PowerLyzer® PowerSoil®
DNA Isolation Kit

Catalog No.	Quantity
12855-50	50 Preps
12855-100	100 Preps

Instruction Manual

PowerLyzer® Products

PowerLyzer® 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 then garnet and when used with the PowerLyzer® 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® 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® DNA and RNA Isolation kits contain either glass or ceramic beads and are compatible with the PowerLyzer® 24 instrument.

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Version: 07272016

Table of Contents

Introduction

The PowerLyzer® PowerSoil® DNA Isolation Kit differs from the original PowerSoil® kit because it contains bead tubes with glass beads that are optimized for robust bead based homogenizers like the PowerLyzer® 24 as well as Fast Prep® and Precellys® instruments. It is comprised of a novel and proprietary method for isolating genomic DNA from environmental samples utilizing our patented Inhibitor Removal Technology® (IRT) in a fraction of the time required by traditional homogenization methods. The PowerLyzer® PowerSoil® DNA Isolation Kit is intended for use with environmental samples containing a high humic acid content including difficult soil types such as compost, sediment, and manure. Other more common soil types have also been used successfully with this kit. The isolated DNA has a high level of purity allowing for more successful PCR amplification of organisms from the sample. PCR analysis has been performed to detect a variety of organisms including bacteria (e.g. *Bacillus subtilis, Bacillus anthracis*), fungi (e.g. yeasts, molds), algae and Actinomycetes (e.g. *Streptomyces*). Homogenization with the PowerLyzer® 24 is faster than traditional vortex methods and also minimizes cross-contamination between samples.

Protocol Overview

The PowerLyzer® PowerSoil® DNA Isolation Kit distinguishes itself from MO BIO's UltraClean® Soil DNA Isolation Kit with a patented humic substance/brown color removal procedure. This kit is effective at removing PCR inhibitors from even the most difficult soil types. Environmental samples are added to a bead beating tube and homogenized on the PowerLyzer® 24 for rapid and thorough homogenization. Cell lysis occurs by mechanical and chemical interaction. Total genomic DNA is captured on a silica membrane in a spin column format. DNA is then washed and eluted from the membrane. DNA is then ready for PCR analysis and other downstream applications.

Optimized for complete homogenization of any sample with the

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

The PowerLyzer® PowerSoil® DNA Isolation Kit contains Bead Tubes with the 0.1 mm glass beads to allow for more options in homogenization methods, including the use of the PowerLyzer® 24 homogenizer. The instrument's velocity and proprietary motion combine to provide the fastest homogenization time possible, minimizing time spent processing samples. The 0.1 mm Glass Bead Tubes are suitable for both high velocity bead beating and vortex beating, depending on the level of homogenization desired. Alternative pre-filled bead tube options are available using harder matrices for grinding. Published references for using the PowerSoil® DNA Isolation Kit with a FastPrep® instrument are available from technical support. For more guidance and data, please contact technical service (technical@mobio.com).

Using the PowerLyzer® PowerSoil® 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.

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.

On the PowerLyzer® 24, a starting point for low biomass and clay soils is 45 seconds at a setting of 4000 RPM. For loamy soils, such as forest soils, settings between 2500-2800 RPM provide the highest yields without compromising integrity. To use the PowerLyzer® 24 in place of the vortex, a setting of 2000 RPM may be used for up to 5 minutes. The optimal setting and time may need to be optimized for the soil and the project for highest yields and integrity.

Using the PowerLyzer® PowerSoil® DNA Isolation Kit with other Homogenizers cont.

A starting point for homogenization is a setting of 5 on the FastPrep® or 5000 RPM on the Precellys® for one pulse of 45 seconds using the PowerLyzer® Glass Bead Tubes provided in this kit. For fungus or other difficult species, a 10 minute 65°C heating step may be performed prior to bead beating. More than one pulse of bead beating, or harder beads may be used, however, keep in mind that the DNA integrity will decrease.

