PowerBiofilm™ DNA Isolation Kit

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

Catalog No.	Quantity
24000-50	50 Preps

Instruction Manual

Inhibitor Removal Technology® (IRT) is a registered trademark of MO BIO Laboratories, Inc. and is covered by US patent protection as well as international patents pending.

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Version: 07092010

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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.

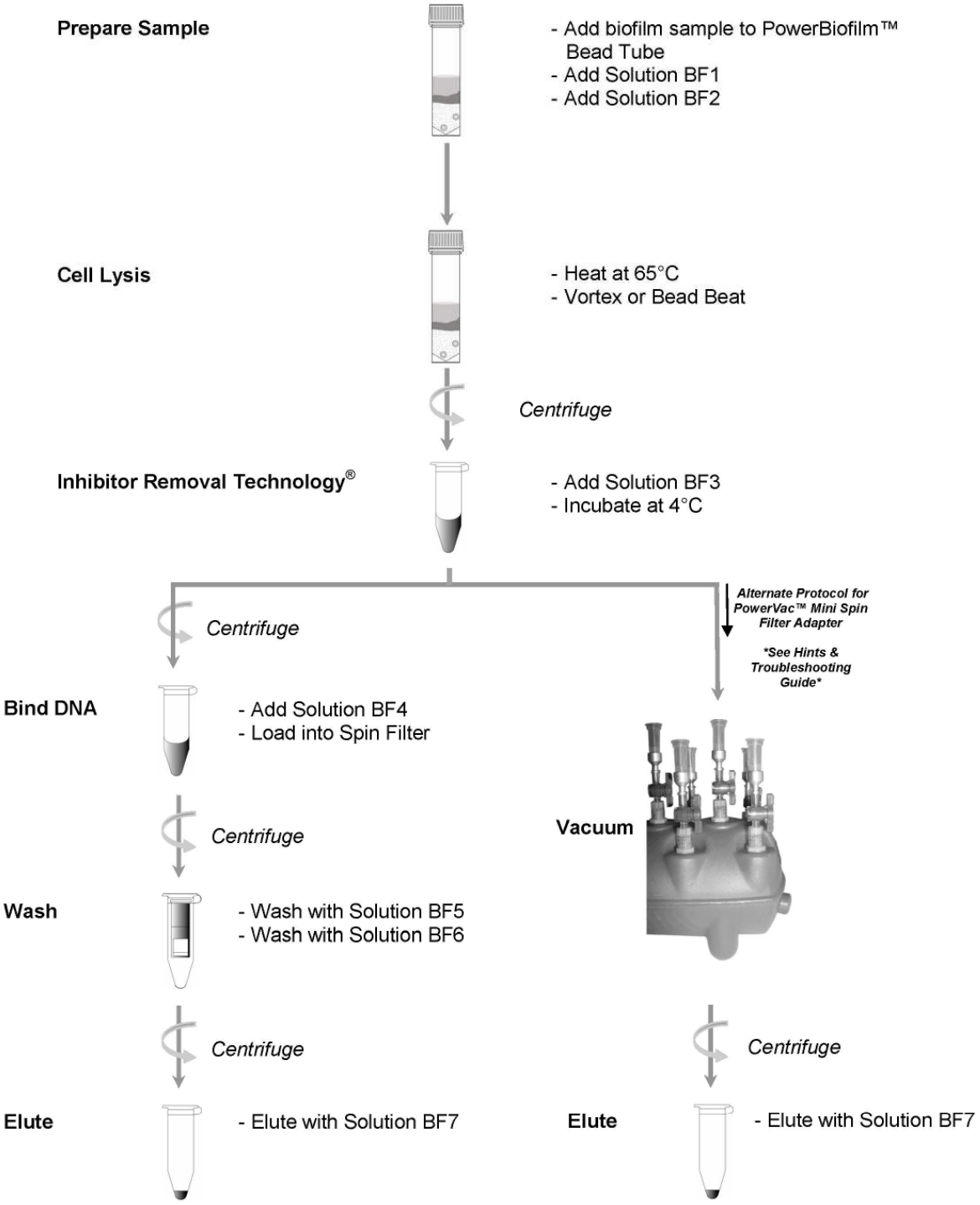
High Throughput Options

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 13000-V1-24	Holds 12 (2 ml) Tubes 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)	17000-1 17000-5 17000-10 17000-11	1 ml 5 x 1 ml 10 x 1 ml 10 ml bottle
PowerBiofilm™ RNA Isolation Kit	25000-20 25000-50	20 Preps 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 or 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 (Catalog# 13000-V1) 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, Catalog# 13000-V1 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:

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

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DNA Purification and Gel Extraction	Catalog No.	Quantity
PowerClean® DNA Clean-Up Kit	12877-50	50 preps
UltraClean® 15 DNA Purification Kit	12100-300	300 preps
UltraClean® PCR Clean-Up Kit	12500-50 12500-100 12500-250	50 preps 100 preps 250 preps
UltraClean®-htp 96 Well PCR Clean-Up Kit	12596-4 12596-12	4 x 96 preps 12 x 96 preps
UltraClean® GelSpin® DNA Extraction Kit	12400-50 12400-100 12400-250	50 preps 100 preps 250 preps
