PowerClean® DNA Clean-Up Kit

Catalog No.	Quantity
12877-50	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, Japan 5112064 and India 246946.

Please recycle

Version: 01152013

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Introduction

The PowerClean® DNA Clean-Up Kit utilizes our patented Inhibitor Removal Technology® (IRT) to provide researchers with a novel and proprietary method for cleaning up previously isolated genomic DNA. Starting DNA may be amber to brown in appearance; an indicator of PCR inhibiting substances, particularly humics and polyphenols. Even samples that appear colorless may contain PCR inhibitors which can be cleaned up with this kit. The PowerClean® DNA Clean-Up Kit will remove this brown color as well as any PCR inhibiting substances, such as heme, polysaccharides, polyphenols fulvic acids and dyes. A high level of purity is achieved with the PowerClean® DNA Clean-Up Kit, allowing for more successful PCR amplification of DNA derived from organisms in the original sample. This kit was validated with DNA isolated from a variety of problematic soils and also with artificially spiked DNA samples with commercial humic acids. However, it performs well on DNA isolated from virtually any sample source.

Protocol Overview

Archived or previously isolated DNA samples are purified when added to our proprietary DNA Clean-Up reagents. Inhibitors are selectively removed from the DNA solution. All DNA including total genomic DNA is captured on a silica membrane in a spin column format. DNA is then washed and eluted from the membrane. Percentage recovery varies depending on the level of inhibitors in the DNA that may be influencing the DNA yield measurement. Purified DNA is ready for PCR analysis and other downstream applications.

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

Other Related Products	Catalog No.	Quantity
PowerSoil® DNA Isolation Kit	12888-50 12888-100	50 preps 100 preps
UltraClean® PCR Clean-Up Kit	12500-50 12500-100 12500-250	50 preps 100 preps 250 preps
UltraClean® GelSpin® DNA Extraction Kit	12400-50 12400-100 12400-250	50 preps 100 preps 250 preps

Equipment Required

Microcentrifuge (13,000 x g)
Pipettor (20 µl - 800 µl)
Vortex-Genie® 2 Vortex (MO BIO Catalog# 13111-V or 13111-V-220)

Kit Contents

	Kit Catalog #12877-50	
Component	**Catalog #**	**Amount**
PowerClean® DNA Solution 1	12877-50-1	4 ml
PowerClean® DNA Solution 2	12877-50-2	1.5 ml
PowerClean® DNA Solution 3	12877-50-3	5 ml
PowerClean® DNA Solution 4	12877-50-4	4 ml
PowerClean® DNA Solution 5	12877-50-5	44 ml
PowerClean® DNA Solution 6	12877-50-6	30 ml
PowerClean® DNA Solution 7	12877-50-7	6 ml
PowerClean® DNA Spin Filters	12877-50-SF	50
PowerClean® DNA 2 ml Collection Tubes	12877-50-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. Reagents labeled flammable should be kept away from open flames and sparks.

WARNING: PowerClean® DNA Solution 6 contains ethanol. It is flammable.

IMPORTANT NOTE FOR USE: Shake to mix PowerClean® DNA Solution 5 before use.

