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DNeasy® PowerClean® Pro Cleanup Kit Handbook

For the removal of PCR inhibitors from purified DNA in just 7 minutes

Sample to Insight

QIAGEN

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Kit Contents

DNeasy PowerClean Pro Cleanup Kit	**(50)**
Catalog no.	**12997-50**
Number of preps	**50**
MB Spin Columns	50
Solution CU	4 ml
Solution IR	2 x 1.5 ml
Solution SB	26 ml
Solution CB	2 x 30 ml
Solution EB	9 ml
Collection Tubes (2 ml)	3 x 50
Quick Start Protocol	1

Storage

The DNeasy PowerClean Pro Cleanup Kit can be stored at room temperature (15–25°C) until the expiration date printed on the label.

Intended Use

All DNeasy products are intended for molecular biology applications. These products are not intended for the diagnosis, prevention or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at **www.qiagen.com/safety** where you can find, view and print the SDS for each QIAGEN kit and kit component.

WARNING 	**Solution CB contains ethanol and is flammable.**

CAUTION 	**DO NOT add bleach or acidic solutions directly to the sample preparation waste**

Solution CU and Solution SB contain guanidine salts, which can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilt, clean with a suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of DNeasy PowerClean Pro Cleanup Kits is tested against predetermined specifications to ensure consistent product quality.

Introduction

The DNeasy PowerClean Pro Cleanup Kit uses Inhibitor Removal Technology® (IRT) to provide researchers with a novel and proprietary method for cleaning up previously isolated genomic DNA. We have significantly streamlined previous versions of the kit, and it now provides improved recoveries with fewer steps.

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 using this kit. The DNeasy PowerClean Pro Cleanup Kit removes brown color as well as any PCR-inhibiting substances, such as heme, polysaccharides, polyphenols, fulvic acids and dyes. The isolated DNA has a high level of purity, which allows for more successful PCR amplification. This kit has been 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.

Principle and procedure

Archived or previously isolated DNA samples are purified when combined with our proprietary DNA clean up reagents, and 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. The DNA is then washed and eluted from the membrane. Percentage recovery may vary depending on the level of inhibitors influencing DNA yield measurements. Purified DNA is ready for PCR analysis and other downstream applications.

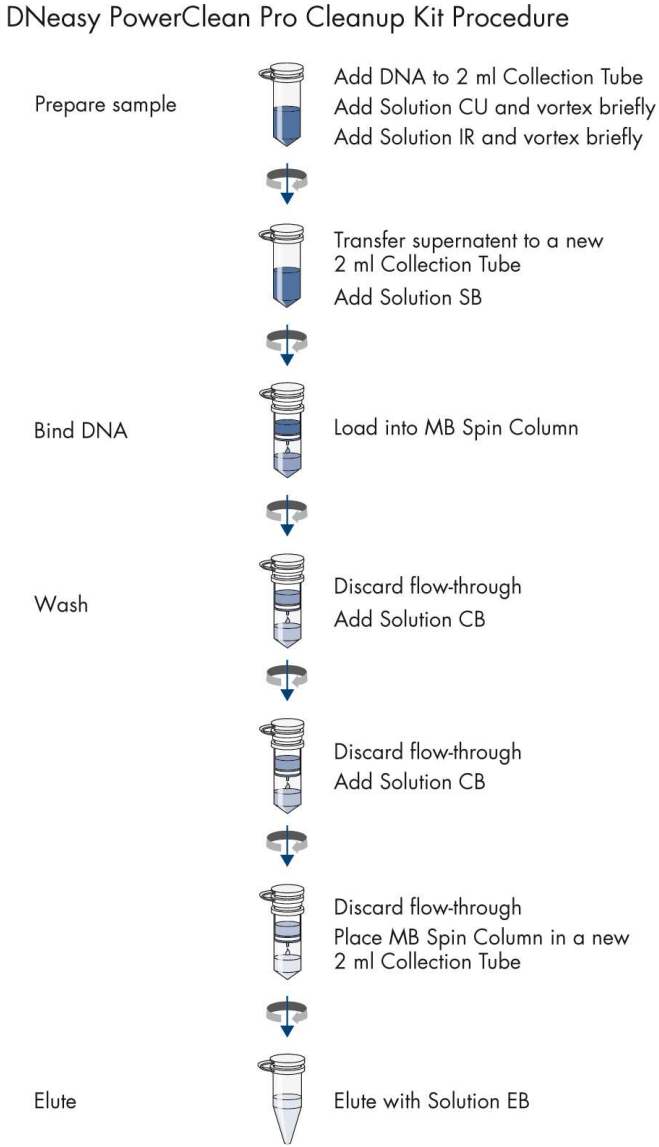

Figure 1. DNeasy PowerClean Pro CleanUp Kit procedure

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, consult the appropriate safety data sheets (SDSs) available from the product supplier.

