PowerBiofilm®
DNA Isolation Kit

(For isolation of genomic DNA from biofilm including microbial mats)

Catalog No.	Quantity
24000-50	50 Preps

Instruction Manual

Please recycle

Version: 11062013

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Introduction

The PowerBiofilm® DNA Isolation Kit is the first of its kind designed for isolating high quality DNA from all types of biofilm samples including microbial mats. Our novel bead tube mix and enhanced lysis buffers help to dissolve polysaccharides to enable lysis of organisms in even the most complex biofilm samples. The bead tube is compatible with both our vortex adapter and PowerLyzer® 24 Bench Top Bead-Based Homogenizer to ensure efficient lysis. Our patented Inhibitor Removal Technology® (IRT) is included which allows for inhibitor free, purified DNA that can be used for a multitude of downstream applications.

Protocol Overview

0.05 to 0.20 g of sample material is added to the PowerBiofilm® Bead Tube then heated to activate lysis components that help to dissolve polysaccharides. Lysis continues through either vortex mixing or bead beating depending on the users' preference. Protein and inhibitor removal follows to precipitate out humic substances as well as polyphenolics and polysaccharides. Total genomic DNA is captured on the novel MO BIO Laboratories flat bottom silica spin column where high quality DNA is then washed and eluted from the spin column membrane for use in downstream applications including PCR and qPCR.

Mechanical Lysis Options

The PowerBiofilm® DNA Isolation Kit may be used with the vortex or the high velocity bead beater, PowerLyzer® 24 homogenizer. The PowerLyzer® 24 is suitable for fast homogenization of biofilm samples including microbial mats.

PowerLyzer® 24
Bench Top Bead-Based Homogenizer
Catalog#13155
(www.mobio.com/powerlyzer)

Using the PowerBiofilm® DNA Isolation Kit with the PowerLyzer® Homogenizer

The PowerLyzer® 24 is a highly efficient bead beating system that allows for optimal DNA extraction from biofilms. The instrument's velocity and proprietary motion combine to provide the fastest homogenization time possible, minimizing the time spent processing samples. The programmable display allows for hands-free, walk-away extraction with up to ten cycles of bead beating for as long as 5 minutes per cycle. This kit provides bead tubes prefilled with a glass and ceramic bead mix for homogenizing biofilm material for optimal DNA isolation.

Using the PowerBiofilm® DNA Isolation Kit with other Homogenizers

For isolation of DNA using this kit with the FastPrep® or Precellys®, the following conversion chart will help you to adapt your current protocol. However, due to the highly efficient motion of beads in the PowerLyzer® 24, we have found that fewer cycles are required to generate the same effect. You may want to perform extractions on the PowerLyzer® 24 at the equivalent speed and number of cycles as your current instrument and compare it to less time or lower speed to determine which settings give the best results.

As a starting point, we recommend that for DNA from biofilm you begin with the settings specified in this manual of 1 cycle at 30 seconds at setting 3200 RPM.

PowerLyzer 24	Fastprep 24 m/s	Precellys 24
500	-	-
600	-	-
700	-	-
800	-	-
900	-	-
1000	-	-
1100	-	-
1200	-	-
1300	-	-
1400	-	-
1500	-	-
1600	-	-
1700	-	-
1800	-	-
1900	-	-
2000	-	-
2100	-	-
2200	-	-
2300	-	-
2400	-	-
2500	4	5000
2600	-	5200
2700	-	5400
2800	4.5	5600
2900	-	5800
3000	-	6000
3100	5	6200
3200	-	6400
3300	-	6600
3400	5.5	6800
3500	-	-
3600	-	-
3700	6	-
3800	-	-
3900	-	-
4000	6.5	-
4100	-	-
4200	-	-
4300	-	-
4400	-	-
4500	-	-
5000	-	-

Equivalent settings slower than 2500 RPM or higher than 4000 RPM on the PowerLyzer® 24 are not obtainable with the Fastprep® or Precellys®.

