NF-4	165 ml
Solution ST5	14600-50-NF-5	3 x 30 ml
Solution ST6	14600-50-NF-6	2 x 22 ml
Solution ST7	14600-50-NF-7	6 ml
Inlet Caps	14600-50-NF-IC	50
Outlet Caps	14600-50-NF-OC	50
PowerWater® Sterivex™ Bead Tubes (0.1 mm glass)	14600-50-NF-BT	50
Binding Columns	14600-50-NF-BC	50
3 ml Syringes	14600-50-NF-SY1	50
20 ml Syringes	14600-50-NF-SY2	50
5 ml Collection Tubes	14600-50-NF-T1	50
2.2 ml Collection Tubes	14600-50-NF-T2	150

Kit Storage

All reagents and kit components should be stored at room temperature (15-30°C). After Solution ST1A is added to make Solution ST1B, store at 4°C.

Precautions

Please wear gloves when using this product. Avoid all skin contact with kit reagents. In case of contact, wash thoroughly with water. Do not ingest. See Material Safety Data Sheets for emergency procedures in case of accidental ingestion or contact. All MSDS information is available upon request (760-929-9911) or at www.mobio.com. Reagents labeled flammable should be kept away from open flames and sparks.

WARNING: Solutions ST5 and ST6 contain alcohol. They are flammable.

Do not use bleach to clean the PowerVac™ Manifold. Bleach should never be mixed with solutions containing guanidine. For more information on cleaning the PowerVac™ Manifold, please refer to the PowerVac™ Manifold manual.

Important Notes Before Starting

It is recommended to use Sterivex™ filter units, Millipore catalog# SVGPL10RC. This unit has a male Luer-Lok™ tip (outlet) that can be easily capped, minimizing the risk of sample loss or leakage. If you have non-luer style Sterivex™ filters, contact technical services for recommendations (technical@mobio.com).

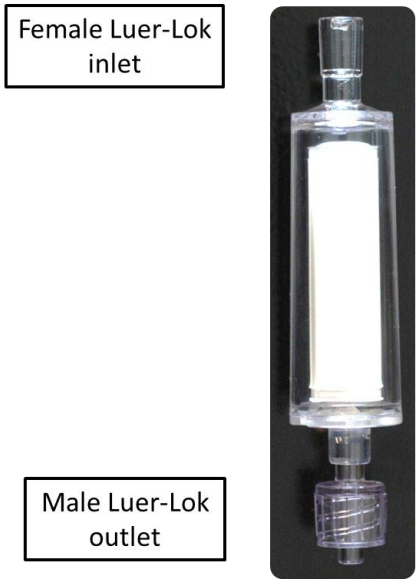
Solution ST1A must be added to the bottle labeled Solution ST1B and mixed well. Solution ST1B should be stored at 4°C.

Solutions ST2 and ST4 must be warmed at 65°C for 5-10 minutes to dissolve precipitates prior to use. Solutions ST2 and ST4 must be used while still warm.

Shake to mix Solution ST6 before use.

For optimum results, we recommend trying a few preps using the protocol as written and a few preps using the Alternative Lysis Method which omits Step 8 and Steps 15-18.

Cat # SVGPL10RC Filter Unit



Experienced User Protocol

Please wear gloves at all times

Add Solution ST1A to bottle ST1B (only required the first time you use ST1B). Mix well and store at 4°C when not in use.
Warm Solutions ST2 and ST4 prior to use at 65°C for 5-10 minutes. Use Solutions ST2 and ST4 while still warm.