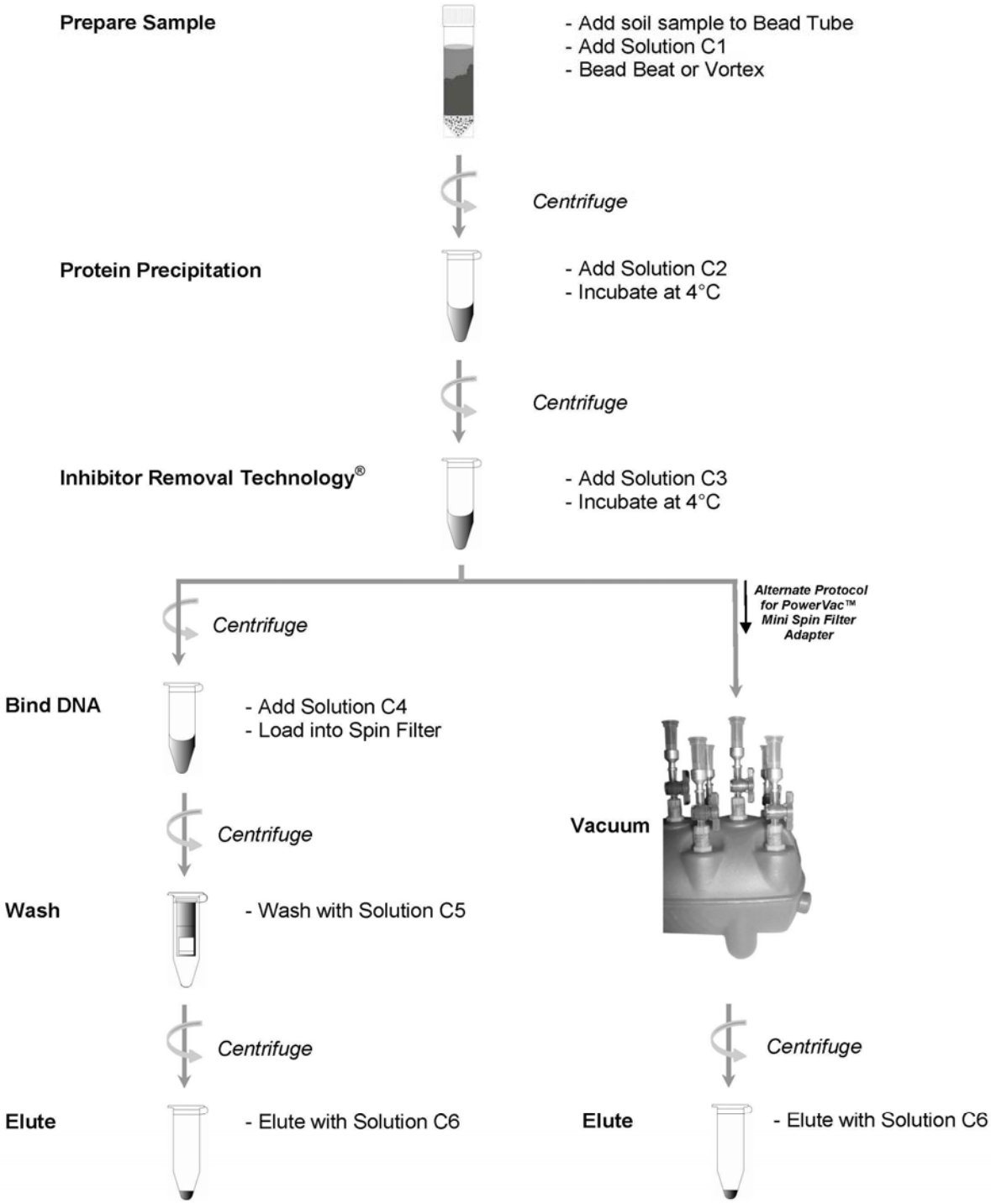
High Throughput Options

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 (Catalog# 11991) allows for processing of up to 20 spin filter preps at a time using the PowerVac™ Mini Spin Filter Adapters (See Other Related Products listed below). For additional high throughput options MO BIO offers the PowerSoil®-htp 96 Well Soil DNA Isolation Kit for processing 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 soil, MO BIO offers the 96 Well Plate Shaker and Plate Adapter Set (MO BIO Catalog# 11996 & 11990, respectively.)