Fastprep® is a registered trademark of MP Biomedical. Precellys® is a registered trademark of Bertin Technologies.

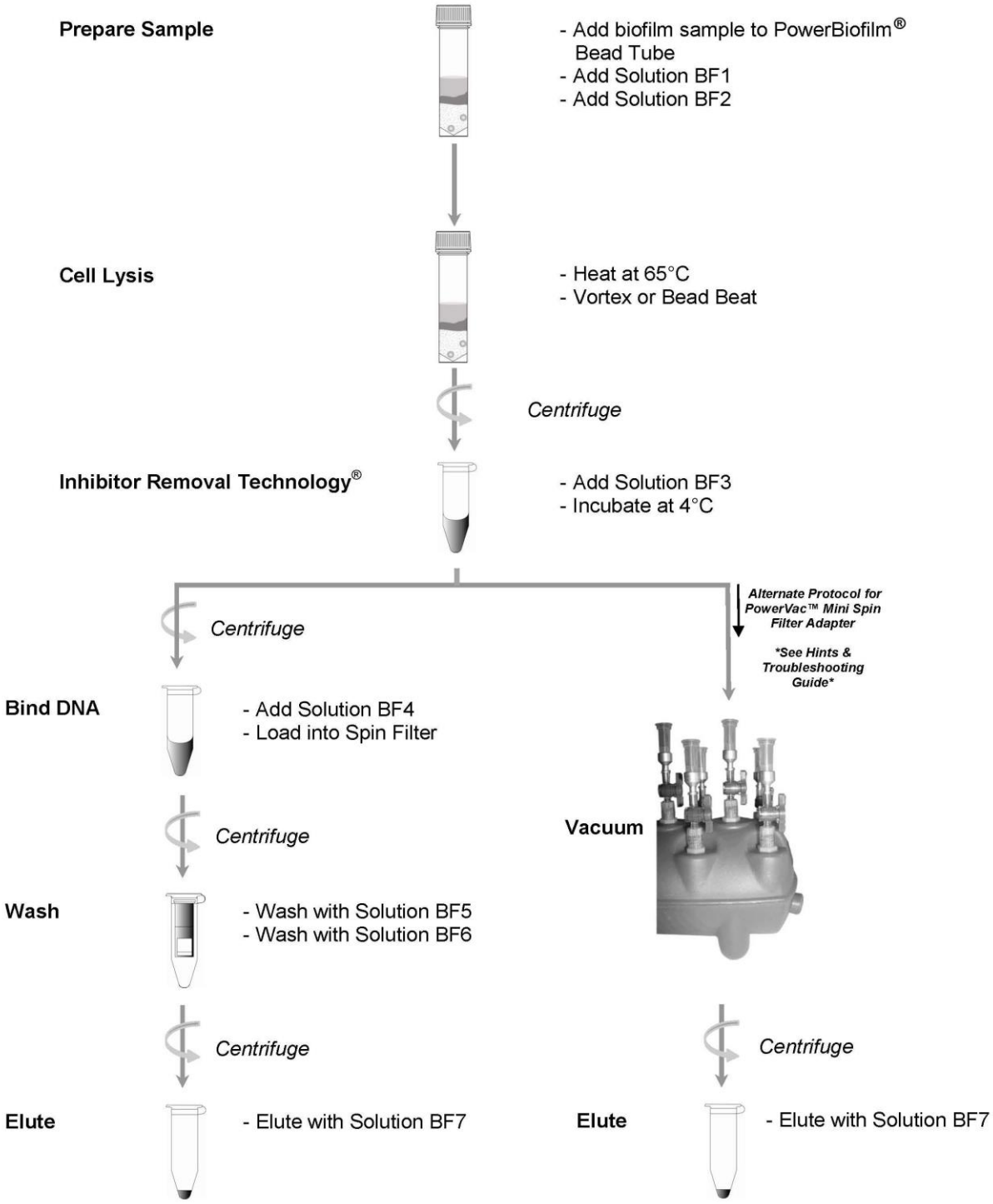
High Throughput Options

MO BIO offers a vacuum based protocol for faster processing without centrifugation for the DNA binding and column washing steps for Spin Filters. The MO BIO PowerVac™ Manifold allows for processing of up to 20 spin filter preps at a time using the PowerVac™ Mini Spin Filter Adapters.

