



# PowerLyzer<sup>®</sup> UltraClean<sup>®</sup> Microbial DNA Isolation Kit

| Catalog No. | Quantity |
|-------------|----------|
| 12255-50    | 50 Preps |

## *Instruction Manual*

New protocol instruction: *Shake Solution MD3 to mix before using to ensure consistent results.*

### **PowerLyzer<sup>®</sup> Products**

**PowerLyzer<sup>®</sup> DNA and RNA Isolation kits combine either, glass, ceramic or metal bead tubes with MO BIO's trusted chemistry as an alternative to our traditional kits utilizing Garnet Bead Tubes for sample homogenization. These materials are much harder than garnet and when used with the PowerLyzer<sup>®</sup> 24 Bench Top Bead-Based Homogenizer or other bead beater, offer more robust mechanical shaking. Optimal lysis conditions will vary with each sample type. By providing more versatility for lysis, MO BIO's PowerLyzer<sup>®</sup> kits are a powerful tool in obtaining higher yields of DNA or RNA from some spores, yeast and fungi as well as some Gram positive strains of bacteria from a wide range of sample types. All PowerLyzer<sup>™</sup> DNA and RNA Isolation kits contain either glass or ceramic beads and are compatible with the PowerLyzer<sup>®</sup> 24 instrument.**



Please recycle

Version: 11142013

Technical Information: Toll free 1-800-606-6246, or 1-760-929-9911 Email: [technical@mobio.com](mailto:technical@mobio.com) Website: [www.mobio.com](http://www.mobio.com)



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## Introduction

The PowerLyzer<sup>®</sup> UltraClean<sup>®</sup> Microbial DNA Isolation Kit differs from the original UltraClean<sup>®</sup> kit. This kit contains 0.5 ml bead tubes with 0.1 mm glass beads that are optimized for quick and efficient cell lysis using robust bead based homogenizers like the PowerLyzer<sup>®</sup> 24 as well as Fast Prep<sup>®</sup> and Precellys<sup>®</sup> instruments. The PowerLyzer<sup>®</sup> UltraClean<sup>®</sup> Microbial DNA Isolation Kit is designed to isolate high-quality genomic DNA from microorganisms in about half the time required by traditional homogenization and vortex methods and enables up to 24 samples to be homogenized simultaneously. A variety of microorganisms in pure cultures and from plates, including bacterial and fungal spores, have been tested successfully with this kit.

## Protocol Overview

Microbial cells, resuspended in bead solution are added to a bead beating tube containing 0.1 mm glass beads, followed by lysis solution. The principal is to lyse the microorganisms by a combination of heat, detergent, and mechanical force using the PowerLyzer<sup>®</sup> 24 bead homogenizer or a specially designed MO BIO Vortex Adapter on a standard vortex. From the lysed cells, the released DNA is bound to a silica Spin Filter. The filter is washed, and the DNA is recovered in certified DNA-free Tris buffer.

## Optimized for complete homogenization of any sample with the



**PowerLyzer<sup>®</sup> 24**  
**Bench Top Bead-Based Homogenizer**  
**Catalog#13155**  
**([www.mobio.com/powerlyzer](http://www.mobio.com/powerlyzer))**

The PowerLyzer<sup>®</sup> UltraClean<sup>®</sup> Microbial DNA Isolation Kit comes with 0.1mm Glass MicroBead Tubes for DNA extraction on high powered bead beating instruments or the vortex. Using the PowerLyzer<sup>®</sup> 24, microbial samples are lysed in 5 minutes at 2000 RPM. The instrument's velocity and proprietary motion combine to provide the fastest homogenization time possible, minimizing the time spent processing samples. For species that are difficult to lyse, such as some fungi or spores, faster settings may be employed (up to 2800 RPM for DNA isolation). Also heating at 65°C before homogenization may be used to enhance lysis. The Glass MicroBead Tubes provided may also be used on the vortex similarly to the Garnet Bead Tubes provided in the original UltraClean<sup>®</sup> Microbial DNA Isolation Kit (MO BIO Catalog #12224).



