MON PowerFood®
Microbial DNA Isolation Kit
(For isolation of genomic DNA from cultured food)

Catalog No.	Quantity
21000-100-MON	100 Preps

Instruction Manual

Please recycle

Version: 04012014

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Introduction

The PowerFood® Microbial DNA Isolation Kit is designed to isolate high-quality genomic DNA from microorganisms cultured from food according to FDA guidelines (Bacteriological Analytical Manual, Edition 8, Revision A /1998). The kit combines our patented Inhibitor Removal Technology® (IRT) with reformulated buffers to provide high quality, inhibitor-free DNA for use in downstream applications including PCR and qPCR.

DNA from a variety of known food pathogens has been isolated using the PowerFood® Microbial DNA Isolation Kit and detected using real-time quantitative PCR.

Pathogen	Enriched Food
Clostridium difficile	Raw ground beef, ready-to-eat salad
Clostridium perfringens	Raw ground beef, carrot juice
Enterococcus faecalis	Ready-to-eat salad
Escherichia coli	Strawberries, orange juice
Lactobacillus acidophilus	Yogurt
Lactobacillus spp.	Yogurt
Listeria monocytogenes	Brie cheese
Salmonella enterica	Strawberries, orange juice, brie cheese
Shigella spp.	Shrimp
Vibrio spp.	Shrimp