1. Filter water sample through a Sterivex™ filter unit, Millipore catalog# SVGPL10RC. After filtering, remove as much of the remaining liquid as possible using a syringe (Not Included) containing air and then cap both ends with the Inlet and Outlet Caps. Typical volumes filtered are 1 – 10 L. The volume of water filtered will depend on the microbial load and turbidity of the water sample.
   Note: It is recommended to use Sterivex™ filter units, Millipore catalog# SVGPL10RC. This unit has a male Luer-Lok™ tip (outlet) that can be easily capped. For use of different Sterivex filter units, see the **Use of Alternate Sterivex™ Filter Units section in the Hints & Troubleshooting Guide.**
   For long term storage, Sterivex™ filter units should be stored capped without excess liquid at -20°C.
   We do not recommend adding SET (Sucrose:EDTA:Tris) buffer to Sterivex™ filter units for storage. SET buffer is not required for this protocol and may interfere with DNA extraction and inhibitor removal. **See the Storage with SET Buffer section under the Hints & Troubleshooting Guide.**
2. Remove the Inlet Cap and add **0.9 ml of Solution ST1B** using a pipette tip. Insert pipette completely into the inlet so that the pipette tip is visible inside the unit just above the membrane.
   Note: Solution ST1A must be added to bottle ST1B prior to first use only.
3. Re-cap the inlet and secure the Sterivex™ filter unit horizontally, with the inlet facing out, to a MO BIO Vortex Adapter, catalog# 13000-V1-15 or 13000-V1-5.
4. Vortex at **minimum** speed for 5 minutes.
5. While still attached to the vortex adapter, rotate the Sterivex™ filter unit 180 degrees from the original position by marking the unit underneath with a marker and turning the unit until the mark faces up. Vortex at **minimum** speed for an additional 5 minutes.
6. Set the Sterivex™ filter unit with the inlet facing up and remove the Inlet Cap.
7. Add **0.9 ml of ST2** using a pipette tip. Re-cap the inlet.
   Note: Solution ST2 must be warmed to dissolve precipitates prior to use. Solution ST2 should be used while still warm. When adding ST2, insert pipette completely into the inlet so that the pipette tip is visible inside the unit just above the membrane.
8. Incubate the Sterivex™ filter unit at 90ºC for 5 minutes. For samples containing easy to lyse organisms or where less DNA shearing is desired, this step can be omitted. **See the Alternative Lysis Methods section under the Hints & Troubleshooting Guide.**
   Note: Place the unit so that the heat is evenly distributed around it. Do not heat at higher temperatures or for longer than 5 minutes.
9. Cool the unit at room temperature for 2 minutes. Check to make sure the caps are on tight.
   Note: The caps at both ends may loosen after heating. It is important to cool the unit before retightening the caps to minimize warping of the inlet.
10. Secure the Sterivex™ filter unit horizontally, with the inlet facing out, to a MO BIO Vortex Adapter, catalog# 13000-V1-15 or 13000-V1-5
11. Vortex at **maximum** speed for 5 minutes.
12. Set the Sterivex™ filter unit with the inlet facing up and remove the Inlet Cap.