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)
PowerLyzer® 24 Bench Top Bead-Based Homogenizer	13155	1 unit
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
PowerBiofilm® RNA Isolation Kit	25000-50	50 Preps

PowerBiofilm® DNA Isolation Kit



Equipment Required
Microcentrifuge (13,000 x *g*)
Pipettors (100 – 1000 µl)

Optional Equipment
PowerVac™ Manifold Mini System (MO BIO Catalog# 11992)
PowerVac™ Mini Spin Filter Adapters (MO BIO Catalog# 11992-10 or 11992-20)

Reagents Required but not Included
100% ethanol (for the PowerVac™ Manifold protocol only)

Sample Disruption and Homogenization for DNA Purification from Biofilms
Depending on sample type the following equipment may be required

- 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)
- PowerLyzer® 24 Bench Top Bead-Based Homogenizer (MO BIO Catalog# 13155)

Kit Contents

	Kit Catalog # 24000-50	
Component	**Catalog #**	**Amount**
PowerBiofilm® Bead Tubes	24000-50-BT	50
Solution BF1	24000-50-1	20 ml
Solution BF2	24000-50-2	6 ml
Solution BF3	24000-50-3	11 ml
Solution BF4	24000-50-4	50 ml
Solution BF5	24000-50-5	2 x 18 ml
Solution BF6	24000-50-6	2 x 18 ml
Solution BF7	24000-50-7	5.5 ml
Spin Filters	24000-50-SF	50
2 ml Collection Tubes	24000-50-T	250

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 technical support) or at www.mobio.com. Reagents labeled flammable should be kept away from open flames and sparks.

WARNING
Solutions BF5 and BF6 are flammable.

Wear gloves when handling PowerBiofilm® Bead Tubes.

Important Notes Before Starting

Solution BF1 must be warmed at 55°C for 5-10 minutes to dissolve precipitates prior to each use. Solution BF1 should be used while still warm.

Solution BF4 may precipitate over time. If precipitation occurs, warm at 55°C for 5-10 minutes. Solution BF4 can be used while still warm.

Shake to mix Solution BF5 before use.

Use only PowerBiofilm® Bead Tubes with this kit.

Experienced User Protocol

Please wear gloves at all times

Warm Solution BF1 prior to use at 55°C for 5-10 minutes. Use Solution BF1 while still warm. Check Solution BF4 and warm at 55°C for 5-10 minutes if necessary. Solution BF4 can be used while still warm. Use only PowerBiofilm® Bead Tubes with this kit.

1. Weigh out **0.05 to 0.20 g** of biofilm material and place it into a 2 ml Collection Tube (provided). Centrifuge at 13,000 x *g* for 1 minute. Remove excess liquid using a pipette tip. For less saturated samples (ex. microbial mats) add directly to the PowerBiofilm® Bead Tube **(For information on selecting the right amount of material to add, see Amount of Starting Material in the Hints and Troubleshooting Guide before continuing).**

   Note: Use only PowerBiofilm® Bead Tubes with this kit.
2. Resuspend the biofilm material in **350 µl of Solution BF1** and transfer to the PowerBiofilm® Bead Tube. For less saturated samples, add **350 µl of Solution BF1** directly to the PowerBiofilm® Bead Tube already containing the biofilm material.