Plasmid DNA Isolation	**Catalog No.**	**Quantity**
UltraClean® 6 Minute Mini Plasmid Prep Kit	12300-50 12300-100 12300-250	50 preps 100 preps 250 preps
UltraClean® Standard Mini Plasmid Prep Kit	12301-50 12301-100 12301-250	50 preps 100 preps 250 preps
UltraClean®-htp 96 Well Plasmid Prep Kit	12396-4 12396-12	4 x 96 preps 12 x 96 preps
UltraClean® Midi Plasmid Prep Kit	12700-20 12700-50	20 preps 50 preps
UltraClean® Maxi Plasmid Prep Kit	12600-10 12600-20	10 preps 20 preps
UltraClean® Endotoxin-Free Mini Plasmid Prep Kit	12311-100 12311-250	100 preps 250 preps
UltraClean® Endotoxin-Free Midi Plasmid Prep Kit	12711-10	10 preps
UltraClean® Endotoxin-Free Maxi Plasmid Prep Kit	12611-10	10 preps
UltraClean® Endotoxin Removal Kit	12615	1 kit
UltraClean® Endotoxin-Free Ethanol Precipitation Kit	12616	1 kit
UltraClean® Endotoxin Removal Reagent	12625-25	25 ml
Endotoxin-Free Sodium Chloride	12626-15	15 ml
Endotoxin-Free Centrifuge Tubes	12617-100 12618-50 12619-25	100 each/2 ml tubes 50 each/15 ml tubes 25 each/50 ml tubes
RNA Isolation	**Catalog No.**	**Quantity**
PowerBiofilm™ RNA Isolation Kit	25000-50	50 preps
LifeGuard™ Soil Stabilization Solution	12868-10 12868-100 12868-1000 12868-7500	10 ml 100 ml 1 L 7.5 L
On-Spin Column DNase I Kit (RNase-Free)	15100-50	50 preps
BiOstic® Stabilized Blood RNA Isolation Kit	12231-20 12231-50 12231-100	20 preps 50 preps 100 preps
BiOstic® Blood Total RNA Isolation Kit	12230-20 12230-50	20 preps 50 preps
RNA PowerSoil® DNA Elution Accessory Kit	12867-25	25 preps
RNA PowerSoil® Total RNA Isolation Kit	12866-25	25 preps
UltraClean® Microbial RNA Isolation Kit	15800-50 15800-250	50 preps 250 preps