## Using the PowerLyzer® UltraClean® Microbial 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 less cycle numbers 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 microorganisms you begin with the settings specified in this manual.

| PowerLyzer 24 | Fastprep 24 m/s | Precellys 24 |
|---------------|-----------------|--------------|
| 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.*



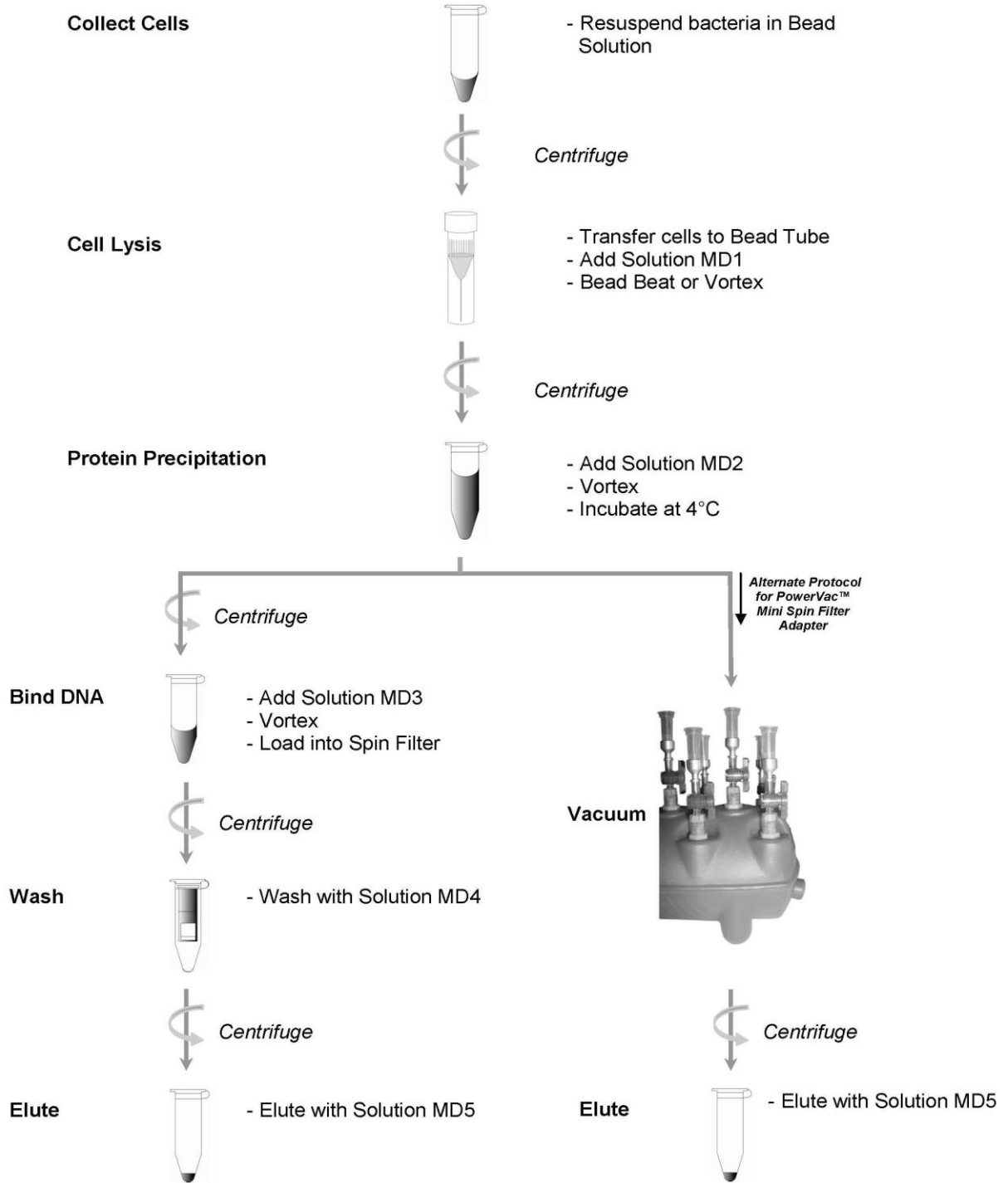
### High Throughput Options

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. The UltraClean®-htp 96 Well Microbial DNA Isolation Kit is available for processing up to 2 x 96 samples using a centrifuge capable of spinning two 96 Well Blocks stacked (13 cm x 8 cm x 5.5 cm) at 2500 x *g*. For 96 well homogenization of bacteria, MO BIO offers the 96 Well Plate Shaker and Plate Adapter Set (MO BIO Catalog# 11996 & 11999, respectively.)

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

| Other Related Products                              | Catalog No. | Quantity             |
|---|-------------|----------------------|
| UltraClean® Microbial RNA Isolation Kit             | 15800-50    | 50 preps             |
| UltraClean® PCR Clean-Up Kit                        | 12500-50    | 50 preps             |
|   | 12500-100   | 100 preps            |
|   | 12500-250   | 250 preps            |
| UltraClean®-htp 96 Well Microbial DNA Isolation Kit | 10196-4     | 4 x 96 preps         |
|   | 10196-12    | 12 x 96 preps        |
| PowerVac™ Manifold                                  | 11991       | 1 manifold           |
| PowerVac™ Mini System                               | 11992       | 1 unit + 20 adapters |
| PowerVac™ Mini Spin Filter Adapters                 | 11992-10    | 10 adapters          |
|   | 11992-20    | 20 adapters          |