- Microcentrifuge (up to16,000 x *g*)
- Pipettor (50–600 µl)
- Vortex-Genie® 2
- Vortex Adapter for vortexing 1.7 or 2 ml tubes (cat. no. 13000-V1-24)

Protocol: Experienced User

Important points before starting

- Shake to mix Solution SB.
- Wear gloves at all times.

Procedure

1. Add up to 100 µl of DNA sample to a clean 2 ml Collection Tube (provided). If there is less than 100 µl of sample, adjust volume with deionized or PCR-grade water.
2. Add 50 µl of Solution CU and vortex briefly to mix.
3. Add 50 µl of Solution IR and vortex briefly to mix.
4. Centrifuge tubes at 13,000 x *g* for 2 min at room temperature.
5. Taking care to avoid the pellet, transfer the entire volume of supernatant to a clean 2 ml Collection Tube (provided).
   Note: Expect 160–190 µl of supernatant.
6. Shake to mix Solution SB. Add 400 µl of Solution SB and vortex briefly to mix.
7. Centrifuge tubes briefly to remove any solution from the cap.
8. Load up to 600 µl onto an MB Spin Column and centrifuge at 10,000 x *g* for 1 min at room temperature. Discard the flow-through.
9. Add 500 µl of Solution CB to the MB Spin Column and centrifuge at 10,000 x *g* for 30 s at room temperature. Discard the flow-through.
10. Repeat Step 9 and then proceed to Step 11.
11. Centrifuge the MB Spin Column at maximum speed for 2 min at room temperature.
12. Carefully place the MB Spin Column in new 2 ml Collection Tube (provided). Avoid splashing any Solution CB onto the MB Spin Column.

13. Add 50–100 µl of Solution EB (depending on starting volume of DNA sample) to the center of the white filter membrane. Incubate for 1 min at room temperature.
    Note: For efficient elution, use a **minimum** of 50 µl of Solution EB, irrespective of starting sample volume. By reducing elution volume, it may be possible to obtain more concentrated DNA.
14. Centrifuge at 10,000 x *g* for 1 min at room temperature.
15. Discard the MB Spin Column. The DNA is now ready for downstream applications.
    Note: We recommend storing DNA frozen (–20°C to –80°C) as Solution EB does not contain EDTA.

Protocol: Detailed

Important points before starting

- Shake to mix Solution SB.
- Wear gloves at all times.

Procedure

1. Add up to 100 µl of DNA sample to a clean 2 ml Collection Tube (provided). If there is less than 100 µl of sample, adjust volume with deionized or PCR-grade water.
   Note: After the sample has been added to the Collection Tube, a disassociation procedure will be performed. The solutions in the DNeasy PowerClean Pro Cleanup Kit 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 CU and vortex briefly to mix.
   Note: Brief vortexing mixes the components in the tube and begins to disassociate DNA from PCR-inhibiting substances.
3. Add 50 µl of Solution IR and vortex briefly to mix.
   Note: Solution IR contains Inhibitor Removal Technology (IRT): 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 tubes at 13,000 x *g* for 2 min at room temperature.
5. Taking care to avoid the pellet, transfer the entire volume of supernatant to a clean 2 ml Collection Tube (provided).
   Note: Expect 160–190 µl of supernatant.
   Note: 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.

6. Shake to mix Solution SB. Add 400 µl of Solution SB and vortex briefly to mix.
   Note: DNA binds tightly to silica at high salt concentrations. Solution SB has a high salt concentration, which allows binding of DNA, but not non-DNA organic and inorganic material that may still be present at low levels, to MB Spin Columns.

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

8. Load up to 600 µl onto an MB Spin Column and centrifuge at 10,000 x *g* for 1 min at room temperature. Discard the flow-through.
   Note: DNA is selectively bound to the silica membrane in the MB Spin Column in the presence of high salt solution. Contaminants pass through the filter membrane, leaving only DNA bound to it.

9. Add 500 µl of Solution CB to the MB Spin Column and centrifuge at 10,000 x *g* for 30 s at room temperature. Discard the flow-through.

10. Repeat Step 9 and then proceed to Step 11.
    Note: Solution CB is an ethanol-based wash solution used to further clean the DNA that is bound to the silica filter membrane in the MB Spin Column. Solution CB removes residues of salt, humic substances and other contaminants while allowing the DNA to stay bound to the silica membrane.

11. Centrifuge the MB Spin Column at maximum speed for 2 min at room temperature.
    Note: This drying spin removes residual Solution CB, which contains ethanol. It is critical to remove all traces of Solution CB because the ethanol can interfere with several downstream applications such as PCR, restriction digests and gel electrophoresis.

12. Carefully place the MB Spin Column in new 2 ml Collection Tube (provided). Avoid splashing any Solution CB onto the MB Spin Column.

13. Add 50–100 µl of Solution EB (depending on starting volume of DNA sample) to the center of the white filter membrane. Incubate for 1 min at room temperature.
    Note: For efficient elution, use a **minimum** of 50 µl of Solution EB, irrespective of starting sample volume. By reducing elution volume, it may be possible to obtain more concentrated DNA.