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

Other Related Products	Catalog No.	Quantity
PowerMax® Soil DNA Isolation Kit	12988-10	10 preps
PowerSoil®-htp 96 Well Soil DNA Isolation Kit	12955-4 12955-12	4 x 96 preps 12 x 96 preps
Ceramic Bead Tubes, 1.4 mm Glass Bead Tubes, 0.5 mm Glass Bead Tubes, 0.1mm	13113-50 13116-50 13118-50	50 tubes 50 tubes 50 tubes
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
PowerLyzer® Tube Holder	13156	1 unit
PowerLyzer® Tube Holder Stand	13157	1 unit

PowerLyzer® PowerSoil® DNA Isolation Kit



Equipment Required

PowerLyzer® 24 or other bead homogenizer
Microcentrifuge (10,000 x *g*)
Pipettors (60 µl - 750 µl)
Vortex-Genie® 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™ Manifold protocol only)

Kit Contents

	Kit Catalog # 12855-50		Kit Catalog # 12855-100	
Component	**Catalog #**	**Amount**	**Catalog #**	**Amount**
PowerLyzer® Glass Bead Tubes, 0.1 mm	13118-50-GBT	50	13118-100-GBT	100
PowerSoil® Bead Solution	12855-50-BS	42 ml	12855-100-BS	84 ml
PowerSoil® Solution C1	12888-50-1	3.3 ml	12888-100-1	7 ml
PowerSoil® Solution C2	12888-50-2	14 ml	12888-100-2	28 ml
PowerSoil® Solution C3	12888-50-3	11 ml	12888-100-3	22 ml
PowerSoil® Solution C4	12888-50-4	72 ml	12888-100-4	144 ml
PowerSoil® Solution C5	12888-50-5	30 ml	12888-100-5	60 ml
PowerSoil® Solution C6	12888-50-6	6 ml	12888-100-6	12 ml
PowerSoil® Spin Filters (units in 2 ml tubes)	12888-50-SF	50	12888-100-SF	100
PowerSoil® 2 ml Collection Tubes	12888-50-T	200	12888-100-T	400

Kit Storage

Kit reagents and components should be stored at room temperature (15-30°C).

Precautions

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

WARNING: Solution C5 contains ethanol. It is flammable. Do not use bleach to clean the inside of the PowerVac™ Manifold or to rinse the PowerVac™ Mini Spin Filter Adapters when attached to the manifold.

IMPORTANT NOTE FOR USE:
Make sure all tubes rotate freely in your centrifuge without rubbing.
Shake to mix Solution C4 before use.

Bead Tube Identification

Due to the high energies of the PowerLyzer® 24, the potential of marring of the tops of the caps is possible, therefore it is recommended to mark the sides of the Glass Bead Tubes as well as the caps to ensure proper sample identification.

Experienced User Protocol

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

1. Properly identify each Glass Bead Tube on both the cap and on the side; See "**Important Notes For Use"** for more information.
2. To the **PowerLyzer® Glass Bead Tube, 0.1 mm** provided, add 0.25 grams of soil sample.
3. Add 750 µl of Bead Solution to the Glass Bead Tube. Gently vortex to mix.
4. **Check Solution C1.** If **Solution C1** is precipitated, heat solution to 60°C until dissolved before use.
5. Add 60 µl of **Solution C1** and invert several times or vortex briefly.
6. Bead Beating Options:

    A. PowerLyzer® 24 homogenizer: Place the PowerLyzer® Glass Bead Tubes into the Tube Holder for the PowerLyzer® 24. The Glass Bead Tubes must be balanced (evenly spaced) on the Tube Holder. Run the samples at a time and RPM suitable for your soil type.

    Note: For clay soils, a setting of 4000 RPM for 45 seconds is the best starting point. For loose, granular, and high organic soils, 2500 RPM for 45 seconds will provide an optimal result with 0.1 mm glass beads.

    Note: For other bead types or mixes, you may want to evaluate several speeds at a set time to determine at what point the integrity of the DNA is compromised.

    B. Vortex: Secure the PowerLyzer® Glass Bead Tubes horizontally using the MO BIO Vortex Adapter tube holder for the vortex or secure tubes horizontally on a flat-bed vortex pad with tape. Vortex at maximum speed for 10 minutes.

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

7. Make sure the Glass Bead Tubes rotate freely in your centrifuge without rubbing. Centrifuge Bead 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.
    Note: For clay soils, you may need to centrifuge the tubes longer to pellet all of the debris and obtain maximal supernatant. Centrifuge for 3 minutes at 10,000 x g for clay soils or if your soil is not completely pelleted after 30 seconds.
8. Transfer the supernatant to a clean **2 ml Collection Tube** (provided).
    Note: Expect between 400 to 500 µl of supernatant. Supernatant may still contain some soil particles.
9. Add 250 µl of **Solution C2** and vortex for 5 seconds. Incubate at 4°C for 5 minutes.
10. Centrifuge the tubes at room temperature for 1 minute at 10,000 x *g.*
11. Avoiding the pellet, transfer up to, but no more than, 600 µl of supernatant to a clean **2 ml Collection Tube** (provided).
12. Add 200 µl of **Solution C3** and vortex briefly. Incubate at 4°C for 5 minutes.
13. Centrifuge the tubes at room temperature for 1 minute at 10,000 x *g.*
14. Avoiding the pellet, transfer up to, but no more than, 750 µl of supernatant into a clean **2 ml Collection Tube** (provided).
15. Shake to mix Solution C4 before use. Add 1200 µl of **Solution C4** to the supernatant and vortex for 5 seconds.