| PowerLyzer® Tube Holder                             | 13156       | 1 unit               |
| PowerLyzer® Tube Holder Stand                       | 13157       | 1 unit               |

## PowerLyzer® UltraClean® Microbial DNA Isolation Kit





## Equipment Required

PowerLyzer<sup>®</sup> 24 or other bead homogenizer  
Microcentrifuge (10,000 x g)  
Pipettor (50 µl – 200 µl, 100 µl – 1000 µl)  
Vortex-Genie<sup>®</sup> 2 Vortex (MO BIO Catalog# 13111-V or 13111-V-220)  
Vortex Adapter (MO BIO Catalog# 13000-V1-24)

## Reagents Required but not Included

100% ethanol (for the PowerVac<sup>™</sup> Manifold protocol only)

## Kit Contents

| Kit Catalog # 12255-50                                |              |         |
|---|--------------|---------|
| Component   | Catalog #    | Amount  |
| PowerLyzer <sup>®</sup> Glass MicroBead Tubes, 0.1 mm | 12255-50-GBT | 50      |
| MicroBead Solution                                    | 12224-50-BS  | 16.5 ml |
| Solution MD1  | 12224-50-1   | 2.75 ml |
| Solution MD2  | 12224-50-2   | 5.5 ml  |
| Solution MD3  | 12224-50-3   | 50 ml   |
| Solution MD4  | 12224-50-4   | 16.5 ml |
| Solution MD5  | 12224-50-5   | 3 ml    |
| Spin Filters Units in 2 ml Tubes                      | 12224-50-SF  | 50      |
| 2 ml Collection Tubes                                 | 12224-50-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](http://www.mobio.com). Reagents labeled flammable should be kept away from open flames and sparks.

**WARNING:** Solution MD4 is flammable. Do not use bleach to clean the inside of the PowerVac<sup>™</sup> Manifold or to rinse the PowerVac<sup>™</sup> Mini Spin Filter Adapters when attached to the manifold.

## IMPORTANT NOTES FOR USE:

Make sure all tubes rotate freely in the centrifuge without rubbing.  
Shake to mix Solution MD3 before use.