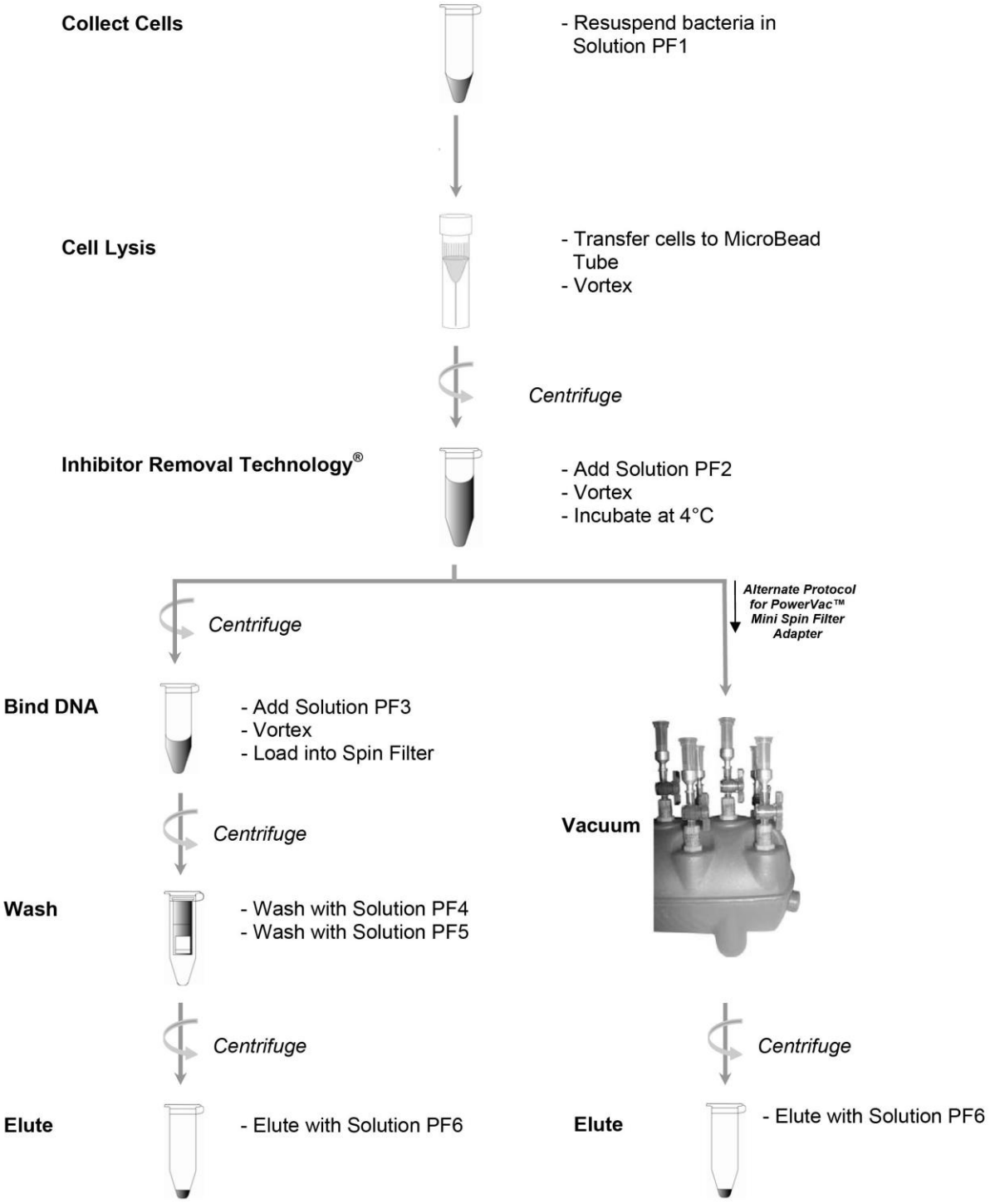
Protocol Overview

1.8 ml of microbial food culture is pelleted by centrifugation and resuspended in lysis buffer. The supernatant is transferred to a bead beating tube containing beads designed for small cell (microbial) lysis and vortex mixed using a specially designed MO BIO Vortex Adapter. After the protein and inhibitor removal steps, total genomic DNA is captured on the MO BIO Laboratories flat bottom silica spin column. The bound DNA is then washed and eluted from the spin column membrane.

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

Other Related Products	Catalog No.	Quantity
Vortex Adapter for Vortex Genie® 2	13000-V1-24	Holds 24 (2 ml) Tubes
Vortex Genie® 2 Vortex	13111-V-220 13111-V	1 unit (220V) 1 unit (120V)
PowerVac™ Manifold	11991	1 manifold
PowerVac™ Mini System	11992	1 unit + 20 adapters
PowerVac™ Mini Spin Filter Adapters	11992-10 11992-20	10 adapters 20 adapters
PCR Water (Certified DNA-free)	17000-5 17000-10 17000-11	5 x 1 ml 10 x 1 ml 10 ml bottle

PowerFood® Microbial DNA Isolation Kit



Equipment Required

BagMixer® 400 VW
BagMixer® 400 Bags
Microcentrifuge (13,000 x *g*)
Pipettors
Vortex-Genie® 2 Vortex (MO BIO Catalog# 13111-V or 13111-V-220)
Vortex Adapter for 2 ml Tubes (MO BIO Catalog# 13000-V1-24)

Kit Contents

	Kit Catalog # 21000-100-MON	
Component	**Catalog #**	**Amount**
MicroBead Tubes (contain 250 mg MicroBeads)	21000-100-MON-BT	100
Solution PF1	21000-100-MON-1	50 ml
Solution PF2	21000-100-MON-2	11 ml
Solution PF3	21000-100-MON-3	100 ml
Solution PF4	21000-100-MON-4	3 x 24 ml
Solution PF5	21000-100-MON-5	3 x 24 ml
Solution PF6	21000-100-MON-6	11 ml
Spin Filters	21000-100-MON-SF	100
2 ml Collection Tubes	21000-100-MON-T	200

Kit Storage

Kit reagents and components should be stored at room temperature (15-30°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 PF4 and PF5 contain alcohol. They are flammable.

IMPORTANT NOTE FOR USE: Solution PF1 must be warmed at 55°C for 5-10 minutes to dissolve precipitates prior to use. Solution PF1 should be used while still warm.

Solution PF3 may precipitate over time. If precipitation occurs, warm at 55°C for 5-10 minutes. Solution PF3 can be used while still warm.

Shake to mix Solution PF4 before use.

Vacuum Protocol using the PowerVac™ Manifold

Please wear gloves at all times

For each sample lysate, use one Spin Filter column. Keep the Spin Filter in the attached 2 ml Collection Tube and continue using the Collection Tube as a Spin Filter holder until needed for the Vacuum Manifold Protocol. Label each Collection Tube top and Spin Filter column to maintain sample identity. If the Spin Filter becomes clogged during the vacuum procedure, you can switch to the procedure for purification of the DNA by centrifugation.

You will need to provide 100% ethanol for step 4 of this protocol

1. For each prep, attach one aluminum **PowerVac™ Mini Spin Filter Adapter** (MO BIO Catalog# 11992-10 or 11992-20) into the Luer-Lok® fitting of one port in the manifold. Gently press a Spin Filter column into the PowerVac™ Mini Spin Filter Adapter until snugly in place. Ensure that all unused ports of the vacuum manifold are closed.
   Note: Aluminum PowerVac™ Mini Spin Filter Adapters are reusable.