13. 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. Hold the 3 ml Syringe and Sterivex™ filter unit vertically with the syringe at the bottom to allow as much of the lysate as possible to be near the inlet.
14. Push the air into the unit until there is resistance and release the plunger. Back pressure withdraws the lysate and fills the syringe. Continue to pull back on the plunger to remove as much of the lysate as possible. Detach the syringe from the Sterivex™ filter unit.
    Note: Do not force the air from the syringe into the Sterivex™ filter unit.
15. Add the lysate to the 5 ml PowerWater® Sterivex™ glass Bead Tube.
    Note: For samples containing easy to lyse organisms or where less DNA shearing is desired, steps 15 -18 can be omitted. **See the Alternative Lysis Methods section under the Hints & Troubleshooting Guide.**
16. Secure the PowerWater® Sterivex™ glass Bead Tube horizontally to a MO BIO Vortex Adapter, catalog# 13000-V1-15 or 13000-V1-5.
17. Vortex at **maximum** speed for 5 minutes.
18. Centrifuge the tube at 4,000 x *g* for 1 minute at room temperature.
19. Transfer all the supernatant to a clean 2.2 ml Collection Tube.
    Note: Placing the pipette tip down into the beads and against the bottom of the tube is required. Pipette more than once to ensure removal of all supernatant. Any carryover of beads will not affect subsequent steps. Expect to recover ~1.5 ml of supernatant.
20. Add **300 µl of Solution ST3** and vortex briefly to mix. Incubate at 4°C for 5 minutes.
21. Centrifuge the tube at 13,000 x *g* for 1 minute.
22. Avoiding the pellet, transfer the supernatant to a clean 5 ml Collection Tube.
23. Remove the plunger from a 20 ml Syringe and place the syringe firmly into the Binding Column.
24. Attach the syringe/binding column unit to a Luer-Lok™ stopcock on the PowerVac™ Manifold, MO BIO Catalog# 11991.
    Note: If you do not have a vacuum manifold, **see the Vacuum Manifold Options section under the Hints & Troubleshooting Guide.**
25. Add **3 ml of Solution ST4** to the 5 ml Collection Tube containing supernatant and vortex to mix.
    Note: Solution ST4 must be warmed to dissolve precipitates prior to use. Solution ST4 must be used while still warm. If Solution ST4 is not used warm then the Binding Column may clog.
26. Load the entire 4.5 ml of supernatant into the syringe attached to the Binding Column.
27. Turn on the vacuum source and open the stopcock of the port. Allow the lysate to pass through the binding column. This should take 5 to 10 minutes.
    Note: If the Binding Columns become clogged, **see the Clogging of Binding Columns section under the Hints & Troubleshooting Guide.**
28. After the lysate has passed through the column completely, close the one-way Luer-Lok™ stopcock of that port.
    Note: Close the ports to samples that have completed filtering to increase the vacuum pressure to the other columns.
29. While keeping the binding column attached to the Luer-Lok™ stopcock , remove the 20 ml Syringe from the Binding Column and discard.
30. Add **0.8 ml of Solution ST5** into the Binding Column. Open the stopcock while holding the column steady. Allow the ethanol to pass through the column completely. Close the stopcock.
31. Shake to mix Solution ST6. Add **0.8 ml of Solution ST6** to the Binding Column. Open the Luer-Lok™ stopcock and apply a vacuum until Solution ST6 has passed through the Binding Column completely. Continue to pull a vacuum for another minute to dry the membrane. Close the port.
32. Add **0.8 ml of Solution ST5** to the Binding Column. Open the Luer-Lok™ stopcock and apply a vacuum until Solution ST5 has passed through the Binding Column completely. Continue to pull a vacuum for another minute to dry the membrane. Close the port.

33. 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. Make certain that all vacuum pressure is released before performing the next step. It is important to turn off the vacuum at the source to prevent backflow into the columns.
34. Remove the binding column and place in a 2.2 ml Collection Tube. Pull the lid of the Collection Tube forward so that the cap fits completely over the Binding Column. Centrifuge the tube at 13,000 × *g* for 2 minutes to completely dry the membrane.
35. Transfer the Binding Column to a new 2.2 ml Collection Tube and add **100 µl of Solution ST7** to the center of the white filter membrane. Pull the lid of the Collection Tube forward so that the cap fits completely over the Binding Column. Alternatively, sterile DNA-Free PCR Grade Water may be used for elution from the silica binding column membrane at this step (MO BIO Catalog# 17000-10).
36. Centrifuge at room temperature for 1 minute at 13,000 x *g*.
37. Discard the Binding Column. The DNA in the tube is now ready for any downstream application. No further steps are required.

    We recommend storing DNA frozen (-20 to -80°C). Solution ST7 contains no EDTA. To concentrate the DNA see the Hints & Troubleshooting Guide.

Thank you for choosing the PowerWater® Sterivex™ DNA Isolation Kit.