## MicroBead Tube Identification

Due to the high energies of the PowerLyzer<sup>®</sup> 24, the potential of marring of the tops of the caps of the MicroBead Tubes is possible, therefore it is recommended to mark the sides of these tubes as well as the caps to ensure proper sample identification.

## Experienced User Protocol

(If this is your first time using this kit please read the Detailed Protocol on the following page)  
Please wear certified RNase-Free gloves (Catalog#1556) at all times.

1. Properly identify each Glass MicroBead Tube on both the cap and on the side; See “**Important Notes For Use**” for more information.
2. Add 1.8 ml of microbial (bacteria, yeast) culture to a **2 ml Collection Tube** (provided) and centrifuge at 10,000 x g for 30 seconds at room temperature. Decant the supernatant and spin the tubes at 10,000 x g for 30 seconds at room temperature and completely remove the media supernatant with a pipette tip.  
**Note:** Based on the type of microbial culture, it may be necessary to centrifuge longer than 30 seconds.
3. Resuspend the cell pellet in 300 µl of **MicroBead Solution** and gently vortex to mix. Transfer resuspended cells to a **PowerLyzer® Glass MicroBead Tube, 0.1 mm**.
4. Check Solution MD1. If Solution MD1 is precipitated, heat the solution at 60°C until the precipitate has dissolved. Add 50 µl of **Solution MD1** to the **Glass MicroBead Tube**.  
**Optional:** To increase yields, to minimize DNA shearing, or for difficult cells, see Alternative Lysis Methods in the “Hints & Troubleshooting Guide” section before continuing.
5. Homogenization options:
  - A. PowerLyzer® 24 homogenizer: Place the PowerLyzer® Glass MicroBead Tubes onto the Tube Holder for the PowerLyzer® 24. The Glass MicroBead Tubes must be balanced (evenly spaced) on the Tube Holder. Homogenize for 5 minutes at 2000 RPM.  
**Note:** Depending on your sample less time at a higher speed may be used.
  - B. Vortex: Secure PowerLyzer® Glass MicroBead Tubes horizontally using the MO BIO Vortex Adapter tube holder for the vortex (MO BIO Catalog# 13000-V1-24) or secure tubes horizontally on a flat-bed vortex pad with tape. Vortex at maximum speed for 10 minutes. (See “Hints & Troubleshooting Guide” for less DNA shearing).
6. Make sure the Glass MicroBead Tubes rotate freely in the centrifuge without rubbing. Centrifuge the tubes at 10,000 x g for 30 seconds at room temperature.  
**CAUTION:** Be sure not to exceed 10,000 x g or tubes may break.
7. Transfer the supernatant to a clean **2 ml Collection Tube** (provided).  
**Note:** Expect 300 to 350 µl of supernatant.
8. Add 100 µl of **Solution MD2**, to the supernatant. Vortex for 5 seconds. Then incubate at 4°C for 5 minutes.
9. Centrifuge the Tubes at room temperature for 1 minute at 10,000 x g.
10. Avoiding the pellet, transfer the entire volume of supernatant to a clean **2 ml Collection Tube** (provided). Expect approximately 450 µl in volume.  
**Note:** A small carryover of glass beads is possible. This will not affect the results.
11. Shake to mix Solution MD3 before use. Add 900 µl of **Solution MD3** to the supernatant and vortex for 5 seconds.
12. Load about 700 µl into the **Spin Filter** and centrifuge at 10,000 x g for 30 seconds at room temperature. Discard the flow through, add the remaining supernatant to the **Spin Filter**, and centrifuge at 10,000 x g for 30 seconds at room temperature.  
**Note:** A total of 2 to 3 loads for each sample processed are required. Discard all flow through liquid.