2. Transfer 650 µl of prepared sample lysate (from step 12) to the **Spin Filter column**.

3. Turn on the vacuum source and open the stopcock of the port. Hold the tube in place when opening the stopcock to keep the spin filter steady. Allow the lysate to pass through the **Spin Filter column**. After the lysate has passed through the column completely, load again with the next 650 µl of lysate. Continue until all of the lysate has been loaded onto the **Spin Filter column**. Close the one-way Luer-Lok® stopcock of that port.
   Note: If Spin Filter Columns are filtering slowly, close the ports to samples that have completed filtering to increase the pressure to the other columns.

4. Load 800 µl of 100% ethanol into the Spin Filter so that it completely fills the column. Open the stopcock while holding the column steady. Allow the ethanol to pass through the column completely. Close the stopcock.

5. Shake to mix Solution PF4. Add 650 µl of **Solution PF4** to each Spin Filter. Open the Luer-Lok® stopcock and apply a vacuum until **Solution PF4** has passed through the Spin Filter completely. Continue to pull a vacuum for another minute to dry the membrane. Close each port.

6. Add 650 µl of **Solution PF5** to each Spin Filter. Open the Luer-Lok® stopcock and apply a vacuum until **Solution PF5** has passed through the Spin Filter completely. Continue to pull a vacuum for another minute to dry the membrane. Close each port.

7. 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.

8. Remove the **Spin Filter column** and place in the original labeled **2 ml Collection Tube**. Place into the centrifuge and spin at 13,000 × *g* for 2 minutes to completely dry the membrane.

9. Transfer the **Spin Filter column** to a new **2 ml Collection Tube** and add 100 µl of **Solution PF6** to the center of the white filter membrane.

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

11. Discard the **Spin Filter column**. The DNA in the tube is now ready for any downstream application. No further steps are required.

Thank you for choosing the MON PowerFood® Microbial DNA Isolation Kit!

Experienced User Protocol

Please wear gloves at all times

Warm Solution PF1 prior to use at 55°C for 5-10 minutes. Use Solution PF1 while still warm. Check Solution PF3 and warm at 55°C for 5-10 minutes if necessary. Solution PF3 can be used while still warm.

1. Homogenize the food sample using a lab blender such as a BagMixer® 400 VW and incubate homogenates according to FDA guidelines (Bacteriological Analytical Manual, Edition 8, Revision A /1998).
2. Add 1.8 ml of microbial food culture to a 2 ml Collection Tube (provided) and centrifuge at 13,000 x *g* for 1 minute at room temperature. Decant the supernatant and spin the tubes at 13,000 x *g* for an additional 1 minute. Completely remove the remaining media supernatant with a pipet tip.
3. Resuspend the cell pellet in **450 µl of Solution PF1**.
   Note: Solution PF1 must be warmed to dissolve precipitates prior to use. Solution PF1 should be used while still warm.
4. Transfer resuspended cells to the MicroBead Tube **(To increase yields or for difficult cells, see Alternative Lysis Methods in the Hints and Troubleshooting Guide before continuing).**
5. Secure the MicroBead Tube horizontally to a MO BIO Vortex Adapter, Catalog# 13000-V1-24.
6. Vortex at maximum speed for 10 minutes **(To reduce DNA shearing, see Alternative Lysis Methods in the Hints and Troubleshooting Guide)**.
7. Centrifuge the tubes at 13,000 x *g* for 1 minute at room temperature.
   CAUTION: Be sure not to exceed 13,000 x *g* or tubes may break.
8. Transfer the supernatant to a clean 2 ml Collection Tube (provided).
   Note: Expect approximately 400 µl of supernatant.
9. Add **100 µl of Solution PF2** and vortex briefly to mix. Incubate at 4°C for 5 minutes.
10. Centrifuge the tubes at 13,000 x *g* for 1 minute at room temperature.
11. Avoiding the pellet, transfer the entire volume of supernatant to a clean 2 ml Collection Tube (provided).
    Note: Expect approximately 450 µl in volume.
12. Add **900 µl of Solution PF3** and vortex to mix.
    Note: Check Solution PF3 for precipitation prior to use. Warm if necessary. Solution PF3 can be used while still warm.
13. Load 650 µl of supernatant onto a Spin Filter and centrifuge at 13,000 x *g* for 1 minute. Discard the flow through and repeat until all the supernatant has been loaded onto the Spin Filter.
    Note: A total of two loads for each sample processed is required.
14. Place the Spin Filter basket into a clean 2 ml Collection Tube (provided).
15. Shake to mix Solution PF4 before use. Add **650 µl of Solution PF4** and centrifuge at 13,000 x *g* for 1 minute at room temperature.
16. Discard the flow through and add **650 µl of Solution PF5** and centrifuge at 13,000 x *g* for 1 minute at room temperature.
17. Discard the flow through and centrifuge again at 13,000 x *g* for 2 minutes to remove residual wash.
18. Place the Spin Filter basket into a clean 2 ml Collection Tube (provided).
19. Add **100 µl of Solution PF6** to the center of the white filter membrane.
20. Centrifuge at 13,000 x *g* for 1 minute.
21. Discard the Spin Filter basket. The DNA is now ready for any downstream application. No further steps are required.

