PowerFecal™
DNA Isolation Kit

Catalog No.	Quantity
12830-50	50 Preps

Instruction Manual

Inhibitor Removal Technology® (IRT) is a registered trademark of MO BIO Laboratories, Inc. and is covered by the following patents USA US 7,459,548 B2, Australia 2005323451 and India 246946.

Please recycle

Version: 11022012

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Introduction

The PowerFecal™ DNA Isolation Kit is designed for fast and easy purification of both microbial and host genomic DNA from stool and feces. Based on the MO BIO PowerSoil® DNA Isolation Kit, the PowerFecal™ DNA Isolation Kit uses the same patented Inhibitor Removal Technology® (IRT) for stool that has worked so well for soil. IRT is very effective at removing inhibitory substances commonly found in stool such as polysaccharides, heme compounds and bile salts. The result is high purity DNA that is ready to use in the most demanding downstream applications.

Protocol Overview

The recommended starting sample is 0.25 grams of stool or biosolid. Each sample is homogenized in a 2 ml bead beating tube containing garnet beads. Cell lysis of host cells as well as microbial cells is facilitated by both mechanical collisions between beads and chemical disruption of cell membranes, ensuring efficient extraction from even the toughest of microorganisms. Patented Inhibitor Removal Technology® (IRT) is then used to remove common substances in fecal samples that interfere with PCR. Total genomic DNA is captured on a silica spin column. DNA is then washed and eluted so that it is ready for PCR analysis and other downstream applications including qPCR and next generation sequencing analysis.

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). Using the PowerVac™ Manifold minimizes the most time consuming steps in the procedure. 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 plate homogenization, we recommend the Retsch 96 Well Plate Shaker (MO BIO Catalog# 11996 in the USA only) and Adapters (MO BIO Catalog# 11990). For information outside the USA, contact technical@mobio.com.

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™ 2 ml Tube Holder	13156	1 unit
PowerLyzer™ Tube Holder Stand	13157	1 unit

PowerFecal™ DNA Isolation Kit

Prepare Sample

- Add sample to Dry Bead Tube
- Add Bead Solution
- Add Solution C1
- Heat Tubes at 65°C
- Attach to Vortex Adapter
- Vortex

Centrifuge

Cell Lysis

- Add Solution C2
- Incubate at 4°C

Centrifuge

Inhibitor Removal Technology®

- Add Solution C3
- Incubate at 4°C

Centrifuge

Bind DNA

- Add Solution C4
- Load into Spin Filter

Centrifuge

Wash

- Wash with Solution C5

Centrifuge

Elute

- Elute with Solution C6

Equipment Required

Microcentrifuge (13,000 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)

Reagents Required but not Included

100% ethanol (for the PowerVac™ Manifold protocol only)

Kit Contents

	Kit Catalog # 12830-50	
Component	**Catalog #**	**Amount**
Dry Bead Tubes	12830-50-BT	50
Bead Solution	12830-50-BS	42 ml
Solution C1	12830-50-1	3.3 ml
Solution C2	12830-50-2	14 ml
Solution C3	12830-50-3	11 ml
Solution C4	12830-50-4	72 ml
Solution C5	12830-50-5	30 ml
Solution C6	12830-50-6	6 ml
Spin Filters (units in 2 ml tubes)	12830-50-SF	50
2 ml Collection Tubes	12830-50-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 on the product page. 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: Shake to mix Solution C4 before use.

Experienced User Protocol

Please wear gloves at all times

1. To the **Dry Bead Tube,** provided, add 0.25 grams of stool or biosolid.
   Note: For fecal samples that are especially high in lipids, polysaccharides and protein (for example: meconium or some bird feces) less material (0.10 grams) may improve the DNA yield and purity.

2. Add **750 µl of Bead Solution** to the **Dry Bead Tube**. Gently vortex to mix.

3. **Check Solution C1.** If **Solution C1** has precipitated, heat solution to 60°C until dissolved before use.

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

5. Heat the tubes at 65°C for 10 minutes.

6. Secure the bead tubes horizontally using the MO BIO Vortex Adapter tube holder for the vortex (MO BIO Catalog# 13000-V1) or secure tubes horizontally on a flat-bed vortex pad with tape. Vortex at maximum speed for 10 minutes.

7. Centrifuge the tubes at 13,000 x *g* for 1 minute.

8. Transfer the supernatant to a clean **2 ml Collection Tube** (provided). Expect between 400 to 500 µl of supernatant.

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

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

11. Avoiding the pellet, transfer up to 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 13,000 x *g* for 1 minute.

14. Avoiding the pellet, transfer the supernatant to a clean **2 ml Collection Tube** (provided). Do not transfer more than 750 µl at this step.

15. Shake to mix Solution C4 before use. Add 1200 µl of **Solution C4** to the supernatant and vortex for 5 seconds.

16. Load 650 µl of supernatant onto a **Spin Filter** and centrifuge at 13,000 x *g* for 1 minute. Discard the flow through and repeat until all the supernatant has been loaded onto the Spin Filter.
    Note: A total of 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 (MO BIO 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 11.**

17. Add 500 µl of **Solution C5** and centrifuge for 1 minute at 13,000 x *g*.

18. Discard the flow through.

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

20. Carefully place the 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 or TE buffer may be used for elution from the silica Spin Filter membrane at this step (MO BIO Catalog# 17000-10).