16. Load approximately 675 µl onto a **Spin Filter** and centrifuge at 10,000 x *g* for 1 minute at room temperature. Discard the flow through and add an additional 675 µl of supernatant to the **Spin Filter** and centrifuge at 10,000 x *g* for 1 minute at room temperature. Load the remaining supernatant onto the **Spin Filter** and centrifuge at 10,000 x *g* for 1 minute at room temperature.
    Note: A total of three loads for each sample processed are required.

    High Throughput Option: Step 16 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 14.**

17. Add 500 µl of **Solution C5** and centrifuge at room temperature for 30 seconds at 10,000 x *g*.
18. Discard the flow through.
19. Centrifuge again at room temperature for 1 minute at 10,000 x *g*.
20. Carefully place spin filter in a clean **2 ml Collection Tube** (provided). Avoid splashing any of **Solution C5** onto the **Spin Filter**.
21. Add 100 µl of **Solution C6** to the center of the white filter membrane. Alternatively, sterile DNA-Free PCR Grade Water may be used for elution from the silica Spin Filter membrane at this step (MO BIO Catalog# 17000-10).
22. Centrifuge at room temperature for 30 seconds at 10,000 x *g*.
23. Discard the **Spin Filter**. 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 C6** contains no EDTA. To concentrate the DNA see the Hints & Troubleshooting Guide.

Thank you for choosing the PowerLyzer® PowerSoil® 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 Bead Tube on both the cap and on the side; See "**Important Notes For Use"** for more information.

2. To the **PowerLyzer® Glass Bead Tube, 0.1 mm** provided, add 0.25 grams of soil sample.

   What's happening: After your sample has been loaded into the Glass Bead Tube, the next step is a homogenization and lysis procedure. The Glass Bead Tube will help disperse the soil particles.

3. Add 750 µl of Bead Solution to the Glass Bead Tube. Gently vortex to mix.

   What's happening: Gentle vortexing mixes the components in the Glass Bead Tube and begins to disperse the sample in the Bead Solution.

4. **Check Solution C1**. If **Solution C1** is precipitated, heat solution to 60°C until the precipitate has dissolved before use.

   What's happening: Solution C1 contains SDS and other disruption agents required for complete 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 form a white precipitate in the bottle. Heating to 60°C will dissolve the SDS and will not harm the SDS or the other disruption agents. Solution C1 can be used while it is still warm.

5. Add 60 µl of **Solution C1** and invert several times or vortex briefly.

6. Bead Beating Options:

   A. PowerLyzer® 24 homogenizer: Place the PowerLyzer® Glass Bead Tubes into the Tube Holder for the PowerLyzer® 24. The Glass Bead Tubes must be balanced (evenly spaced) on the Tube Holder. Run the samples at a time and RPM suitable for your soil type.

      Note: For clay soils, a setting of 4000 RPM for 45 seconds is the best starting point. For loose, granular, and high organic soils, 2500 RPM for 45 seconds will provide an optimal result with 0.1 mm glass beads.

      Note: For other bead types or mixes, you may want to evaluate several speeds at a set time to determine at what point the integrity of the DNA is compromised.

   B. Vortex: Secure the PowerLyzer® Glass Bead Tubes horizontally using the MO BIO Vortex Adapter tube holder for the vortex or secure tubes horizontally on a flat-bed vortex pad with tape. Vortex at maximum speed for 10 minutes.

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

   What's happening: The bead beating or vortexing step is critical for complete homogenization and cell lysis. Cells are lysed by a combination of chemical agents from steps 1-4 and mechanical shaking introduced at this step. By shaking the beads in the presence of disruption agents, collision of the beads with microbial cells will cause the cells to break open.