RNA Isolation …*Continued*	Catalog No.	Quantity
UltraClean® Tissue & Cells RNA Isolation Kit	15000-50 15000-250	50 preps 250 preps
UltraClean® Plant RNA Isolation Kit	13300-20 13300-50	20 preps 50 preps
Genomic DNA Isolation	**Catalog No.**	**Quantity**
PowerBiofilm™ DNA Isolation Kit	24000-50	50 preps
PowerFood™ Microbial DNA Isolation Kit	21000-50 21000-100	50 preps 100 preps
BiOstic® Bacteremia DNA Isolation Kit	12240-50	50 preps
BiOstic® FFPE Tissue DNA Isolation Kit	12250-50	50 preps
BiOstic® Paraffin Removal Reagent	12251-50	2 x 25 ml
PowerMax® Soil DNA Isolation Kit	12988-10	10 preps
PowerSoil® DNA Isolation Kit	12888-50 12888-100	50 preps 100 preps
PowerSoil®-htp 96 Well Soil DNA Isolation Kit	12955-4 12955-12	4 x 96 preps 12 x 96 preps
UltraClean® Soil DNA Isolation Kit	12800-50 12800-100	50 preps 100 preps
UltraClean®-htp 96 Well Soil DNA Isolation Kit	12896-4 12896-12	4 x 96 preps 12 x 96 preps
UltraClean® Mega Soil DNA Isolation Kit	12900-10	10 preps
PowerClean® DNA Clean-Up Kit	12877-50	50 preps
UltraClean® Fecal DNA Isolation Kit	12811-50 12811-100	50 preps 100 preps
PowerMicrobial® Midi DNA Isolation Kit	12225-25	25 preps
PowerMicrobial® Maxi DNA Isolation Kit	12226-25	25 preps
UltraClean® Microbial DNA Isolation Kit	12224-50 12224-250	50 preps 250 preps
UltraClean®-htp 96 Well Microbial DNA Isolation Kit	10196-4 10196-12	4 x 96 preps 12 x 96 preps
PowerPlant® DNA Isolation Kit	13200-50 13200-100	50 preps 100 preps
UltraClean® Plant DNA Isolation Kit	13000-50 13000-250	50 preps 250 preps
UltraClean®-htp 96 Well Plant DNA Isolation Kit	13096-4 13096-12	4 x 96 preps 12 x 96 preps
UltraClean® Tissue & Cells DNA Isolation Kit	12334-50 12334-250	50 preps 250 preps
UltraClean®-htp 96 Well Tissue DNA Isolation Kit	12996-4 12996-12	4 x 96 preps 12 x 96 preps
UltraClean® Blood DNA Isolation Kit (Non-Spin)	12000-100	100 preps
UltraClean® Blood DNA Isolation Kit (Processes 1,000 ml of Blood)	12000-1000	1 kit
UltraClean® Blood DNA Isolation Kit Plus RNase (Processes 1,000 ml of Blood)	12002-1000	1 kit

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Genomic DNA Isolation *...Continued*	Catalog No.	Quantity
UltraClean® BloodSpin® DNA Isolation Kit	12200-50 12200-250	50 preps 250 preps
UltraClean®-htp 96 Well BloodSpin® DNA Isolation Kit	12296-4 12296-12	4 x 96 preps 12 x 96 preps
UltraClean® Forensic DNA Isolation Kit	14000-10 14000-20	10 isolations 20 isolations
PowerWater® DNA Isolation Kit	 14900-50-NF 14900-50-22 14900-50-45 14900-100-NF 14900-100-22 14900-100-45	50 preps (No filters) (0.22 µm) (0.45 µm) 100 preps (No filters) (0.22 µm) (0.45 µm)
RapidWater™ DNA Isolation Kit	 14810-50-NF 14810-50-22 14810-50-45 14810-100-NF 14810-100-22 14810-100-45	50 preps (No filters) (0.22 µm) (0.45 µm) 100 preps (No filters) (0.22 µm) (0.45 µm)
UltraClean® Water DNA Isolation Kit (0.45µm filters)	14800-10 14800-25	10 preps 25 preps
UltraClean® Water DNA Isolation Kit (0.22 µm filters)	14880-10 14880-25	10 preps 25 preps
UltraClean® Water DNA Isolation Kit (No filters)	14800-10-NF 14800-25-NF	10 preps 25 preps
Microbiological Culture Media	**Catalog No.**	**Quantity**
TB DRY® Powder Growth Media	12105-05 12105-1 12105-5	500 g 1 kg 5 kg
LB Broth Powder Growth Media, pH 7	12106-05 12106-1 12106-5	500 g 1 kg 5 kg
LB Agar Powder Growth Media, pH 7	12107-05 12107-1 12107-5	500 g 1 kg 5 kg
LB Broth (Lennox) Powder Growth Media, pH 7	12108-05 12108-1 12108-5	500 g 1 kg 5 kg
LB Agar (Lennox) Powder Growth Media, pH 7	12109-05 12109-1 12109-5	500 g 1 kg 5 kg
Soybean-Casein Digest Medium (TSB), USP	12114-05 12114-1 12114-5	500 g 1 kg 5 kg
Soybean-Casein Digest Agar Medium (TSA), USP	12115-05 12115-1 12115-5	500 g 1 kg 5 kg
Yeast Extract	12110-05 12110-1 12110-5	500 g 1 kg 5 kg
Tryptone	12111-05 12111-1 12111-5	500 g 1 kg 5 kg
Agar, Bacteriological Grade	12112-05 12112-1 12112-5	500 g 1 kg 5 kg