    Note: Eluting with 100 µl of Solution C6 will maximize DNA yield. For more concentrated DNA, a minimum of 50 µl of Solution C6 can be used. Do not use less than 50 µl of Solution C6.

22. Centrifuge at 13,000 x *g* for 1 minute and discard the Spin Filter basket.

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 PowerFecal™ DNA Isolation Kit.

Detailed Protocol (Describes what is happening at each step)

Please wear gloves at all times

1. To the **Dry Bead Tube,** provided, add 0.25 grams of stool or biosolid.
   Note: For fecal samples that are especially high in lipids, polysaccharides and protein (for example: meconium or some bird feces) less material (0.10 grams) may improve the DNA yield and purity.

2. Add **750 µl of Bead Solution** to the **Dry Bead Tube**. Gently vortex to mix.

   What's happening: After your sample has been loaded into the bead tube, the next steps will be homogenization and cell lysis. The garnet beads and Bead Solution will help to dissolve and disperse the sample particles.

3. **Check Solution C1.** If **Solution C1** has precipitated, heat solution to 60°C until dissolved before use.

   What's happening: Solution C1 contains SDS. If it gets cold, it will form a white precipitate in the bottle. Heating to 60°C will dissolve the SDS while not harming the SDS or the other disruption agents. Solution C1 can be used while it is still warm.

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

   What's happening: Solution C1 contains SDS and other disruption agents that aid cell lysis. SDS is an anionic detergent that breaks down fatty acids and lipids associated with the cell membrane of several organisms.

5. Heat the tubes at 65°C for 10 minutes.

   What's happening: Fecal samples contain a complex array of polysaccharides, lipids, salts and cells. Heating the sample increases the reaction rate between the lysis buffer and these substances and aids cell lysis.

6. Secure the bead tubes horizontally using the MO BIO Vortex Adapter tube holder for the vortex (MO BIO Catalog# 13000-V1) or secure tubes horizontally on a flat-bed vortex pad with tape. Vortex at maximum speed for 10 minutes.

   What's happening: 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 MO BIO Vortex Adapter is a simple platform to keep the bead tubes tightly attached to the vortex during shaking. While one can attach the tubes with tape, the tape can become loose so that the tubes shake unevenly and inefficiently resulting in inconsistent results or lower yields.

7. Centrifuge the tubes at 13,000 x *g* for 1 minute.

8. Transfer the supernatant to a clean **2 ml Collection Tube** (provided). Expect between 400 to 500 µl of supernatant.

   What's happening: Expect between 400 to 500 µl of lysate at this step. The exact volume depends on the absorbency of your starting material and is not critical for the procedure to be effective.

9. Add **250 µl of Solution C2** and vortex briefly to mix. 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 polysaccharides, 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 13,000 x *g* for 1 minute.

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 polysaccharides, cell debris, and proteins. For best DNA yield 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.

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

14. Avoiding the pellet, transfer the supernatant to a clean **2 ml Collection Tube** (provided). Do not transfer more than 750 µl at this step.

    What's happening: The pellet at this point contains additional non-DNA organic and inorganic material. For the best DNA yield and quality avoid transferring any of the pellet. It is important that no more than 750 µl be transferred to the next step to ensure proper binding of the DNA to the silica spin column.

15. Shake to mix Solution C4 before use. Add 1200 µl of **Solution C4** to the supernatant 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 650 µl of supernatant onto a **Spin Filter** and centrifuge at 13,000 x *g* for 1 minute. Discard the flow through and repeat until all the supernatant has been loaded onto the Spin Filter.
    Note: A total of 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 (MO BIO 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 11.**

    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 for 1 minute at 13,000 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 and other contaminants while allowing the DNA to stay bound to the silica membrane.

18. Discard the flow through.

    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 again for 1 minute at 13,000 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 the 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 or TE buffer may be used for elution from the silica Spin Filter membrane at this step (MO BIO Catalog# 17000-10).

    Note: Eluting with 100 µl of Solution C6 will maximize DNA yield. For more concentrated DNA, a minimum of 50 µl of Solution C6 can be used. Do not use less than 50 µl of Solution C6.

    What's happening: Placing 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 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.

22. Centrifuge at 13,000 x *g* for 1 minute and discard the Spin Filter basket.

    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 PowerFecal™ 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 x *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 PowerFecal™ DNA Isolation Kit.

Hints & Troubleshooting Guide

Amount of Sample to Process
This kit is designed to process 0.25 grams of fecal or stool sample per prep. We do not recommend using greater than 0.25 grams. Because of very high levels of proteins, lipids and polysaccharides contained in some types of fecal samples, less material (0.10 grams) may actually improve the extracted DNA yield and purity in these samples.

Wet Fecal Sample
If the fecal sample is high in water content, add the sample to Dry 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 PCR.
- Diluting the template DNA should not be necessary with DNA isolated with the PowerFecal™ 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.

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.

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

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

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

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

Products recommended for you

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

Description	Catalog No.	Quantity
PowerMicrobiome™ RNA Isolation Kit	26000-50	50 preps
PowerMag™ Microbiome RNA/DNA Isolation Kit	27500-4-EP	4 x 96 preps
Vortex Genie® 2 Vortex	13111-V 13111-V-220	1 unit (120V) 1 unit (220V)
Vortex Adapter for Vortex Genie® 2	13000-V1-24	Holds 24 (1.5-2.0 ml) tubes
UltraClean® PCR Clean-Up Kit	12500-50 12500-100 12500-250	50 preps 100 preps 250 preps