Note: As Solution EB (sterile elution buffer) passes through the silica membrane of the MB Spin Column, the DNA is released because it only stays bound to the silica membrane in the presence of high concentration of salt.

Note: Placing Solution EB in the center of the small white membrane will make sure the entire membrane is wetted. This will result in more efficient release of DNA from the silica MB Spin Column membrane. Alternatively, sterile DNA-free PCR-grade Water (cat. no. 17000-10) may be used for elution. If DNA degradation is a concern, sterile TE may be used instead.

14. Centrifuge at 10,000 x *g* for 1 min at room temperature.
15. Discard the MB Spin Column. The DNA is now ready for downstream applications.
    Note: We recommend storing DNA frozen (–20°C to –80°C) as Solution EB does not contain EDTA.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: **www.qiagen.com/FAQ/FAQList.aspx**. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and/or protocols in this handbook or sample and assay technologies. For contact information, visit **www.qiagen.com**.

		Comments and suggestions
Sample Processing/DNA		
a)	Amount of DNA to process	This kit is designed to process up to 100 µl of DNA (20 µg maximum). For inquiries about using larger amounts of sample, please contact QIAGEN Technical Services.
b)	DNA does not amplify	Check DNA yields by gel electrophoresis or spectrophotometer reading. Template DNA concentrations and other reaction conditions, such as enzyme activity and copy number of the target sequence, can influence PCR outcomes. If DNA does not amplify after altering the concentration of template DNA, please contact QIAGEN Technical Services.
c)	Eluted sample is brown	We have not observed any coloration in DNA isolated using the DNeasy PowerClean Pro Cleanup Kit. If you observe coloration in your samples, please contact QIAGEN Technical Services.
d)	DNA floats out of a well when loading a gel	This usually occurs because residual ethanol remains in the final sample. Avoid transferring any Solution CB to the elution step. Ethanol precipitation (described in "Concentrating eluted DNA") is the best way to remove residual ethanol.
e)	Concentrating eluted DNA	The final volume of eluted DNA will be 50–100 µl. The DNA may be concentrated by adding 5–10 µl of 3 M NaCl and inverting 3–5 times to mix. Next, add 100 µl of 100% cold ethanol and invert 3–5 times to mix. Incubate at –20°C for 30 minutes and centrifuge at 10,000 x *g* for 5 minutes at room temperature. Decant all liquid. Briefly dry residual ethanol in a speed vac or ambient air. Avoid over-drying the pellet or resuspension may be difficult. Resuspend precipitated DNA in desired volume of 10 mM Tris (Solution EB).

		Comments and suggestions
f)	Storing DNA	DNA is eluted in Solution EB (10 mM Tris) and must be stored at –20°C 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 in DNA-free PCR-grade water (cat. no. 17000-10).

Ordering Information

Product	Contents	Cat. no.
DNeasy PowerClean Pro Cleanup Kit (50)	For 50 preps: Remove PCR inhibitors from purified DNA in just 7 minutes	12997-50
DNeasy PowerSoil® Kit (50)	For 50 preps: Isolate microbial genomic DNA from all soil types	12888-50
DNeasy PowerSoil Kit (100)	For 100 preps: Isolate microbial genomic DNA from all soil types	12888-100
DNeasy PowerMax® Soil Kit (10)	For 10 preps: Isolate microbial DNA from large quantities of soil; great for samples with low microbial load	12988-10
DNeasy PowerLyzer® PowerSoil Kit (50)	For 50 preps: Isolate DNA from tough soil microbes; optimized for use with bead-based homogenizers	12855-50
DNeasy PowerLyzer PowerSoil Kit (100)	For 100 preps: Isolate DNA from tough soil microbes; optimized for use with bead-based homogenizers	12855-100
DNeasy PowerPlant® Pro Kit (50)	For 50 preps: Isolate genomic DNA from plant and seed samples, removes polyphenolics and polysaccharides	13400-50
DNeasy PowerWater® Kit (50)	For 50 preps: Isolate genomic DNA from filtered water samples, including turbid water	14900-50-NF
DNeasy PowerWater Kit (100)	For 100 preps: Isolate genomic DNA from filtered water samples, including turbid water	14900-100-NF

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

Notes

Limited License Agreement for DNeasy PowerClean Pro Cleanup Kit

Use of this product signifies the agreement of any purchaser or user of the product to the following terms:

1. The product may be used solely in accordance with the protocols provided with the product and this handbook and for use with components contained in the kit only. QIAGEN grants no license under any of its intellectual property to use or incorporate the enclosed components of this kit with any components not included within this kit except as described in the protocols provided with the product, this handbook, and additional protocols available at www.qiagen.com. Some of these additional protocols have been provided by QIAGEN users for QIAGEN users. These protocols have not been thoroughly tested or optimized by QIAGEN. QIAGEN neither guarantees them nor warrants that they do not infringe the rights of third-parties.
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