Other Reagents and Lab Accessories	Catalog No.	Quantity
20 bp DNA Ladder	17020-40	40 µg
100 bp DNA Ladder	17100-40	40 µg
1 kb DNA Ladder	17200-100	100 µg
UltraClean® Agarose, Molecular Biology Grade	15003-50 15003-100 15003-500 15003-1000	50 g 100 g 500 g 1 kg
UltraClean® MS-8 Agarose	15515-50 15515-100 15515-500	50 g 100 g 500 g
UltraClean® Forensic Agarose	15505-50 15505-100 15505-500	50 g 100 g 500 g
UltraClean® Low Melt Agarose	15005-50 15005-100 15005-500	50 g 100 g 500 g
UltraClean® Low Melt Sieve Agarose	15004-50 15004-100 15004-500	50 g 100 g 500 g
Ethidium Bromide Solution	15006-1 15006-10	1 ml 10 ml
Ethidium Bromide Destaining Tea Bags	15007-25	25 bags
Bromophenol Blue Gel Loading Buffer	15008-1 15008-5	1 ml 5 x 1 ml
Bromophenol Blue/Xylene Cyanol Gel Loading Buffer	15009-1 15009-5	1 ml 5 x 1 ml
TAE Buffer, 50X (Tris-acetate-EDTA)	15001-100 15001-500 15001-1000	100 ml 500 ml 1 liter
TBE Buffer, 10X (Tris-borate-EDTA)	15002-100 15002-500 15002-1000	100 ml 500 ml 1 liter
RNase-Free Gloves	1555-XS 1555-S 1555-M 1555-L	bag of 100 bag of 100 bag of 100 bag of 100
UltraClean® Lab Cleaner	12095-250 12095-500 12095-1000	250 ml squeeze bottle 500 ml spray bottle 1 liter bottle
KAPA PROBE FAST qPCR Kits	51220-100 51220-500 51220-1000	100 reactions 500 reactions 1000 reactions
KAPA SYBR® FAST Universal 2X qPCR Master Mix	51230-100 51230-500 51230-1000	100 reactions 500 reactions 1000 reactions
KAPA2G Robust HotStart ReadyMix	51240-100 51240-500	100 reactions 500 reactions