The PowerLyzer® 24 can homogenize soils at high acceleration in only 45 seconds using the glass beads to achieve lysis in less time. The time and speed for each soil may vary. Glass Bead Tubes may also be used on the MO BIO Vortex with the Vortex Adapter. The Vortex Adapter is designed to be a simple platform to facilitate keeping the tubes tightly attached to the vortex. It should be noted that although you can attach tubes with tape, often the tape becomes loose and not all tubes will shake evenly or efficiently. This may lead to inconsistent results or lower yields. Therefore, the use of the MO BIO Vortex Adapter is a highly recommended and cost effective way to obtain maximum DNA yields if you do not have the PowerLyzer® 24 or similar bead beating instrument.

7. Make sure the Glass Bead Tubes rotate freely in your centrifuge without rubbing. Centrifuge 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.
   Note: For clay soils, you may need to centrifuge the tubes longer to pellet all of the debris and obtain maximal supernatant. Centrifuge for 3 minutes at 10,000 x g for clay soils or if your soil is not completely pelleted after 30 seconds.

8. Transfer the supernatant to a clean **2 ml Collection Tube** (provided).
   Note: Expect between 400 to 500 µl of supernatant at this step. The exact recovered volume depends on the absorbancy of your starting material and is not critical for the procedure to be effective. The supernatant may be dark in appearance and still contain some soil particles. The presence of carry over soil or a dark color in the mixture is expected in many soil types at this step. Subsequent steps in the protocol will remove both carry over soil and coloration of the mixture.

9. Add 250 µl of **Solution C2** and vortex for 5 seconds. Incubate at 4°C for 5 minutes.

   What's happening: Solution C2 is patented Inhibitor Removal Technology® (IRT). It contains a reagent to precipitate non-DNA organic and inorganic material including humic substances, 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. Centrifuge the tubes at room temperature for 1 minute at 10,000 x *g.*

11. Avoiding the pellet, transfer up to 600 µl of supernatant to a clean **2 ml Collection Tube** (provided).

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

12. Add 200 µl of **Solution C3** and vortex briefly. Incubate at 4°C for 5 minutes.

    What's happening: Solution C3 is patented Inhibitor Removal Technology® (IRT) and is a second reagent to precipitate additional non-DNA organic and inorganic material including humic acid, cell debris, and proteins. It is important to remove contaminating organic and inorganic matter that may reduce DNA purity and inhibit downstream DNA applications.

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

14. Transfer up to 750 µl of supernatant to a clean **2 ml Collection Tube** (provided).

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

15. Shake to mix Solution C4 before use. Add 1.2 ml of **Solution C4** to the supernatant (be careful solution doesn't exceed rim of tube) and vortex for 5 seconds.

    What's happening: Solution C4 is a high concentration salt solution. Since DNA binds tightly to silica at high salt concentrations, this will adjust the DNA solution salt concentrations to allow binding of DNA, but not non-DNA organic and inorganic material that may still be present at low levels, to the Spin Filters.

16. Load approximately 675 µl onto a **Spin Filter** and centrifuge at 10,000 x *g* for 1 minute at room temperature. Discard the flow through and add an additional 675 µl of supernatant to the **Spin Filter** and centrifuge at 10,000 x *g* for 1 minute at room temperature. Load the remaining supernatant onto the **Spin Filter** and centrifuge at 10,000 x *g* for 1 minute at room temperature.

    Note: A total of three loads for each sample processed are required.

    High Throughput Option: Step 16 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 14.**

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

17. Add 500 µl of **Solution C5** and centrifuge at room temperature for 30 seconds at 10,000 x *g*.

    What's happening: Solution C5 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 residual salt, humic acid, and other contaminants while allowing the DNA to stay bound to the silica membrane.

18. Discard the flow through from the **2 ml Collection Tube**.

    What's happening: This flow through fraction is just non-DNA organic and inorganic waste removed from the silica Spin Filter membrane by the ethanol wash solution.

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

    What's happening: This second spin removes residual Solution C5 (ethanol wash solution). It is critical to remove all traces of wash solution because the ethanol in Solution C5 can interfere with many downstream DNA applications such as PCR, restriction digests, and gel electrophoresis.

20. Carefully place Spin Filter in a clean **2 ml Collection Tube** (provided). Avoid splashing any **Solution C5** onto the **Spin Filter**.

    Note: It is important to avoid any traces of the ethanol based wash solution.