Detailed Protocol (Describes what is happening at each step)

Please wear gloves at all times

Add Solution ST1A to bottle ST1B (only required the first time you use ST1B). Mix well and store at 4°C when not in use.
Warm Solutions ST2 and ST4 prior to use at 65°C for 5-10 minutes. Use Solutions ST2 and ST4 while still warm.

1. Filter water sample through a Sterivex™ filter unit, Millipore catalog# SVGPL10RC. After filtering, remove as much of the remaining liquid as possible using a syringe (Not Included) containing air and then cap both ends with the Inlet and Outlet Caps. Typical volumes filtered are 1 – 10 L. The volume of water filtered will depend on the microbial load and turbidity of the water sample.
   Note: It is recommended to use Sterivex™ filter units, Millipore catalog# SVGPL10RC. This unit has a male Luer-Lok™ tip (outlet) that can be easily capped. For use of different Sterivex filter units, see the **Use of Alternate Sterivex™ Filter Units section in the Hints & Troubleshooting Guide.**
   For long term storage, Sterivex™ filter units should be stored capped without excess liquid at -20°C.
   We do not recommend adding SET (Sucrose:EDTA:Tris) buffer to Sterivex™ filter units for storage. SET buffer is not required for this protocol and may interfere with DNA extraction and inhibitor removal. **See the Storage with SET Buffer section under the Hints & Troubleshooting Guide.**

2. Remove the inlet cap and add **0.9 ml of Solution ST1B** using a pipette tip. Insert pipette completely into the inlet so that the pipette tip is visible inside the unit just above the membrane.
   Note: Solution ST1A must be added to bottle ST1B prior to first use only.

3. Re-cap the inlet and secure the Sterivex™ filter unit horizontally, with the inlet facing out, to a MO BIO Vortex Adapter, catalog# 13000-V1-15 or 13000-V1-5.

4. Vortex at **minimum** speed for 5 minutes.

5. While still attached to the vortex adapter, rotate the Sterivex™ filter unit 180 degrees from the original position by marking the unit underneath with a marker and turning the unit until the mark faces up. Vortex at **minimum** speed for an additional 5 minutes.

   What's happening: Solution ST1B is a cell release solution that helps to pull microbes from the membrane into solution so they can be lysed. After Solution ST1A is added to bottle ST1B, it should be stored at 4°C.

6. Set the Sterivex™ filter unit with the inlet facing up and remove the Inlet Cap.

7. Add **0.9 ml of ST2** using a pipette tip. Re-cap the inlet.
   Note: Solution ST2 must be warmed to dissolve precipitates prior to use. Solution ST2 should be used while still warm. When adding ST2, insert pipette completely into the inlet so that the pipette tip is visible inside the unit just above the membrane.

   What's happening: Solution ST2 is a strong lysing reagent that includes a detergent to help break cell walls and will remove non-DNA organic and inorganic material. It is also part of the patented Inhibitor Removal Technology® (IRT). When cold, this solution will form a white precipitate in the bottle. Heating to 65°C will dissolve the components without harm. Solution ST2 should be used while it is still warm.

8. Incubate the Sterivex™ filter unit at 90ºC for 5 minutes. For samples containing easy to lyse organisms or where less DNA shearing is desired, this step can be omitted. **See the Alternative Lysis Methods section under the Hints & Troubleshooting Guide.**
   Note: Place the unit so that the heat is evenly distributed around it. Do not heat at higher temperatures or for longer than 5 minutes.

   What's happening: Heating the Sterivex™ filter units containing the bacterial release solution (ST1B) and lysis reagent (ST2) improves cell lysis of organisms that may be resistant to lysis reagents and mechanical bead beating.

9. Cool the unit at room temperature for 2 minutes. Check to make sure the caps are on tight.
   Note: The caps at both ends may loosen after heating. It is important to cool the unit before retightening the caps to minimize warping of the inlet.

10. Secure the Sterivex™ filter unit horizontally, with the inlet facing out, to a MO BIO Vortex Adapter, catalog# 13000-V1-15 or 13000-V1-5.