Experienced User Protocol

Please wear gloves at all times

1. Add up to 150 µl of DNA sample to **2 ml Collection Tube** (provided). If less than 150 µl is added, adjust volume with distilled water.
2. Add 70 µl of **PowerClean® DNA Solution 1** to DNA. Gently invert 3-5 times to mix.
3. Check **PowerClean® DNA Solution 2.** If it has precipitated, heat to 60°C and gently invert the tube periodically until it has completely dissolved. Vigorous shaking will result in foaming. This solution may be used while still warm.
4. Add 20 µl of **PowerClean® DNA Solution 2** and invert 3-5 times to mix.
5. Add 85 µl of **PowerClean® DNA Solution 3** and invert 3-5 times to mix. Incubate at 4°C for 5 minutes.
6. Centrifuge tubes at 10,000 x *g* for 1 minute at room temperature.
7. Avoiding pellet, transfer the entire volume of supernatant to a clean **2 ml Collection Tube** (provided).
8. Add 70 µl of **PowerClean® DNA Solution 4** and invert 3-5 times to mix. Incubate at 4°C for 5 minutes.
9. Centrifuge tubes at 10,000 x *g* for 1 minute at room temperature.
10. Avoiding pellet, transfer the supernatant into a clean **2 ml Collection Tube** (provided).
11. Shake to mix **PowerClean® DNA Solution 5**. Add 800 µl of **PowerClean® DNA Solution 5** to the supernatant and vortex for 5 seconds.
12. Load approximately 600 µl onto **Spin Filter** and centrifuge at 10,000 x *g* for 1 minute at room temperature. Discard flow through. Add remaining 600 µl supernatant to **Spin Filter** and centrifuge at 10,000 x *g* for 1 minute at room temperature.
    Note: A total of two loads for each sample processed may be required.
13. Add 500 µl of **PowerClean® DNA Solution 6** to **Spin Filter** and centrifuge at 10,000 x *g* for 30 seconds at room temperature
14. Discard flow through.

    Optional: An additional wash of the Spin Filter with 100% ethanol may be performed prior to the final dry spin. This may enhance the purity for some sample types. After decanting the flow through from step 14, add 650µl of 100% ethanol to the spin column and centrifuge at 10,000 x g for 30 seconds. To completely remove residual ethanol, decant the flow through and increase the dry spin (step 15) to 2 minutes at 13,000 x g or max speed. Continue with step 16.

15. Centrifuge **Spin Filter** at 13,000 x *g* for 1 minute at room temperature.
16. Carefully place **Spin Filter** in new **2 ml Collection Tube** (provided). Avoid splashing any **PowerClean® DNA Solution 6** onto **Spin Filter**.
17. If starting with 50 µl of genomic DNA, add 50 µl of **PowerClean® DNA Solution 7** to center of white filter membrane. If starting with 100 or 150 µl of genomic DNA, add 100 µl of **PowerClean® DNA Solution 7** to center of white filter membrane.
    Note: For efficient elution, use a minimum of 50 µl of **PowerClean® DNA Solution 7**, irrespective of starting volume. By reducing elution volume, it is possible to obtain DNA in a more concentrated form.
18. Centrifuge at 10,000 x *g* for 30 seconds at room temperature.
19. Discard **Spin Filter**. DNA in **2 ml Collection Tube** is now application ready. No further steps are required. We recommend storing DNA frozen (-20° to -80°C). **PowerClean® DNA Solution** 7 does not contain EDTA.

Thank you for choosing the PowerClean® DNA Clean-Up Kit.

Detailed Protocol (Describes what is happening at each step)

Please wear gloves at all times

1. Add up to 150 µl of DNA sample to a **2 ml Collection Tube** (provided). If less than 150 µl is added, adjust the volume with distilled water.

   What's happening: After the sample has been added to the Collection Tube, a disassociation procedure is performed. The PowerClean® DNA Solutions contain reagents that will (a) help disperse molecular interactions, (b) begin to dissolve humic substances and (c) protect nucleic acids from degradation.

2. Add 70 µl of **PowerClean® DNA Solution 1** to the DNA. Gently invert 3-5 times to mix.

   What's happening: Gentle inversion of the tube mixes the components in the tube and begins to disassociate DNA from PCR inhibiting substances.

3. Check **PowerClean® DNA Solution 2.** If it has precipitated, heat to 60°C and gently invert the tube periodically until it has completely dissolved. Vigorous shaking will result in foaming. This solution may be used while it is still warm.

   What's happening: PowerClean® DNA Solution 2 contains detergents and other agents required for complete disassociation. The chemicals in PowerClean® DNA Solution 2 will precipitate under cold storage conditions. Heating to 60°C will dissolve the reagent or the other disassociation agents. PowerClean® DNA Solution 2 can be used while it is warm.