We recommend storing DNA frozen (-20°C).

Thank you for choosing the MON PowerFood® Microbial DNA Isolation Kit!

Detailed Protocol
Please wear gloves at all times

Warm Solution PF1 prior to use at 55°C for 5-10 minutes. Use Solution PF1 while still warm. Check Solution PF3 and warm at 55°C for 5-10 minutes if necessary. Solution PF3 can be used while still warm.

1. Homogenize the food sample using a lab blender such as a BagMixer® 400 VW and incubate homogenates according to FDA guidelines (Bacteriological Analytical Manual, Edition 8, Revision A /1998).

2. Add 1.8 ml of microbial food culture to a 2 ml Collection Tube (provided) and centrifuge at 13,000 x *g* for 1 minute at room temperature. Decant the supernatant and spin the tubes at 13,000 x *g* for an additional 1 minute. Completely remove the remaining media supernatant with a pipet tip.

   What's happening: This step concentrates and pellets the microbial cells. It is important to pellet the cells completely and remove all the culture media in this step.

3. Resuspend the cell pellet in **450 µl of Solution PF1**.

   Note: Solution PF1 must be warmed to dissolve precipitates prior to use. Solution PF1 should be used while still warm.

   What's happening: Solution PF1 is a strong lysing reagent that includes a detergent to break cell walls and help remove non-DNA organic and inorganic material, it is also an important part of our patented IRT technology. When cold, this solution will form a white precipitate in the bottle. Heating to 55°C will dissolve the components without harm. Solution PF1 should be used while it is still warm.

4. Transfer resuspended cells to the MicroBead Tube **(To increase yields or for difficult cells, see Alternative Lysis Methods in the Hints and Troubleshooting Guide before continuing).**

5. Secure the MicroBead Tube horizontally to a MO BIO Vortex Adapter, Catalog# 13000-V1-24.

6. Vortex at maximum speed for 10 minutes **(To reduce DNA shearing, see Alternative Lysis Methods in the Hints and Troubleshooting Guide)**.

   What's happening: Microbial cells are lysed using a combination of chemical (lysis buffer) and mechanical (vortex mixing) lysis conditions. Many cell types will not lyse without this chemically enhanced bead beating process. The PowerFood® Bead Tube is secured onto a MO BIO Vortex Adapter which holds tubes horizontally at an equal distance from the center of rotation for even and efficient homogenization across samples. Tape should not be used to secure tubes to the vortex because it can become loose and result in reduced or uneven homogenization efficiency. More robust bead beaters may also be used. In most cases the times may be shorter with other devices but you may run the risk of increased DNA shearing. This process is compatible with the PowerLyzer® and fast prep machines.

7. Centrifuge the tubes at 13,000 x *g* for 1 minute at room temperature.
   CAUTION: Be sure not to exceed 13,000 x *g* or tubes may break.

   What's happening: The cell debris is pelleted along the side of the tube while the DNA remains in the supernatant. This step is important for the removal of any remaining contaminating non-DNA organic and inorganic matter that may reduce the DNA purity and inhibit downstream applications.

8. Transfer the supernatant to a clean 2 ml Collection Tube (provided).

   Note: Expect approximately 400 µl of supernatant.

