



# PowerClean<sup>ä</sup> DNA Clean-Up Kit

Catalog No.	Quantity
12877-50	50 Preps

## *Instruction Manual*

### **Introduction**

The PowerClean™ DNA Clean-Up Kit\* provides researchers with a novel and proprietary method for cleaning up genomic DNA previously isolated from environmental samples. 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. A high level of purity is achieved with the PowerClean™ DNA Clean-Up Kit allows 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.

The PowerClean™ DNA Clean-Up Kit distinguishes itself with a **NEW** Clean-Up procedure. This new procedure is effective at removing PCR inhibitors, and humic substance/brown color from even the most difficult DNA samples tested as well as from DNA samples spiked with humic acids at different concentrations.

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 DNA sample type. DNA is ready for PCR analysis and other downstream applications.

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

**\*PATENT PENDING**

Version: 07282006

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### Equipment Required

Microcentrifuge (10,000 x g)  
Pipettor (50 µl - 500 µl)  
Vortex

### Kit Contents

Component	Kit Catalog #12877-50	
	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	22 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 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 any 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 on our web site at [www.mobio.com](http://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.**

## Experienced User Protocol

Please wear gloves at all times

1. Add up to 150µl of DNA sample to Collection Tube (provided). If less than 150µl is added, adjust volume with distilled water.
2. Add 70µl 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 20µl of PowerClean™ DNA Solution 2 and invert 3-5 times to mix.
5. Add 85µl 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 Collection Tube (provided).
8. Add 70µl 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 Collection Tube (provided).
11. Add 400µl of PowerClean™ DNA Solution 5 to the supernatant and vortex for 5 seconds.
12. Load approximately 650µl onto Spin Filter and centrifuge at 10,000 x g for 1 minute at room temperature. Discard flow through. Add remaining 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 500µl 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.
15. Centrifuge Spin Filter at 10,000 x g for 1 minute at room temperature.
16. Carefully place Spin Filter in new Collection Tube (provided). Avoid splashing any PowerClean™ DNA Solution 6 onto Spin Filter.
17. If starting with 50µl of genomic DNA, add 50µl of PowerClean™ DNA Solution 7 to center of white filter membrane. If starting with 100 or 150µl of genomic DNA, add 100µl 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 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

Please wear gloves at all times

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

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

*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.

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

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

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

*This solution 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 250-325ml 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 Collection Tube (provided).

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

*This solution 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.*

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

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

*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. Add 400µl of PowerClean™ DNA Solution 5 to the supernatant (be careful that solution doesn't exceed rim of Collection Tube) and vortex for 5 seconds.

*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 650µl 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 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.

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

*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 Collection Tube.

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

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

*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 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 50µl of genomic DNA, add 50µl of PowerClean™ DNA Solution 7 to the center of the white filter membrane. If starting with 100 or 150µl of genomic DNA, add 100µl 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 No. 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 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.**



## **Additional Information**

### ***Amount of DNA to Process***

This kit is designed to process up to 150µl 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 150µl depending on the amount of starting material. The DNA may be concentrated by adding 1/10<sup>th</sup> volume of 5M NaCl and inverting 3-5 times to mix. Next, add 200µl 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 Solution 6. (See "Concentrating the DNA" above)

### ***Storing DNA***

DNA is eluted in PowerClean™ DNA Solution 7 (10mM 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 Laboratories Inc. Catalog No. 17000-10).



## Other Quality Products Available from MO BIO Laboratories, Inc.

<u>Product Description</u>	<u>Catalog No.</u>
<b>DNA Isolation Kits</b>	
PowerSoil™ DNA Isolation Kit (50 preps)	12888-50
PowerSoil™ DNA Isolation Kit (100 preps)	12888-100
PowerMax™ Soil DNA Isolation Kit (10 preps)	12988-10
PowerSoil™ htp 96 Well Soil DNA Isolation Kit (4 x 96 preps)	12955-4
UltraClean™ Soil DNA Isolation Kit (50 preps)	12800-50
UltraClean™ Mega Soil DNA Isolation Kit (10 preps)	12900-10
UltraClean-htp™ 96 Well Soil DNA Isolation Kit (4 x 96 preps)	12896-4
UltraClean™ Fecal DNA Isolation Kit (50 preps)	12811-50
UltraClean™ Microbial DNA Isolation Kit (50 preps)	12224-50
<b>RNA Isolation Kits</b>	
RNA PowerSoil™ Total RNA Isolation Kit (25 preps)	12866-25
UltraClean™ Microbial RNA Isolation Kit (50 preps)	15800-50
<b>DNA Purification Kits</b>	
UltraClean™ 15 DNA Purification Kit (300 preps)	12100-300
UltraClean™ GelSpin™ DNA Purification Kit (100 preps)	12400-100
UltraClean™ PCR Clean-Up™ Kit (100 preps)	12500-100

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