11. Vortex at **maximum** speed for 5 minutes.

    What's happening: Vortexing at maximum speed helps to further free microbes and lyse cells within the Sterivex™ filter membrane.

12. Set the Sterivex™ filter unit with the inlet facing up and remove the Inlet Cap.

13. Pull back the plunger of a 3 ml Syringe to fill the barrel with 1 ml of air and attach to the inlet of Sterivex™ filter unit. Hold the 3 ml Syringe and Sterivex™ filter unit vertically with the syringe at the bottom to allow as much of the lysate as possible to be near the inlet.

14. Push the air into the unit until there is resistance and release the plunger. Back pressure withdraws the lysate and fills the syringe. Continue to pull back the on the plunger to remove as much of the lysate as possible. Detach the syringe from the Sterivex™ filter unit.
    Note: Do not force the air from the syringe into the Sterivex™ filter unit.

    What's happening: the lysate containing both intact and lysed microbes is removed from the Sterivex™ filter unit for further processing.

15. Add the lysate to the 5 ml PowerWater® Sterivex™ glass Bead Tube.
    Note: For samples containing easy to lyse organisms or where less DNA shearing is desired, steps 15 -18 can be omitted. **See the Alternative Lysis Methods section under the Hints & Troubleshooting Guide.**

16. Secure the PowerWater® Sterivex™ glass Bead Tube horizontally to a MO BIO Vortex Adapter, catalog# 13000-V1-15 or 13000-V1-5.

17. Vortex at **maximum** speed for 5 minutes.

    What's happening: The bead beating or vortexing step is critical for complete homogenization and cell lysis. Cells are lysed by a combination of chemical agents (ST1B, ST2) and mechanical shaking introduced at this step. By shaking the beads in the presence of disruption agents, collision of the beads with microbial cells will cause the remaining cells to break open.

18. Centrifuge the tube at 4000 x *g* for 1 minute at room temperature.

19. Transfer all the supernatant to a clean 2.2 ml Collection Tube.
    Note: Placing the pipette tip down into the beads and against the bottom of the tube is required. Pipette more than once to ensure removal of all supernatant. Any carryover of beads will not affect subsequent steps. Expect to recover ~1.5 ml of supernatant.

    What's happening: The supernatant is separated and removed from sample debris and beads at this step.

20. Add **300 µl of Solution ST3** and vortex briefly to mix. Incubate at 4°C for 5 minutes.

    What's happening: Solution ST3 is another part of the patented Inhibitor Removal Technology® (IRT) and is a second reagent to remove additional non-DNA organic and inorganic material including humic acid, cell debris, and proteins. It is important to remove contaminating organic and inorganic matter that may reduce DNA purity and inhibit downstream DNA applications.

21. Centrifuge the tube at 13,000 x *g* for 1 minute.

22. Avoiding the pellet, transfer the supernatant to a clean 5 ml Collection Tube.

    What's happening: The pellet at this point contains additional non-DNA organic and inorganic material. For best DNA yield and purity, avoid transferring any of the pellet.

23. Remove the plunger from a 20 ml Syringe and place the syringe firmly into the Binding Column.

    What's happening: The 20 ml Syringe serves as a binding column extender that allows the one-step addition of all sample lysate (4.5 ml) without the use of a midi or maxi column and centrifugation.

24. Attach the syringe/binding column unit to a Luer-Lok™ stopcock on the PowerVac™ Manifold, MO BIO Catalog# 11991.
    Note: If you do not have a vacuum manifold, **see the Vacuum Manifold Options section under the Hints & Troubleshooting Guide.**

25. Add **3 ml of Solution ST4** to the 5 ml Collection Tube containing supernatant and vortex to mix.
    Note: Solution ST4 must be warmed to dissolve precipitates prior to use. Solution ST4 must be used while still warm. If Solution ST4 is not used warm then the Binding Column may clog.