   Note: Solution BF1 must be warmed to dissolve precipitates prior to use. Solution BF1should be used while still warm.
3. Add **100 µl of Solution BF2**. Vortex briefly to mix
4. Incubate the PowerBiofilm® Bead Tube at 65°C for 5 minutes.
5. Bead beat the sample following one of the methods described below (Bead Beater or Vortex Adapter).

a) PowerLyzer® 24 Homogenizer

1) Properly identify each PowerBiofilm® Bead Tube on both the cap and on the side.
   Note: Due to the high energies of the PowerLyzer® 24, the potential of marring the tops of the caps is possible, therefore, it is recommended to mark the side of the PowerBiofilm® Bead Tube, as well as the cap, to ensure proper sample identification.
2) Place Bead Tubes into the Tube Holder of the PowerLyzer® 24. The Bead Tubes must be balanced (evenly spaced) on the Tube Holder. Homogenize for 1 cycle at speed 3200 rpm for 30 seconds.
3) Centrifuge the tube at 13,000 x *g* for 1 minute. Transfer the supernatant to a new 2 ml Collection Tube (provided).
   Note: Expect approximately 325 - 400 µl of supernatant depending on sample material. If the volume falls below this range, use less starting material.

b) Vortex Adapter

1) Secure the PowerBiofilm® Bead Tube horizontally to a MO BIO Vortex Adapter and vortex at maximum speed for 10 minutes.
   Note: If you are using the 24 place Vortex Adapter for more than 12 preps, increase the time by 5 – 10 minutes.
2) Centrifuge the tube at 13,000 x *g* for 1 minute at room temperature. Transfer the supernatant to a clean 2 ml Collection Tube (provided).
   Note: Expect approximately 400 - 450 µl of supernatant depending on sample material. If the volume falls below this range, use less starting material.

6. Add **100 µl of Solution BF3** and vortex briefly to mix. Incubate at 4°C for 5 minutes.
   Note: Use 200 µl of Solution BF3 if the sample is known to contain excessive amounts of inhibitors or the supernatant is very darkly colored. See "DNA Does Not Amplify..." in the Hints and Troubleshooting Guide before continuing.
7. Centrifuge the tube at 13,000 x *g* for 1 minute at room temperature.
8. Avoiding the pellet, transfer the entire volume of supernatant to a clean 2 ml Collection Tube (provided).
   Note: Expect approximately 375 - 450 µl in volume depending on sample material.
9. Add **900 µl of Solution BF4** and vortex briefly to mix.
   Note: Check Solution BF4 for precipitation prior to use. Warm if necessary. Solution BF4 can be used while still warm.
10. 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 minimum of two loads for each sample processed are required. Depending on the sample and amount of BF3 used, up to three loads may be necessary.
11. Place the Spin Filter basket into a clean 2 ml Collection Tube (provided).
12. Shake to mix Solution BF5 before use. Add **650 µl of Solution BF5** and centrifuge at 13,000 x *g* for 1 minute at room temperature.
13. Discard the flow through and add **650 µl of Solution BF6** and centrifuge at 13,000 x *g* for 1 minute at room temperature.
14. Discard the flow through and centrifuge again at 13,000 x *g* for 2 minutes to remove residual wash.
15. Place the Spin Filter basket into a clean 2 ml Collection Tube (provided).
16. Add **100 µl of Solution BF7** to the center of the white filter membrane.
17. Centrifuge at 13,000 x *g* for 1 minute.
18. 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). Solution BF7 contains no EDTA.

Thank you for choosing the PowerBiofilm® DNA Isolation Kit!

Detailed Protocol

Please wear gloves at all times

Warm Solution BF1 prior to use at 55°C for 5-10 minutes. Use Solution BF1 while still warm. Check Solution BF4 and warm at 55°C for 5-10 minutes if necessary. Solution BF4 can be used while still warm. Use only PowerBiofilm® Bead Tubes with this kit.