**High Throughput Option:** Step 12 can become tedious when many samples need to be processed. For this reason, MO BIO has developed a vacuum protocol. It does require the purchase of our aluminum Spin Filter Adapters (catalog # 11992-10) which will allow you to fit our flat bottom spin filters on to any vacuum manifold with Luer lock fittings. Please read **Vacuum Protocol using the PowerVac™ Manifold on page 13.**

13. Add 300  $\mu$ l of **Solution MD4** and centrifuge at room temperature for 30 seconds at 10,000 x *g*.
14. Discard the flow through.
15. Centrifuge at room temperature for 1 minute at 10,000 x *g*.
16. Being careful not to splash liquid on the spin filter basket, place **Spin Filter** in a new **2 ml Collection Tube** (provided).
17. Add 50  $\mu$ l of **Solution MD5** to the center of the white filter membrane.
18. Centrifuge at room temperature for 30 seconds at 10,000 x *g*.
19. Discard **Spin Filter column**. The DNA in the tube is now ready for any downstream application. No further steps are required.

We recommend storing DNA frozen (-20°C). **Solution MD5** contains no EDTA.

**Thank you for choosing the PowerLyzer® UltraClean® Microbial DNA Isolation Kit.**



## Detailed Protocol (Describes what is happening at each step)

Please wear certified RNase-Free gloves (Catalog#1556) at all times.

1. Properly identify each Glass MicroBead Tube on both the cap and on the side; See “**Important Notes For Use**” for more information.
2. Add 1.8 ml of microbial (bacteria, yeast) culture to a **2 ml Collection Tube** (provided) and centrifuge at 10,000 x g for 30 seconds at room temperature. Decant the supernatant and spin the tubes at 10,000 x g for 30 seconds at room temperature and completely remove the media supernatant with a pipette tip.

**Note:** Based on the type of microbial culture, it may be necessary to centrifuge longer than 30 seconds.

*What's happening:* This step concentrates and pellets the microbial cells. In some cases it may take longer to completely pellet the cells. It is important to pellet the cells completely and remove all the culture media in this step.

3. Resuspend the cell pellet in 300 µl of **MicroBead Solution** and gently vortex to mix. Transfer resuspended cells to a **PowerLyzer<sup>®</sup> Glass MicroBead Tube, 0.1 mm**.

*What's happening:* The MicroBead Solution contains salts and a buffer which stabilizes and homogeneously disperses the microbial cells prior to lysis.

4. Check Solution MD1. If Solution MD1 is precipitated, heat the solution at 60°C until the precipitate has dissolved. Add 50 µl of **Solution MD1** to the **Glass MicroBead Tube**.

*What's happening:* Solution MD1 contains SDS and other disruption agents required for cell lysis. In addition to aiding in cell lysis, SDS is an anionic detergent that breaks down fatty acids and lipids associated with the cell membrane of several organisms. If it gets cold, it will precipitate. Heating at 60°C will dissolve the SDS and will not harm the SDS or the other disruption agents. In addition, Solution MD1 can be used while it is still warm.

**Optional:** To increase yields, to minimize DNA shearing, or for difficult cells, see Alternative Lysis Methods in the “Hints & Troubleshooting Guide” section before continuing.

*What's happening:* This optional step can lead to better performance in some cases. We recommend using only one of these methods for any individual prep.

5. Homogenization options:
  - A. PowerLyzer<sup>®</sup> 24 homogenizer: Place the PowerLyzer<sup>®</sup> Glass MicroBead Tubes onto the Tube Holder for the PowerLyzer<sup>®</sup> 24. The Glass MicroBead Tubes must be balanced (evenly spaced) on the Tube Holder. Homogenize for 5 minutes at 2000 RPM.

**Note:** Depending on your sample less time at a higher speed may be used.
  - B. Vortex: Secure PowerLyzer<sup>®</sup> Glass MicroBead Tubes horizontally using the MO BIO Vortex Adapter tube holder for the vortex (MO BIO Catalog# 13000-V1-24) or secure



tubes horizontally on a flat-bed vortex pad with tape. Vortex at maximum speed for 10 minutes. (See "Hints & Troubleshooting Guide" for less DNA shearing).