    What's happening: Solution ST4 is a high concentration salt solution. DNA binds tightly to silica at high salt concentrations. Solution ST4 adjusts the salt concentration to selectively allow binding of the DNA to the silica filter membrane, while non-DNA organic and inorganic material that may still be present at low levels is prevented from binding. .

26. Load the entire 4.5 ml of supernatant into the syringe attached to the Binding Column.

27. Turn on the vacuum source and open the stopcock of the port. Allow the lysate to pass through the binding column. This should take 5 to 10 minutes.
    Note: If the Binding Columns become clogged, **see the Clogging of Binding Columns section under the Hints & Troubleshooting Guide.**

    What's happening: The DNA is selectively bound to the silica filter membrane in the binding column basket while contaminants pass through the silica filter membrane.

28. After the lysate has passed through the column completely, close the one-way Luer-Lok™ stopcock of that port.

    Note: Close the ports to samples that have completed filtering to increase the vacuum pressure to the other columns.

29. While keeping the binding column attached to the Luer-Lok™ stopcock, remove the 20 ml Syringe from the binding column and discard.

    What's happening: The column extender is discarded so that the Binding Column can be washed.

30. Add **0.8 ml of Solution ST5** into the Binding Column. Open the stopcock while holding the column steady. Allow the ethanol to pass through the column completely. Close the stopcock.

    What's happening: Solution ST5 is a prewash to help remove residual contaminants which will result in higher DNA purity and yield.

31. Shake to mix Solution ST6. Add **0.8 ml of Solution ST6** to the Binding Column. Open the Luer-Lok™ stopcock and apply a vacuum until Solution ST6 has passed through the Binding Column completely. Continue to pull a vacuum for another minute to dry the membrane. Close the port.

    What's happening: Solution ST6 is an alcohol based wash solution used to further clean the DNA that is bound to the silica filter membrane in the binding column. This wash solution removes residual salt and other contaminants while allowing the DNA to stay bound to the silica filter membrane.

32. Add **0.8 ml of Solution ST5** to the Binding Column. Open the Luer-Lok™ stopcock and apply a vacuum until Solution ST5 has passed through the binding column completely. Continue to pull a vacuum for another minute to dry the membrane. Close the port.

    What's happening: Solution ST5 ensures complete removal of Solution ST6 which will result in higher DNA purity and yield.

33. 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. Make certain that all vacuum pressure is released before performing the next step. It is important to turn off the vacuum at the source to prevent backflow into the columns.

34. Remove the Binding Column and place in a 2.2 ml Collection Tube. Pull the lid of the Collection Tube forward so that the cap fits completely over the Binding Column. Centrifuge the tube at 13,000 × *g* for 2 minutes to completely dry the membrane.

    What's happening: The second spin removes residual Solution ST5. It is critical to remove all traces of wash solution because the ethanol in Solution ST5 can interfere with many downstream DNA applications such as PCR, restriction digests, and gel electrophoresis.

35. Transfer the Binding Column to a new 2.2 ml Collection Tube and add **100 µl of Solution ST7** to the center of the white filter membrane. Pull the lid of the Collection Tube forward so that the cap fits completely over the Binding Column. Alternatively, sterile DNA-Free PCR Grade Water may be used for elution from the silica binding column membrane at this step (MO BIO Catalog# 17000-10).

What's happening: Placing Solution ST7 (sterile elution buffer) in the center of the small white membrane will ensure the entire membrane is wetted. This will result in a more efficient and complete release of the DNA from the silica filter membrane. As Solution ST7 passes through the silica filter membrane, the DNA that was bound in the presence of high salt is selectively released by Solution ST7 (10 mM Tris) which lacks salt.

Alternatively, sterile DNA-Free PCR Grade Water may be used for DNA elution from the silica filter membrane at this step. Solution ST7 contains no EDTA. If DNA degradation is a concern, sterile TE may also be used instead of ST7 for elution of DNA from the binding column.