4. Add 20 µl of **PowerClean® DNA Solution 2** and gently invert 3-5 times to mix.

5. Add 85 µl of **PowerClean® DNA Solution 3** and gently invert 3-5 times to mix. Incubate at 4°C for 5 minutes.

   What's happening: PowerClean® DNA Solution 3 is patented Inhibitor Removal Technology® (IRT). It contains a reagent that precipitates non-DNA organic and inorganic materials, including humic substances and proteins. It is important to remove contaminating organic and inorganic matter that may reduce DNA purity and inhibit downstream DNA applications.

   ***Note**: Expect between 250-325 µl of supernatant at this step. The exact recovered volume depends on the nature of your starting material and is not critical for the procedure to be effective. The supernatant may still be dark in appearance. The presence of a dark color in the mixture is expected in many sample types at this step. Subsequent steps in the protocol will remove the coloration of the mixture.*

6. Centrifuge the tube at 10,000 x *g* for 1 minute at room temperature.

7. Avoiding the pellet, transfer the entire supernatant to a clean **2 ml Collection Tube** (provided).

   What's happening: The pellet contains non-DNA organic and inorganic materials, including humic substances and proteins. For the best DNA yield and quality, avoid transferring any of the pellet.

8. Add 70 µl of **PowerClean® DNA Solution 4** and gently invert 3-5 times to mix. Incubate at 4°C for 5 minutes.

   What's happening: PowerClean® DNA Solution 4 is patented Inhibitor Removal Technology® (IRT) and is the

second reagent to precipitate additional non-DNA organic and inorganic materials, including humic substances and proteins. It is important to remove contaminating organic and inorganic matter that may reduce DNA purity and inhibit downstream DNA applications.

9. Centrifuge the tube at 10,000 x *g* for 1 minute at room temperature.

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

    What's happening: The pellet contains additional non-DNA organic and inorganic materials, including humic substances and proteins. For the best DNA yield and quality, avoid transferring any of the pellet.

11. Shake to mix **PowerClean® DNA Solution 5**. Add 800 µl of **PowerClean® DNA Solution 5** to the supernatant (be careful that solution doesn't exceed rim of **2 ml Collection Tube**) and vortex for 5 seconds.

    What's happening: PowerClean® DNA Solution 5 is a high salt concentration solution. Since DNA binds tightly to silica at high salt concentrations, this solution will adjust the 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.

12. Load approximately 600 µl onto a **Spin Filter** and centrifuge at 10,000 x *g* for 1 minute at room temperature. Discard the flow through and load the remaining 600 µl supernatant onto the **Spin Filter** and centrifuge at 10,000 x *g* for 1 minute at room temperature. **Note**: A total of two loads for each sample processed may be required.

    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 the DNA bound to the membrane.

13. Add 500 µl of **PowerClean® DNA Solution 6** and centrifuge at 10,000 x *g* for 30 seconds at room temperature.

    What's happening: This solution 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, humic substances, and other contaminants while allowing the DNA to stay bound to the silica membrane.

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

    What's happening: The flow through fraction is non-DNA organic and inorganic waste removed from the silica spin filter membrane by the ethanol wash solution.

    Optional: An additional wash of the Spin Filter with 100% ethanol may be performed prior to the final dry spin. This may enhance the purity for some sample types. After decanting the flow through from step 14, add 650µl of 100% ethanol to the spin column and centrifuge at 10,000 x g for 30 seconds. To completely remove residual ethanol, decant the flow through and increase the dry spin (step 15) to 2 minutes at 13,000 x g or max speed. Continue with step 16.

15. Centrifuge at 13,000 x *g* for 1 minute at room temperature.

    What's happening: This second spin removes residual ethanol wash solution. It is critical to remove all traces of wash solution because the ethanol in PowerClean® DNA Solution 6 can interfere with many downstream applications such as PCR, restriction digests and gel electrophoresis.

16. Carefully place the **Spin Filter** in a new **2 ml Collection Tube** (provided). Avoid splashing any **PowerClean® DNA Solution 6** onto the **Spin Filter**.