*What's happening: This step creates the combined chemical/ mechanical lysis conditions required to release desired nucleic acids from microbial cells. Many cell types will not lyse without this chemically enhanced bead beating process. The vortex action is typically all that is required, however, 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 fast prep machines.*

6. Make sure the 2 ml Glass MicroBead Tubes rotate freely in the centrifuge without rubbing. Centrifuge the tubes at 10,000 x g for 30 seconds at room temperature.

*What's happening: The cell debris is sent to the bottom of the tube while DNA remains in the supernatant.*

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

**Note:** Expect 300 to 350  $\mu$ l of supernatant.

*What's happening: The volume to expect will vary depending on the size of the original cell pellet from step 1.*

8. Add 100  $\mu$ l of **Solution MD2**, to the supernatant. Vortex for 5 seconds. Then incubate at 4°C for 5 minutes.
9. Centrifuge the Tubes at room temperature for 1 minute at 10,000 x g.

*What's happening: Solution MD2 contains a reagent to precipitate non-DNA organic and inorganic material including cell debris and proteins. It is important to remove contaminating organic and inorganic matter that may reduce DNA purity and inhibit downstream DNA applications.*

10. Avoiding the pellet, transfer the entire volume of supernatant to a clean **2 ml Collection Tube** (provided). Expect approximately 450  $\mu$ l in volume.

**Note:** A small carryover of glass beads is possible. This will not affect the results.

*What's happening: The pellet at this point contains non-DNA organic and inorganic materials, including cell debris and proteins. For the best DNA quality and yield, avoid transferring any of the pellet.*

11. Shake to mix Solution MD3 before use. Add 900  $\mu$ l of **Solution MD3** to the supernatant and vortex for 5 seconds.

*What's happening: Solution MD3 is a highly concentrated salt solution. It sets up the high salt condition necessary to bind DNA to the Spin Filter membrane in the following step.*

12. Load about 700  $\mu$ l into the **Spin Filter** and centrifuge at 10,000 x g for 30 seconds at room temperature. Discard the flow through, add the remaining supernatant to the **Spin Filter**, and centrifuge at 10,000 x g for 30 seconds at room temperature.

**Note:** A total of 2 to 3 loads for each sample processed are required. Discard all flow through liquid.



**High Throughput Option:** Step 12 can become tedious when many samples need to be processed. For this reason, MO BIO has developed a vacuum protocol. It does require the purchase of our aluminum Spin Filter Adapters (catalog # 11992-10) which will allow you to fit our flat bottom spin filters on to any vacuum manifold with Luer lock fittings. Please read **Vacuum Protocol using the PowerVac™ Manifold on page 13.**

*What's happening: DNA is selectively bound to the silica membrane in the Spin Filter device. Contaminants pass through the silica filter membrane, leaving only the DNA bound to the membrane.*

13. Add 300 µl of **Solution MD4** and centrifuge at room temperature for 30 seconds at 10,000 x g.

*What's happening: Solution MD4 is an ethanol 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 residues of salt, and other contaminants while allowing the DNA to stay bound to the silica filter membrane.*

14. Discard the flow through.

*What's happening: This flow through is waste containing ethanol wash solution and contaminants that did not bind to the silica filter membrane.*

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

*What's happening: This step removes residual Solution MD4 (ethanol wash solution). It is critical to remove all traces of wash solution because it can interfere with down stream DNA applications.*

16. Being careful not to splash liquid on the spin filter basket, place **Spin Filter** in a new **2 ml Collection Tube** (provided).

*What's happening: It is important to avoid any traces of the ethanol based wash solution.*

17. Add 50 µl of **Solution MD5** to the center of the white silica filter membrane.

*What's happening: Placing the Solution MD5 (elution buffer) in the center of the small white silica filter membrane will make sure the entire membrane is wetted. This will result in more efficient release of bound DNA*

18. Centrifuge at room temperature for 30 seconds at 10,000 x g.

*What's happening: As the Solution MD5 (elution buffer) passes through the silica filter membrane, DNA is released, and it flows through the membrane, and into the Collection Tube. The DNA is released because it can only bind to the silica filter membrane in the presence of salt. Solution MD5 is 10mM Tris pH 8 and does not contain salt.*

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

We recommend storing DNA frozen (-20°C). **Solution MD5** contains no EDTA.