1. Weigh out **0.05 to 0.20 g** of biofilm material and place it into a 2 ml Collection Tube (provided). Centrifuge at 13,000 x *g* for 1 minute. Remove excess liquid using a pipette tip. For less saturated samples (ex. microbial mats) add directly to the PowerBiofilm® Bead Tube **(For information on selecting the right amount of material to add, see Amount of Starting Material in the Hints and Troubleshooting Guide before continuing).**

   Note: Use only PowerBiofilm® Bead Tubes with this kit.

   What's happening: Biofilm samples will vary in their moisture content. It is important to remove residual liquid to prevent dilution of the lysis components which could result in reduced DNA yield. Some biofilm samples, such as microbial mats may be added directly to the PowerBiofilm® Bead Tube without an initial centrifugation step.

2. Resuspend the biofilm material in **350 µl of Solution BF1** and transfer to the PowerBiofilm® Bead Tube. For less saturated samples, add **350 µl of Solution BF1** directly to the PowerBiofilm® Bead Tube already containing the biofilm material.

   Note: Solution BF1 must be warmed to dissolve precipitates prior to use. Solution BF1 should be used while still warm.

   What's happening: Solution BF1 is a component of patented Inhibitor Removal Technology® (IRT). It is a strong lysing reagent that includes a detergent to help break cell walls and stabilizes and protects DNA from degradation. When cold, this solution will form a white precipitate in the bottle. Heating to 55°C will dissolve the components without harm. Solution BF1 can be used while it is still warm.

3. Add **100 µl of Solution BF2**. Vortex briefly to mix.

   What's happening: Solution BF2 contains a chaotropic agent that aids in lysis. BF2 also stabilizes and protects DNA integrity.

4. Incubate the PowerBiofilm® Bead Tube at 65°C for 5 minutes.

   What's happening: Lysis components are heat activated to aid in the breakdown of extracellular polymeric substances (EPS).

5. Bead beat the sample following one of the methods described below.

   a) PowerLyzer® 24 Homogenizer

   1) Properly identify each PowerBiofilm® Bead Tube on both the cap and on the side.

      Note: Due to the high energies of the PowerLyzer® 24, the potential of marring the tops of the caps is possible, therefore, it is recommended to mark the side of the PowerBiofilm® Bead Tube, as well as the cap, to ensure proper sample identification.

2) Place the PowerBiofilm® Bead Tubes into the Tube Holder of the PowerLyzer® 24. The Bead Tubes must be balanced (evenly spaced) on the Tube Holder. Homogenize at 3200 RPM for 30 seconds.

3) Centrifuge the tube at 13,000 x *g* for 1 minute. Transfer the supernatant to a new 2 ml Collection Tube (provided).

Note: Expect approximately 325 - 400 µl of supernatant depending on sample material. If the volume falls below this range, use less starting material.

b) Vortex Adapter

1) Secure the PowerBiofilm® Bead Tube horizontally to a MO BIO Vortex Adapter and vortex at maximum speed for 10 minutes.

Note: If you are using the 24 place Vortex Adapter for more than 12 preps, increase the time by 5 – 10 minutes.

2) Centrifuge the tube at 13,000 x *g* for 1 minute at room temperature. Transfer the supernatant to a clean 2 ml Collection Tube (provided).

Note: Expect approximately 400 - 450 µl of supernatant depending on sample material. . If the volume falls below this range, use less starting material.

What is happening: Dissolution of the biofilm matrix and lysis of microbial cells occurs using a combination of chemical (lysis buffers) and mechanical (bead beating) lysis conditions. The resulting 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 contaminating non-DNA organic and inorganic matter that may reduce the DNA purity and inhibit downstream applications.

6. Add **100 µl of Solution BF3** and vortex briefly to mix. Incubate at 4°C for 5 minutes.

Note: Use 200 µl of Solution BF3 if the sample is known to contain excessive amounts of inhibitors or the supernatant is very darkly colored. See “DNA Does Not Amplify...” in the Hints and Troubleshooting Guide before continuing.