21. Add 100 µl of **Solution C6** to the center of the white filter membrane.

    Note: Placing the Solution C6 (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 C6 (elution buffer) passes through the silica membrane, DNA that was bound in the presence of high salt is selectively released by Solution C6 (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 (MO BIO Catalog# 17000-10). Solution C6 contains no EDTA. If DNA degradation is a concern, Sterile TE may also be used instead of Solution C6 for elution of DNA from the Spin Filter.

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

23. Discard the **Spin Filter**. 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 C6** does not contain any EDTA. To concentrate DNA see the Hints & Troubleshooting Guide.

Thank you for choosing the PowerLyzer® PowerSoil® 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 15) 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 500 µl of **Solution C5** to each Spin Filter. Open the Luer-Lok® stopcock and apply a vacuum until **Solution C5** 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 100 µl of **Solution C6** to the center of the white filter membrane. Alternatively, sterile DNA-Free PCR Grade Water may be used for elution from the silica **Spin Filter** membrane at this step (MO BIO Catalog # 17000-10).

9. Centrifuge at room temperature for 30 seconds at 10,000 x *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 C6** contains no EDTA. To concentrate the DNA see the Hints & Troubleshooting Guide.

Thank you for choosing the PowerLyzer® PowerSoil® DNA Isolation Kit.

Hints & Troubleshooting Guide

Amount of Soil to Process

This kit is designed to process 0.25 grams of soil. For inquiries regarding the use of larger sample amounts, please contact technical support for suggestions. For wet soils, see information under "Wet Soil Sample" below.

Wet Soil Sample

If soil sample is high in water content, add the soil sample to Glass Bead Tube and centrifuge at room temperature for 30 seconds at 10,000 x *g*. Remove as much liquid as possible with a pipet tip. Continue with the protocol at step 2.

If DNA Does Not Amplify

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

Eluted DNA Sample Is Brown

We have not observed any coloration in DNAs isolated using the PowerLyzer® PowerSoil® DNA Isolation Kit. If you observe coloration in your samples, please contact technical support for suggestions.

Alternative Lysis Methods

- **For less DNA shearing**: After adding Solution C1, vortex 3-4 seconds, then heat to 70°C for 5 minutes. Vortex 3-4 seconds. Heat another 5 minutes. Vortex 3-4 seconds. This alternative procedure will reduce shearing but may also reduce yield.
- **If cells are difficult to lyse**: Incubation at 70°C for 10 minutes, after adding Solution C1. Follow by continuing with the protocol at step 6.

Concentrating the DNA

The final volume of eluted DNA will be 100 µl. The DNA may be concentrated by adding 10 µl of 5 M NaCl and inverting 3-5 times to mix. Next, add 200 µl of 100% cold ethanol and invert 3-5 times to mix. Centrifuge at 10,000 x *g* for 5 minutes at room temperature. Decant all liquid. Remove residual ethanol in a speed vac, dessicator, or air dry. Resuspend precipitated DNA in sterile water or sterile 10 mM Tris.

DNA Floats Out of Well When Loaded on a Gel

This usually occurs because residual Solution C5 remains in the final sample. Prevent this by being careful in step 20 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 C5.

Storing DNA

DNA is eluted in Solution C6 (10 mM Tris) and must be stored at -20° to -80°C to prevent degradation. DNA can be eluted in TE without loss, but the EDTA may inhibit downstream reactions such as PCR and automated sequencing. DNA may also be eluted with sterile DNA-Free PCR Grade Water (MO BIO Catalog# 17000-10).

Hints & Troubleshooting Guide cont.

Cleaning of the PowerVac™ Mini Spin Filter Adapters

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

Do not use bleach to clean the PowerVac™ Mini Spin Filter Adapters while attached to the PowerVac™ Manifold. Bleach should never be mixed with solutions containing guanidine and should not be used to clean the PowerVac™ Manifold. For more information on cleaning the PowerVac™ Manifold, please refer to the PowerVac™ Manifold manual.

Contact Information

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

Products recommended for you

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

Description	Catalog No.	Quantity
PowerLyzer® 24 Bench Top Bead-Based Homogenizer	13155	1 unit
PowerSoil® DNA Isolation Kit	12888-50 12888-100	50 preps 100 preps
PowerMax® Soil DNA Isolation Kit	12988-10	10 preps
PowerSoil®-htp 96 Well DNA Isolation	12955-4 12955-12	4 preps 12 preps
RNA PowerSoil® Total RNA Isolation Kit	12866-25	25 preps
PowerClean® DNA Clean-Up Kit	12877-50	50 preps