9. Add **100 µl of Solution PF2** and vortex briefly to mix. Incubate at 4°C for 5 minutes.

   What's happening: Solution PF2 is the second step of our patented Inhibitor Removal Technology® (IRT) and further removes additional non-DNA organic and inorganic material including cell debris, and proteins. It is important to remove contaminating organic and inorganic matter that may reduce the DNA purity and inhibit downstream applications.

10. Centrifuge the tubes at 13,000 x *g* for 1 minute at room temperature.

11. Avoiding the pellet, transfer the entire volume of supernatant to a clean 2 ml Collection Tube (provided).

    Note: Expect approximately 450 µl in volume.

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

12. Add **900 µl of Solution PF3** and vortex to mix.

    Note: Check Solution PF3 for precipitation prior to use. Warm if necessary. Solution PF3 can be used while still warm.

    What's happening: Solution PF3 is a highly concentrated salt solution. Since DNA binds tightly to silica at high salt concentrations this will adjust the DNA solution salt concentration to allow binding of DNA, but not non-DNA organic and inorganic material that may still be present at low levels, to the Spin Filter.

13. Load 650 µl of supernatant onto a Spin Filter and centrifuge at 13,000 x *g* for 1 minute. Discard the flow through and repeat until all the supernatant has been loaded onto the Spin Filter.

    Note: A total of two loads for each sample processed is required.

    What's happening: DNA is selectively bound to the silica membrane in the Spin Filter basket and the flow through containing non-DNA components is discarded.

14. Place the Spin Filter basket into a clean 2 ml Collection Tube (provided).

    What's happening: Due to the high concentration of salt in solution PF3, it is important to place the Spin Filter basket into a clean 2 ml Collection Tube to aid in the subsequent wash steps and improve DNA purity and yield.

15. Shake to mix Solution PF4 before use. Add **650 µl of Solution PF4** and centrifuge at 13,000 x *g* for 1 minute at room temperature.

    What's happening: Solution PF4 is an alcohol based wash solution used to further clean the DNA that is bound to the silica filter membrane in the Spin Filter. This wash solution removes residual salt and other contaminants while allowing the DNA to stay bound to the silica membrane.

16. Discard the flow through and add **650 µl of Solution PF5** and centrifuge at 13,000 x *g* for 1 minute at room temperature.

    What's happening: Solution PF5 ensures complete removal of Solution PF4 which will result in higher DNA purity and yield.

17. Discard the flow through and centrifuge again at 13,000 x *g* for 2 minutes to remove residual wash.

    What's happening: The second spin removes residual Solution PF5. It is critical to remove all traces of wash solution because the ethanol in Solution PF5 can interfere with many downstream DNA applications such as PCR, restriction digests, and gel electrophoresis.

18. Place the Spin Filter basket into a clean 2 ml Collection Tube (provided).

19. Add **100 µl of Solution PF6** to the center of the white filter membrane.

    What's happening: Placing Solution PF6 (sterile elution buffer) in the center of the small white membrane will make sure the entire membrane is wetted. This will result in a more efficient and complete release of the DNA from the silica Spin Filter membrane. As Solution PF6 passes through the silica membrane, DNA that was bound in the presence of high salt is selectively released by Solution PF6 (10 mM Tris, 1 mM EDTA.

20. Centrifuge at 13,000 x *g* for 1 minute.

21. Discard the Spin Filter basket. The DNA is now ready for any downstream application. No further steps are required.

    We recommend storing DNA frozen (-20°C).

Thank you for choosing the MON PowerFood® Microbial DNA Isolation Kit!

Hints and Troubleshooting Guide

Isolation of DNA Directly From Food Without Culturing

DNA can be isolated directly from small quantities of both liquid and solid food. Microbial yields may be low but are still detectable by PCR. For both solid food homogenates and liquids, microbes will pellet underneath all other food components. To get to the pellet, remove as much of the fat and food homogenates as possible before adding PF1. For further assistance contact Technical Service at 800-606-6246.

- **For solid foods**: Homogenize 0.25 g of food in 0.75 ml of PBS (not provided) in a 2 ml Collection Tube (provided). Centrifuge for 1 to 3 minutes at 13,000 x *g*. Remove as much of the residual food and liquid as possible with a pipet tip without disturbing the microbial pellet at the bottom of the tube. Resuspend the pellet in 450 µl of PF1 and proceed with the protocol.
- **For liquid foods:** Centrifuge 1 - 1.8 ml of liquid for 1 to 3 minutes at 13,000 x *g.* Remove as much of the residual food, fat and liquid as possible with a pipet tip without disturbing the microbial pellet at the bottom of the tube. Resuspend the pellet in 450 µl of PF1 and proceed with the protocol.