What’s happening: Solution BF3 is a component of patented Inhibitor Removal Technology® (IRT) and is a second reagent to remove additional non-DNA organic and inorganic material including humic acid, cell debris, polyphenolics, polysaccharides and proteins. The system works by using changes in pH to precipitate insoluble large macromolecules. The nucleic acids do not precipitate and are cleared of inhibitors. It is important to remove contaminating organic and inorganic matter that may reduce the DNA purity and inhibit downstream DNA applications.

7. Centrifuge the tube at 13,000 x *g* for 1 minute at room temperature.

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

Note: Expect approximately 375 - 450 µl in volume depending on sample material and bead beating method.

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.

9. Add **900 µl of Solution BF4** and vortex briefly to mix.

   Note: Check Solution BF4 for precipitation prior to use. Warm if necessary. Solution BF4 can be used while still warm.

   What's happening: Solution BF4 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.

10. 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 minimum of two loads for each sample processed are required. Depending on the sample and amount of BF3 used, up to three loads may be necessary.

    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.

11. 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 BF4, 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.

12. Shake to mix Solution BF5 before use. Add **650 µl of Solution BF5** and centrifuge at 13,000 x *g* for 1 minute at room temperature.

    What's happening: Solution BF5 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.

13. Discard the flow through and add **650 µl of Solution BF6** and centrifuge at 13,000 x *g* for 1 minute at room temperature.

    What's happening: Solution BF6 ensures complete removal of Solution BF5 which will result in higher DNA purity and yield.

14. 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 BF6. It is critical to remove all traces of wash solution because the ethanol in Solution BF6 can interfere with many downstream DNA applications such as PCR, restriction digests, and gel electrophoresis.

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

16. Add **100 µl of Solution BF7** to the center of the white filter membrane.

    Note: A reduction in yield will occur if less than 50 µl of Solution BF7 is used for elution. For the highest yields elute in the recommended 100 µl volume of Solution BF7.

    What's happening: Placing Solution BF7 (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 BF7 passes through the silica membrane, DNA

that was bound in the presence of high salt is selectively released by Solution BF7 (10 mM Tris) which lacks salt.

Alternatively, sterile DNA-Free PCR Grade Water may be used for DNA elution from the silica Spin Filter membrane at this step. Solution BF7 contains no EDTA. If DNA degradation is a concern, sterile TE may also be used instead of BF7 for elution of DNA from the Spin Filter.

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

18. 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). Solution BF7 contains no EDTA.

Thank you for choosing the PowerBiofilm® DNA Isolation Kit!

Vacuum Protocol using the PowerVac™ Manifold

Please wear gloves at all times

For this protocol you will need a PowerVac™ Manifold Mini System (MO BIO Catalog# 11992).
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 9) 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 BF5. Add 650 µl of **Solution BF5** to each Spin Filter. Open the Luer-Lok® stopcock and apply a vacuum until **Solution BF5** 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 BF6** to each Spin Filter. Open the Luer-Lok® stopcock and apply a vacuum until **Solution BF6** 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 BF7** to the center of the white filter membrane. Alternatively, sterile DNA-Free PCR Grade Water (MO BIO Catalog# 17000-10) may be used for elution from the silica **Spin Filter** membrane at this step.

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 PowerBiofilm® DNA Isolation Kit!

Hints and Troubleshooting Guide

Amount of Starting Material
This kit is designed to process 0.05 to 0.2 g of biofilm or microbial mat material. The actual amount to use will depend on the type of biofilm and microbial density. If supernatant amounts fall under the range provided in Step 5 of the protocol then DNA yields will not be optimal and less sample material should be used for processing. A recommended starting amount is 0.1 - 0.15 g. For examples of expected yields, see the table under "Expected DNA Yields" below.

Forgetting to Warm Solution BF1
If BF1 is not warmed prior to use, continue with the protocol. You will still obtain DNA, but the yields may not be optimal.