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Other Reagents and Lab Accessories…*Continued*	Catalog No.	Quantity
KAPA HiFi HotStart ReadyMix	51250-100 51250-500	100 reactions 500 reactions
KAPA2G FAST HotStart DNA Polymerase with dNTPs	51260-100 51260-250 51260-500	100 reactions 250 reactions 500 reactions
KAPA2G FAST HotStart ReadyMix	51270-100 51270-500	100 reactions 500 reactions
KAPA Long Range HotStart DNA Polymerase with dNTPs	51280-100 51280-250 51280-500	100 reactions 250 reactions 500 reactions
KAPA Taq Polymerase ReadyMix	51290-250	250 reactions
OmniTaq™ DNA Polymerase Enzyme	1224-250	250 reactions (10 U/µl)
OmniTaq™ DNA Polymerase 2x Master Mix	1226-250	250 reactions (5 x 1.25 ml/tube)
Omni KlenTaq™ DNA Polymerase Enzyme	1225-250	250 reactions (25 U/µl)
Omni KlenTaq™ DNA Polymerase 2x Master Mix	1227-250	250 reactions (5 x 1.25 ml/tube)
DNase (RNase-Free)	15600-5 15601-100	5 mg 2500 units
Proteinase K	1223-100 1222-2	100 mg 2 ml (20 mg/ml)
Ribonuclease A (25 mg/ml)	1202-1 1202-5	1 ml 5 ml
PCR Water	17000-1 17000-5 17000-10 17000-11	1 ml 5 x 1 ml 10 x 1 ml 10 ml bottle
Molecular Biology Grade Water	17012-200 17012-5200	200 ml 5 x 200 ml
DEPC Treated Water	17011-200 17011-5200	200 ml 5 x 200 ml
Endotoxin-Free Water	17013-10 17013-50 17013-100 17013-500	10 ml 50 ml 100 ml 500 ml
Instrumentation and Accessories	**Catalog No.**	**Quantity**
PowerLyzer™ 24 Bench Top Bead-Based Homogenizer (110/220V)	13155	1 unit
PowerLyzer™ Tube Holder	13156	1 unit
PowerLyzer™ Tube Holder Stand	13157	1 unit
PowerVac™ Mini System	11992	1 unit + 20 adapters
PowerVac™ Manifold	11991	1 unit
PowerVac™ Mini Spin Filter Adapters	11992-10 11992-20	10 adapters 20 adapters
Ceramic Bead Tubes, 1.4 mm	13113-50	50 bead tubes
Ceramic Bead Tubes, 2.8 mm	13114-50	50 bead tubes

Instrumentation and Accessories…*Continued*	Catalog No.	Quantity
Glass Bead Tubes, 0.5 mm	13116-50	50 bead tubes
Glass Bead Tubes, 0.1 mm	13118-50	50 bead tubes
Metal Bead Tubes, 2.38 mm	13117-50	50 bead tubes
2.0 ml Tough Tubes with Cap	13119-500 13119-1000	500 1000
Carbide Bead Tubes, 0.25 mm	13121-50	50 x 0.5 ml tubes
Garnet Bead Tubes, 0.15 mm	13122-50	50 x 0.5 ml tubes
Garnet Bead Tubes, 0.70 mm	13123-50	50 x 2 ml tubes
Garnet + ¼ Ceramic 15 ml Bead Tubes, 0.70 mm	13134-50	50 tubes
Garnet + ¼ Ceramic 50 ml Bead Tubes, 0.70 mm `	13144-10 13144-50 13144-100 13144-500	10 tubes 50 tubes 100 tubes 500 tubes
Glass 15 ml Bead Tubes, 0.1 mm	13135-50	50 tubes
Glass 50 ml Bead Tubes, 0.1 mm	13145-10 13145-50 13145-100 13145-500	10 tubes 50 tubes 100 tubes 500 tubes
Glass 15 ml Bead Tubes, 1.0 mm	13136-50	50 tubes
Ceramic 15 ml Bead Tubes, 1.4 mm	13137-50	50 tubes
Ceramic 50 ml Bead Tubes, 1.4 mm	13147-10 13147-50	10 tubes 50 tubes
Metal 50 ml Bead Tubes, 2.38 mm	13149-10 13149-50	10 tubes 50 tubes
PowerMix 15 ml Bead Tubes	13138-50	50 tubes
PowerMix 50 ml Bead Tubes	13148-10 13148-50	10 tubes 50 tubes
2 ml Collection Tubes	1200-100-T 1200-150-T 1200-250-T	100 tubes 150 tubes 250 tubes
2 ml Screw Cap Tubes	12800-200-E	200 tubes & caps
15 ml Collection Tubes	12700-T	25 tubes
50 ml Centrifuge Tubes	12600-T	25 tubes
Spin Filters (in 1.9 ml tubes)	1200-50-SF 1200-100-SF 1200-250-SF	50 filters 100 filters 250 filters
Endotoxin-Free Centrifuge Tubes	12617-100 12618-50 12619-25	100 each/2 ml tubes 50 each/15 ml tubes 25 each/50 ml tubes
15 ml Midi Spin Filters	12700-SF	25 spin filters