36. Centrifuge at room temperature for 1 minute at 13,000 x *g*.

37. Discard the Binding Column. The DNA in the tube is now ready for any downstream application. No further steps are required.

    We recommend storing DNA frozen (-20 to -80°C). Solution ST7 contains no EDTA. To concentrate the DNA see the Hints & Troubleshooting Guide.

Thank you for choosing the PowerWater® Sterivex™ DNA Isolation Kit.

Hints & Troubleshooting Guide

Types of Water Samples

A. Clear Water Samples: Water samples may vary from clear to highly turbid. Larger volumes of clear water can be processed because there is less chance of filter clogging. Potable drinking water and ocean samples will generally allow for very high volumes depending on the quality and particulate count. In most cases, 100 ml to 10 liters can be processed. Some users report processing even higher volumes.

B. Turbid Water Samples: Turbid samples with high levels of suspended solids or sediments will tend to clog Sterivex™ filter units (0.22 micron) so less water will be able to be filtered per unit. 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 prefilter consisting of a larger pore size is recommended.

Use of Alternate Sterivex™ Filter Units

The use of Sterivex™ filter units, Millipore catalog# SVGPL10RC, is recommended. These units have a male Luer-Lok™ tip (outlet) that can be easily capped, minimizing the risk of sample loss or leakage. Other commonly used units have a male tip only. If units containing a male tip are used instead of the male Luer-Lok™ tip, an alternate cap will need to be used. Capping methods include a male luer plug, clay, or wax. Parafilm can also be used to help seal the unit and prevent leakage.

Storage with SET Buffer

SET buffer (Sucrose:EDTA:Tris) should not be added to the Sterivex™ filter units for storage. Sterivex™ filter units can be stored frozen at -20°C without any solutions. If Sterivex™ filter units contain SET buffer then the buffer should be pushed through the unit with a syringe and the units washed with a balanced salt solution such as sterile phosphate buffered saline (PBS) or 0.85% saline. Removal of the SET buffer and washing of the unit may cause a reduction in DNA yield due to premature lysis of labile organisms.

Alternative Lysis Methods

For samples containing easy to lyse organisms or where less DNA shearing is desired the heating step (Step 8) and the bead beating step (Steps 15 - 18) can be omitted. The heating and bead beating steps are critical for samples that contain difficult to lyse organisms such as spores, fungi, archae, and cyanobacteria.

Vacuum Manifold Options

The vacuum manifold is highly recommended for this protocol. If a vacuum manifold is not available alternative methods can be used for processing a few samples at a time.

1) The syringe plunger can be reinserted and used to manually push the lysate through the column. Once all the lysate has been pushed through, the column can be added to a 2.2 ml tube (provided for Step 34). Wash with **0.7 ml of Solution ST6** and centrifuge for 1 minute at 13,000 x *g*. Discard the flow through and wash with **0.7 ml of Solution ST5** and centrifuge for 1 minute at 13,000 x *g*. Continue with Step 34.

2) Place the binding column in a 2.2 ml tube (provided for Step 34) and load 700 µl of supernatant onto the binding column and centrifuge for 1 minute at 13,000 x *g*. Discard the flow through and repeat until all the supernatant has been loaded onto the binding column. 7 loads for each sample processed are required. Wash with **0.7 ml of Solution ST6** and centrifuge for 1 minute at 13,000 x *g*. Discard the flow through and wash with **0.7 ml of Solution ST5.** Centrifuge for 1 minute at 13,000 x *g*. Continue with Step 34.

Hints & Troubleshooting Guide cont.

Clogging of Binding Columns

Check to make sure there are no cracks or breaks in the Luer-Lok™ fittings which would result in loss of vacuum pressure at that port. Solution ST4 must be heated and used while still warm to ensure a steady flow through the column under vacuum. If columns become clogged, the syringe plunger can be reinserted and used to manually push the lysate through the column. Alternatively, the columns can be added to a 2.2 ml tube (provided for Step 34) and centrifuged at 13,000 x *g* until all the lysate has been loaded and the DNA bound to the column. The columns can then be placed back onto the vacuum for washing or kept in the 2.2 ml tube and washed using centrifugation.