Expected DNA Yields
DNA yields will vary depending on the type of biofilm. Yields may also vary between samples of the same biofilm due to their structure. Examples of expected yields are provided as a reference. Due to the diversity of biofilm sample types, yields may fall outside of the examples provided.

Biofilm Type	Sample Amount (g)	DNA Yield (ng/µl)
Sink Pipe	0.20	94 -198
Lagoon Rocks	0.15	100 -150
Phototrophic Biofilm (Microbial Mat)	0.15 0.10 0.05	54 -130 70 -76 37 - 50
Stream Rocks	<0.05	4 - 11
Bioreactor	0.25	56 - 130
Button Thrombolites (Microbial Mat)	0.25	1 - 15
Gypsum Crust	0.20	15 - 28

Low or No DNA Yield
Yields may be significantly reduced if too much starting material is used, samples are bead beat for too long or the PowerBiofilm® Bead Tubes are not used. To avoid sample loss:

- Do not use more sample than the specified range (0.05 – 0.20 g).
- Reduce time when using a bead beater for homogenization. For most biofilms 30 seconds is optimal. Tougher samples, such as microbial mats, may require longer bead beating times and should be user determined. When using the Vortex Adapter, no time adjustment is necessary unless you are processing more than 12 preps on the 24 place Vortex Adapter. In this case run for an additional 5 – 10 minutes.
- Do not use any other bead tube except the ones provided in this kit. The PowerBiofilm® Bead Tubes have been specially designed for use in this kit.

Hints and Troubleshooting Guide cont.

DNA Does Not Amplify or Has Reduced Amplification Efficiency
Biofilms with high concentrations of humic substances and other contaminants may yield DNA with some inhibitor carryover, which can prevent target sequences from amplifying in PCR. Under such circumstances, the template DNA can be diluted one to several fold for successful PCR. For additional preps of the same or similar sample type, use 200 µl of BF3 at step 6 to eliminate inhibitor carry over.

DNA Floats Out of Well When Loaded On a Gel
Residual BF6 Wash Buffer may be in the final sample. To ensure complete drying of the membrane after BF6, 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 BF6. (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.

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. Ethanol precipitation with resuspension into a smaller volume to concentrate the DNA may help to improve the $A_{260/230}$ ratio.

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 (Solution BF7).

Storing DNA
DNA is eluted in Solution BF7 (10mM Tris) 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. 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).

Spin Filter Column Becomes Clogged When Using the Vacuum Manifold Protocol
Some sample lysates may be too viscous to move through the spin filter column under vacuum. If this occurs switch to the original protocol which uses centrifugation.

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

Technical Support:
Phone MO BIO Laboratories, Inc. Toll Free 800-606-6246, or 760-929-9911
Email: technical@mobio.com
Fax: 760-929-0109
Mail: MO BIO Laboratories, Inc, 2746 Loker Ave West, Carlsbad, CA 92010

Ordering Information:

Direct: Phone MO BIO Laboratories, Inc. Toll Free 800-606-6246, or 760-929-9911
Email: orders@mobio.com
Fax: 760-929-0109
Mail: MO BIO Laboratories, Inc, 2746 Loker Ave West, Carlsbad, CA 92010

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
PowerBiofilm® RNA Isolation Kit	25000-50	50 preps
PowerPlant® Pro DNA Isolation Kit	13400-50	50 preps
PowerPlant® RNA Isolation Kit	13500-50	50 preps
PowerPlant® RNA Isolation Kit with DNase	13550-50	50 preps
Vortex Genie® 2 Vortex	13111-V 13111-V-220	1 unit (120V) 1 unit (220V)
Vortex Adapter for Vortex Genie® 2	13000-V1-24	Holds 24 (2 ml) Tubes
PowerVac™ Mini System 11992	11992	1 unit + 20 adapters
PowerLyzer® 24 Bench Top Bead- Based Homogenizer	13155	1 unit
UltraClean® PCR Clean-Up Kit	12500-50 12500-100 12500-250	50 preps 100 preps 250 preps