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

17. If starting with 50 µl of genomic DNA, add 50 µl of **PowerClean® DNA Solution 7** to the center of the white filter membrane. If starting with 100 or 150 µl of genomic DNA, add 100 µl of **PowerClean® DNA Solution 7** to the center of the white filter membrane. For efficient elution, use a minimum of 50 µl of **PowerClean® DNA Solution 7**, irrespective of the starting volume. By reducing the elution volume, it is possible to obtain DNA in a more concentrated form. For example, you can concentrate the DNA present in 150 µl initially, after going through the PowerClean® DNA Clean-Up protocol.

    Note: *Placing this Solution (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 release of the DNA from the silica Spin Filter membrane. As PowerClean® DNA Solution 7 (sterile elution buffer) passes through the silica membrane, DNA is released because it only stays bound to the silica Spin Filter membrane in the presence of high concentration of salt. PowerClean® DNA Solution 7 is 10mM Tris pH 8 and does not contain EDTA. Alternatively, sterile DNA-Free PCR Grade Water (MO BIO Laboratories Catalog# 17000-10) may be used for elution from the silica Spin Filter membrane at this step.*

18. Centrifuge the **Spin Filter** at 10,000 x *g* for 30 seconds at room temperature.

19. Discard the **Spin Filter**. The DNA in the **2 ml Collection Tube** is now application ready. No further steps are required. We recommend storing DNA frozen (-20° to -80°C). **PowerClean® DNA Solution 7** does not contain EDTA.

    Note: *If DNA degradation is a concern, sterile TE may also be used instead for elution of DNA from the Spin Filter.*

Thank you for choosing the PowerClean® DNA Clean-Up Kit.

Hints and Troubleshooting Guide

Amount of DNA to Process
This kit is designed to process up to 150 µl of DNA (20 µg maximum). For inquiries regarding the use of larger sample amounts, please contact technical support for suggestions.

If DNA Does Not Amplify
Make sure to check DNA yields by gel electrophoresis or spectrophotometer reading. Template DNA concentration could influence the outcome of PCR along with other the reaction conditions, enzyme activity, and copy number of the target sequence. If DNA does not amplify after altering the concentration of template DNA, please call our technical support for suggestions.

Eluted DNA Sample Is Brown
We have not observed any coloration in DNA isolated using the PowerClean® DNA Clean-Up Kit. If you observe coloration in your samples, please contact technical support for suggestions.

Concentrating the DNA
The final volume of eluted DNA will be up to 150 µl depending on the amount of starting material. The DNA may be concentrated by adding 1/10th volume of 5 M NaCl and inverting 3-5 times to mix. Next, add 200 µl of 100% cold ethanol and mix. Centrifuge at 10,000 x *g* for 15 minutes at room temperature. Decant all liquid. Remove residual ethanol in a speed vac, dessicator, or ambient air. Resuspend precipitated DNA in sterile water or 10 mM Tris.

DNA Floats Out of Well When Loaded on a Gel
Residual PowerClean® DNA Solution 6 remains in the final sample. Prevent this by being careful not to transfer liquid onto the bottom of the Spin Filter basket. Ethanol precipitation is the best way to remove residual PowerClean® DNA Solution 6. (See "Concentrating the DNA" above)

Storing DNA
DNA is eluted in PowerClean® DNA Solution 7 (10 mM Tris) and must be stored at -20°C to -80°C to prevent degradation. DNA can be eluted in TE but the EDTA may inhibit reactions such as PCR and automated sequencing. DNA may also be eluted with sterile DNA-Free PCR Grade Water (MO BIO Catalog# 17000-10).

Technical Tips
Visit MO BIO's *The Culture Dish* at http://www.mobio.com/blog/ for the latest in technical tips for frequently asked questions. Use this valuable resource to share your suggestions and optimization techniques for difficult or problematic samples.

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
PowerSoil® DNA Isolation Kit	12888-50 12888-100	50 preps 100 preps
PowerWater® DNA Isolation Kit	14900-50-NF 14900-100-NF	50 preps 100 preps
PowerMax® Soil DNA Isolation Kit	12988-10	10 preps
PowerPlant® Pro DNA Isolation Kit	13400-50 13400-100	50 preps 100 preps
PowerLyzer™ PowerSoil® DNA Isolation Kit	12855-50 12855-100	50 preps 100 preps