PowerClean® Pro DNA Clean-Up Kit

Catalog No.	Quantity
12997-50	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: 11052013

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Introduction

The PowerClean® Pro 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. This kit is a significantly streamlined improvement over the original PowerClean® DNA Clean-Up Kit providing for fewer steps with improved recoveries. 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® Pro 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. The resulting high purity DNA allows for more successful PCR amplification. This kit was validated with DNA isolated from a variety of problematic soils and also with DNA samples spiked 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 combined with 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

PowerClean® Pro DNA Isolation Kit

Prepare Sample

- Add DNA to 2 ml Collection Tube
- Add Solution DC1 and vortex briefly
- Add Solution DC2 and vortex briefly

Centrifuge

- Transfer supernatant to a new 2 ml Collection Tube
- Add Solution DC3

Centrifuge briefly

Bind DNA

- Load into Spin Filter

Centrifuge

Wash

- Discard flow through
- Add Solution DC4

Centrifuge

- Discard flow through
- Add Solution DC4

Centrifuge

- Discard flow through
- Place Spin Filter in a new 2 ml Collection Tube
- Add Solution DC5
- Incubate 1 minute

Elute

Centrifuge

Equipment Required

Microcentrifuge (16,000 x *g*)
Pipettor (50 µl - 600 µl)
Vortex-Genie® 2 Vortex (MO BIO Catalog# 13111-V or 13111-V-220)

Kit Contents

	Kit Catalog #12997-50	
Component	**Catalog #**	**Amount**
Solution DC1	12997-50-1	3 ml
Solution DC2	12997-50-2	3 ml
Solution DC3	12997-50-3	22 ml
Solution DC4	12997-50-4	2 x 28 ml
Solution DC5	12997-50-5	6 ml
Spin Filters	12997-50-SF	50
2 ml Collection Tubes	12997-50-T	150

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 DC4 contains ethanol. It is flammable.

IMPORTANT NOTE FOR USE: Shake to mix Solution DC3 before use.

Experienced User Protocol

Please wear gloves at all times

1. Add up to 100 µl of DNA sample to **2 ml Collection Tube** (provided). If less than 100 µl is added, adjust volume with distilled or deionized water.

2. Add 50 µl of **Solution DC1** to DNA. Vortex briefly to mix.

3. Add 50 µl of **Solution DC2** and vortex briefly to mix.

4. Centrifuge tubes at 13,000 x *g* for 2 minutes at room temperature.

5. Avoiding pellet, transfer the entire volume (expect 160 – 190 µl) of supernatant to a clean **2 ml Collection Tube** (provided).

6. Shake to mix **Solution DC3**. Add 400 µl of **Solution DC3** and vortex briefly to mix.

7. Centrifuge tubes briefly to remove any solution from the cap.

8. Load up to 600 µl onto **Spin Filter** and centrifuge at 10,000 x *g* for 1 minute at room temperature. Discard flow through.

9. Add 500 µl of **Solution DC4** to **Spin Filter** and centrifuge at 10,000 x *g* for 30 seconds at room temperature. Discard flow through.

10. Once again add 500 µl of **Solution DC4** to **Spin Filter** and centrifuge at 10,000 x *g* for 30 seconds at room temperature. Discard flow through.

11. Centrifuge **Spin Filter** at maximum speed for 2 minutes at room temperature to remove any residual ethanol from the wash in steps 9 & 10.

12. Carefully place **Spin Filter** in new **2 ml Collection Tube** (provided). Avoid splashing any **Solution DC4** onto **Spin Filter**.

13. If starting with 50 µl of genomic DNA, add 50 µl of **Solution DC5** to center of white filter membrane. If starting with 100 µl of genomic DNA, add 100 µl of **Solution DC5** to center of white filter membrane. Incubate 1 minute at room temperature.
    Note: For efficient elution, use a minimum of 50 µl of **Solution DC5**, irrespective of starting volume. By reducing elution volume, it is possible to obtain DNA in a more concentrated form.
    Centrifuge at 10,000 x *g* for 1 minute at room temperature.

14. Discard the **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). **Solution DC5** does not contain EDTA.

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

Detailed Protocol (Describes what is happening at each step)

Please wear gloves at all times

1. Add up to 100 µl of DNA sample to a **2 ml Collection Tube** (provided). If less than 100 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® Pro 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 50 µl of **Solution DC1** to DNA. Vortex briefly to mix.

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

3. Add 50 µl of **Solution DC2** and vortex briefly to mix.

   What's happening: Solution DC2 is patented Inhibitor Removal Technology® (IRT). It contains reagents that precipitate 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.

4. Centrifuge the tube at 13,000 x *g* for 2 minutes at room temperature.

5. 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.

   ***Note**: Expect between 160-190 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.*

6. Shake to mix **Solution DC3**. Add 400 µl of **Solution DC3** and vortex briefly to mix.

   What's happening: Solution DC3 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 to the Spin Filters, but not non-DNA organic and inorganic material that may still be present at low levels.

7. Centrifuge tubes briefly to remove any solution from the cap.

8. Load up to 600 µl onto **Spin Filter** and centrifuge at 10,000 x *g* for 1 minute at room temperature. Discard flow through.

   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.

9. Add 500 µl of **Solution DC4** to **Spin Filter** and centrifuge at 10,000 x *g* for 30 seconds at room temperature. Discard flow through.

10. Once again add 500 µl of **Solution DC4** to **Spin Filter** and centrifuge at 10,000 x *g* for 30 seconds at room temperature. Discard flow through.

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.

11. Centrifuge **Spin Filter** at maximum speed for 2 minutes at room temperature to remove any residual ethanol from the wash in steps 9 & 10.

    What's happening: This "drying" spin removes residual ethanol wash solution. It is critical to remove all traces of wash solution because the ethanol in Solution DC4 can interfere with many downstream applications such as PCR, restriction digests and gel electrophoresis.

12. Carefully place **Spin Filter** in new **2 ml Collection Tube** (provided). Avoid splashing any **Solution DC4** onto **Spin Filter**.

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

13. If starting with 50 µl of genomic DNA, add 50 µl of **Solution DC5** to center of white filter membrane. If starting with 100 µl of genomic DNA, add 100 µl of **Solution DC5** to center of white filter membrane. Incubate 1 minute at room temperature.
    Note: For efficient elution, use a minimum of 50 µl of **Solution DC5**, irrespective of starting volume. By reducing elution volume, it is possible to obtain DNA in a more concentrated form.
    Centrifuge at 10,000 x *g* for 1 minute at room temperature.

    What's happening: As Solution DC5 (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.

    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. Solution DC5 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. If DNA degradation is a concern, sterile TE may also be used instead for elution of DNA from the Spin Filter.*

14. 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). **Solution DC5** does not contain EDTA.

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

Hints and Troubleshooting Guide

Amount of DNA to Process
This kit is designed to process up to 100 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® Pro 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 100 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. Incubate at -20 C for 20 minutes. Centrifuge at 16,000 x *g* for 20 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 Solution DC4 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 Solution DC4. (See “Concentrating the DNA” above)

Storing DNA
DNA is eluted in Solution DC5 (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	50 preps
PowerLyzer® PowerSoil® DNA Isolation Kit	12855-50 12855-100	50 preps 100 preps