**Thank you for choosing the PowerLyzer® UltraClean® Microbial DNA Isolation Kit.**



## 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 **PowerVac™ Manifold** (MO BIO Catalog# 11991). 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 11) 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. Add 300 µl of **Solution MD4** to each Spin Filter. Open the Luer-Lok® stopcock and apply a vacuum until **Solution MD4** has passed through the Spin Filter completely. Continue to pull a vacuum for another minute to dry the membrane. Close each port.
6. 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.
7. 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 1 minute to completely dry the membrane.
8. Transfer the **Spin Filter column** to a new **2 ml Collection Tube** and add 50 µl of **Solution MD5** to the center of the white silica filter membrane. Alternatively, sterile DNA-Free PCR Grade Water may be used for elution from the silica filter membrane at this step (MO BIO Catalog# 17000-10).
9. Centrifuge at room temperature for 30 seconds at 10,000 × *g*.



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

We recommend storing DNA frozen (-20° to -80°C). **Solution MD5** contains no EDTA. To concentrate the DNA see the Hints & Troubleshooting Guide.

**Thank you for choosing the PowerLyzer<sup>®</sup> UltraClean<sup>®</sup> Microbial DNA Isolation Kit.**

## Hints and Troubleshooting Guide

**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 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 step 5 and go to step 6. 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 for some organisms.
- **If cells are difficult to lyse:** Heat the preps after the addition of MD1 at 70°C for 10 minutes. Follow by continuing with the protocol at step 5. Homogenization in the PowerLyzer® 24 may be performed at higher speeds (up to 2800 RPM) and for up to 5 minutes to increase the lysis of tough organisms. Higher speeds may result in significant DNA shearing.

### **Concentrating the DNA**

The final volume of eluted DNA will be 50 µl. The DNA may be concentrated by adding 5 µl of 5M NaCl and inverting 3-5 times to mix. Next, add 100 µl of 100% cold ethanol and invert 3-5 times to mix. Incubate at -20°C for 30 minutes and centrifuge at 10,000 x *g* for 15 minutes at room temperature. Decant all liquid. Remove residual ethanol in a speed vac or dessicator or air dry. Resuspend precipitated DNA in sterile water or Solution MD5 (10 mM Tris).

### **DNA Floats Out of Well When Loaded on a Gel**

This usually occurs because residual Solution MD4 remains in the final sample. Prevent this by being careful in step 16 not to transfer liquid onto the bottom of the spin filter basket. Ethanol precipitation (described in “Concentrating the DNA”) is the best way to remove residual Solution MD4.

### **Storing DNA**

DNA is eluted in Solution MD5 (10 mM 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).

### **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

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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](http://www.mobio.com/distributors)





## Products recommended for you

For a complete list of products available from MO BIO Laboratories, Inc., visit [www.mobio.com](http://www.mobio.com)

| Description                                     | Catalog No.           | Quantity                      |
|---|-----------------------|-------------------------------|
| PowerLyzer® 24 Bench Top Bead-Based Homogenizer | 13155                 | 1 unit                        |
| UltraClean® Microbial DNA Isolation Kit         | 12224-50<br>12224-250 | 50 preps<br>250 preps         |
| UltraClean®-htp 96 Well Microbial DNA Kit       | 10196-4<br>10196-12   | 4 x 96 preps<br>12 x 96 preps |
| PowerMag® Microbial DNA Isolation Kit           | 27200-4               | 4 x 96 preps                  |
| PowerMicrobial® Maxi DNA Isolation Kit          | 12226-25              | 25 preps                      |
| UltraClean® Microbial RNA Isolation Kit         | 15800-50              | 50 preps                      |