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Instrumentation and Accessories… *Continued*	Catalog No.	Quantity
Vortex-Genie® 2 Vortex (120V)	13111-V	1 unit
Vortex-Genie® 2 Vortex (220V)	13111-V-220	1 unit
Vortex Adapter, holds 12 (1.5-2.0 ml) tubes	13000-V1	1 unit
Vortex Adapter, holds 6 (5 ml) tubes	13000-V1-5	1 unit
Vortex Adapter, holds 4 (15 ml) tubes	13000-V1-15	1 unit
Vortex Adapter, holds 2 (50 ml) tubes	13000-V1-50	1 unit
Vortex Adapter, holds 24 (1.5-2.0 ml) tubes	13000-V1-24	1 unit
BagMixer® 400 VW	23112	1 unit
BagFilter® 400 P	23113-500	Box of 500
BagPage® 400	23114-500	Box of 500
Whirl-Pak® Collection Bag, Small (532 ml)	23210-500	500 bags
Whirl-Pak® Collection Bag, Medium (1,627 ml)	23211-500	500 bags
Whirl-Pak® Collection Bag, Large (3,637 ml)	23212-250	250 bags
Whirl-Pak® Stand up Bag, Small (118 ml)	23220-500	500 bags
Whirl-Pak® Stand up Bag, Medium (532 ml)	23221-500	500 bags
Whirl-Pak® Stand up Bag, Large (1,242 ml)	23222-250	250 bags
Whirl-Pak® Stand up Bag, Extra-Large (2,041 ml)	23223-250	250 bags
Whirl-Pak® Scoop Bag, 60 ml	23240-50	50 bags
Anti-Static Funnels, Micro	23301-96	Pack of 96
Anti-Static Funnels, Small	23302-50	Pack of 50
Anti-Static Funnels, Medium	23303-50	Pack of 50
Anti-Static Funnels, Large	23304-20	Pack of 20

Instrumentation and Accessories… *Continued*	Catalog No.	Quantity
Mini Horizontal Gel System	16001	1 each
Mini Horizontal Gel Caster, 3 place	16003	1 each
Mini Horizontal Gel Tray	16004	1 each
Polycarbonate Single-sided Comb	16005 16006 16007 16008	1 mm x 3 well 1 mm x 8 well 1 mm x 10 well 1 mm x 12 well
Polycarbonate Dual-sided Comb	16013 16014 16015 16016	1 mm x 8 well/16 well 1 mm x 10 well/14 well 2 mm x 8 well/16 well 2 mm x 10 well/14 well
Teflon Single-sided Comb	16009 16010 16011 16012	1 mm x 3 well 1 mm x 8 well 1 mm x 10 well 1 mm x 12 well
Teflon Dual-sided Comb	16017 16018 16019 16020	1 mm x 8 well/16 well 1 mm x 10 well/14 well 2 mm x 8 well/16 well 2 mm x 10 well/14 well
Power Supply w/Timer, (120V)	16023	1 unit
Power Supply w/Timer, (220V)	16023-220	1 unit
96 Well Plate Shaker (120V)	11996	1 unit
96 Well Plate Shaker (220V)	11996-220	1 unit
Plate Adapter Set	11999	1 set
Vacuum Pump (120V)	11998	1 unit
Vacuum Pump (220V)	11998-220	1 unit
UltraVac™ Manifold	11997	1 unit