Low $A_{260/230}$ Ratios are Obtained

$A_{260/230}$ readings are one measure of DNA purity. For samples with low biomass, which would lead to low DNA yields (<20 ng/µl), this ratio may fall below 1.5. This ratio is not an indicator of amplification ability or DNA integrity and may be a result of DNA detection method. Ethanol precipitation with resuspension into a smaller volume to concentrate the DNA may help to improve the appearance of the $A_{260/230}$ ratio.

If DNA Does Not Amplify

- Make sure to check DNA yields by gel electrophoresis or spectrophotometer reading. An excess amount of DNA will inhibit a PCR reaction.
- Diluting the template DNA should not be necessary with DNA isolated with the PowerWater® Sterivex™ DNA Isolation Kit; however, it should still be attempted.
- If DNA will still not amplify after trying the steps above, then PCR optimization (changing reaction conditions and primer choice) may be needed.

Concentrating the DNA

The final volume of eluted DNA will be 100 µl. The DNA may be concentrated by adding 10 µl of 5M NaCl and inverting 3-5 times to mix. Next, add 200 µl of 100% cold ethanol and invert 3-5 times to mix. Incubate at -20° for an hour (or longer). Centrifuge at 13,000 x *g* for 15 minutes at room temperature. Decant all liquid. Wash the DNA pellet with 70% ethanol and centrifuge at 13,000 x *g* for 10 minutes. Decant the ethanol and remove residual ethanol in a speed vacuum, dessicator, or air dry. Resuspend precipitated DNA in sterile water or sterile 10 mM Tris.

DNA Floats Out of Well When Loaded on a Gel

This usually occurs because residual Solution ST5 remains in the final sample. Prevent this by drying under vacuum in step 32 and being careful in step 35 not to transfer liquid onto the bottom of the binding column basket. Ethanol precipitation (described in “Concentrating the DNA”) is the best way to remove residual Solution ST5.

Storing DNA

DNA is eluted in Solution ST7 (10 mM Tris) and must be stored at -20°C to -80°C to prevent degradation. DNA can be eluted in TE without loss, but the EDTA may inhibit downstream reactions such as PCR and automated sequencing. DNA may also be eluted with sterile DNA-Free PCR Grade Water (MO BIO Catalog# 17000-10).

Hints & Troubleshooting Guide cont.

Cleaning of the PowerVac™ Manifold

It is recommended to rinse the PowerVac™ Manifold Luer-Lok™ fittings promptly after use to avoid salt build up. To clean the PowerVac™ Manifold Luer-Lok™ fittings, rinse each opening with DI water followed by 70% ethanol and flush into the manifold base under vacuum. Alternatively, remove the fittings and wash in laboratory detergent and DI water.

Do not use bleach to clean the PowerVac™ Manifold. Bleach should never be mixed with solutions containing guanidine. For more information on cleaning the PowerVac™ Manifold, please refer to the PowerVac™ Manifold manual.

Contact Information

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For the distributor nearest you, visit our web site at www.mobio.com/distributors

Products recommended for you

For a complete list of products available from MO BIO Laboratories, Inc., visit www.mobio.com

Description	Catalog No.	Quantity
PowerWater® DNA Isolation Kit	14900-50-NF 14900-100-NF	50 preps 100 preps
PowerWater® RNA Isolation Kit	14700-50-NF	50 preps
PowerClean® DNA Clean-Up Kit	12877-50	50 preps
PowerVac™ Manifold	11991	1 manifold
Vortex-Genie® 2 Vortex	13111-V 13111-V-220	1 unit (120V) 1 unit (220V)
Vortex Adapter for 1.5 to 2.0 ml tubes	13000-V1-24	1 unit