Microbial DNA has been isolated directly and PCR amplified from the following spiked foods: cream (12%, 25% fat), Brie cheese (37% fat), orange juice, strawberries, lettuce, asparagus, and milk/dark chocolate.

Presence of Food Homogenates in the Microbial Pellet

When pelleting the microbial culture in step 2 of the protocol, food components may pellet as well. Depending on the type of food homogenate this may enlarge the pellet. Resuspend the entire pellet as indicated in the protocol.

Alternative Lysis Methods (*We recommend using only one of these methods for any individual prep.)*

- **To increase yields**: Heating can aid in lysis for some organisms and it can lead to increased yields. Heat preps at 65°C for 10 minutes during step 4 and continue with step 5.
- **For less DNA shearing**: We recommend heating the preps at 65°C for 10 minutes with occasional bump vortexing for a few seconds every 2-3 minutes. Skip steps 5 and 6 and go to step 7. This helps prevent unwanted damage to large DNA. This procedure will reduce DNA shearing and at the same time can increase the yield of total DNA from some organisms.
- **If cells are difficult to lyse**: Heat the preps at 70°C for 10 minutes during step 4. Follow by continuing with the protocol at step 5.

DNA Floats Out of Well When Loaded On a Gel

Residual PF5 Wash Buffer may be in the final sample. To ensure complete drying of the membrane after PF5, centrifuge the spin filter in a clean 2 ml Collection Tube for an additional minute.

- Ethanol precipitation is the best way to remove residual Solution PF5. (See “Concentrating the DNA” below.)
- If you live in a humid climate, you may experience increased difficulty with drying of the membrane in the centrifuge. Increase the centrifugation time at step 17 by another minute or until no visible moisture remains on the membrane.

Hints and Troubleshooting Guide (cont.)

Concentrating the DNA

Your final volume will be 100 µl. If this is too dilute for your purposes, add 5 µl of 3M Sodium Acetate and mix. Then add 2 volumes of 100% cold ethanol. Mix, and incubate at -70°C for 15 minutes or -20°C for 2 hours to overnight. Centrifuge at 13,000 x *g* for 10-15 minutes at 4°C. Decant all liquid. Briefly dry residual ethanol in a speed vac or ambient air. Avoid over-drying the pellet or resuspension may be difficult. Resuspend precipitated DNA in desired volume of 10 mM Tris, 1 mM EDTA (Solution PF6).

Storing DNA

DNA is eluted in Solution PF6 (10 mM Tris, 1 mM EDTA) and must be stored at -20°C to -80°C to prevent degradation. For long term storage, we recommend aliquoting DNA into appropriate volumes and store at -80°C.

Cleaning of the PowerVac™ Mini Spin Filter Adapters

It is recommended to rinse the PowerVac™ Mini Spin Filter Adapters promptly after use to avoid salt build up. To clean the PowerVac™ Mini Spin Filter Adapters, rinse each adapter with DI water followed by 70% ethanol and flush into the manifold base. Alternatively, remove the adapters and wash in laboratory detergent and DI water. PowerVac™ Mini Spin Filter Adapters may be autoclaved.

Do not use bleach to clean the PowerVac™ Mini Spin Filter Adapters while attached to the PowerVac™ Manifold. Bleach should never be mixed with solutions containing guanidine and should not be used to clean the PowerVac™ Manifold. 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
UltraClean® Microbial DNA Isolation Kit	12224-50 12224-250	50 preps 250 preps
PowerLyzer® UltraClean® Microbial DNA Isolation Kit	12255-50	50 preps
PowerMicrobial® Maxi DNA Isolation Kit	12226-25	25 preps
UltraClean® -htp 96 Well Microbial DNA Kit	10196-4 10196-12	4 x 96 preps 12 x 96 preps
UltraClean® Microbial RNA Isolation Kit	15800-50	50 preps
PowerClean® DNA Clean-Up Kit	12877-50	50 preps
Vortex Adapter for Vortex Genie® 2	13000-V1-24	1 unit
PowerVac™ Manifold Mini System	11992	1 unit + adapters
PowerLyzer® 24 Bench Top Bead-Based Homogenizer	13155	1 unit