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DNeasy® PowerClean® Cleanup Kit Handbook

For secondary DNA cleanup and removal of heparin-related inhibitors

Sample to Insight

QIAGEN

Contents

Kit Contents

DNeasy PowerClean Cleanup Kit	**(50)**
Catalog no.	**12877-50**
Number of preps	**50**
Solution CU	4 ml
Solution SL	1.5 ml
Solution AA	5 ml
Solution IRS	15 ml
Solution SB	50 ml
Solution CB	30 ml
Solution EB	9 ml
MB Spin Columns	50
Collection Tubes (2 ml)	4 x 50
Quick Start Protocol	1

Storage

The DNeasy PowerClean Cleanup Kit reagents and components should be stored at room temperature (15–25°C).

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 and Solution SB contain alcohol and are 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 Cleanup Kits is tested against predetermined specifications to ensure consistent product quality.

Introduction

The DNeasy PowerClean Cleanup Kit utilizes Inhibitor Removal Technology® (IRT) to provide researchers with a method to clean 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 DNeasy PowerClean Cleanup Kit will remove colors and PCR-inhibiting substances including heme, polysaccharides, polyphenols, fulvic acids and dyes. Resultant DNA is of high purity, allowing for more successfully amplified DNA through PCR. This kit has been successfully used with DNA isolated from a range of problematic soils as well as samples artificially spiked with commercial humic acids. The DNeasy PowerClean Cleanup Kit performs well on DNA isolated from any sample source.

Principle and procedure

Archived or previously isolated DNA samples are purified when combined with our proprietary DNA Cleanup 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.

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 (SDS) available from the product supplier.

- Microcentrifuge (13,000 x *g*)
- Pipettor (20–800 µl)
- Vortex-Genie® 2 Vortex

Protocol: Experienced User

Notes before starting

- Shake to mix Solution SB.
- If Solution SL has precipitated, heat at 60°C, gently inverting the tube periodically, until the precipitate has dissolved. Solution SL may be used while still warm.
- Wear gloves at all times.

Procedure

1. Add up to 150 µl of DNA sample to a clean 2 ml Collection Tube (provided). If there is less than 150 µl of sample, adjust volume with distilled or deionized water.
2. Add 70 µl of Solution CU to DNA. Gently invert 3–5 times to mix.
3. Add 20 µl of Solution SL and invert 3–5 times to mix.
4. Add 85 µl of Solution AA and invert 3–5 times to mix. Incubate at 2–8°C for 5 min.
5. Centrifuge tubes at 10,000 x *g* for 1 min at room temperature.
6. Taking care to avoid the pellet, transfer the entire volume of the supernatant to a clean 2 ml Collection Tube (provided).
7. Add 70 µl of Solution IRS and invert 3–5 times to mix. Incubate at 2–8°C for 5 min.
8. Centrifuge tubes at 10,000 x *g* for 1 min at room temperature.
9. Taking care to avoid the pellet, transfer the entire volume of the supernatant to a clean 2 ml Collection Tube (provided).
10. Add 800 µl of Solution SB and vortex for 5 s.
11. Load approximately 600 µl into an MB Spin Column and centrifuge at 10,000 x *g* for 1 min at room temperature. Discard flow-through.
12. Add the remaining 600 µl supernatant to the MB Spin Column and centrifuge at 10,000 x *g* for 1 min at room temperature.
    Note: A total of two loads may be required for each sample processed.

13. 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 flow-through.
    Note: An additional wash of the MB Spin Column with 100% ethanol may be performed prior to the final dry spin. This may enhance the purity of the DNA obtained from some sample types. After discarding the flow-through in step 13, add 650 µl of 100% ethanol to the spin column and centrifuge at 10,000 x *g* for 30 s. To completely remove residual ethanol, discard the flow-through and increase the dry spin (step 14) to 2 min at 13,000 x *g* or max speed. Then continue with step 15.
14. Centrifuge the MB Spin Column at 13,000 x *g* for 1 min at room temperature.
15. Carefully place the MB Spin Column in new 2 ml Collection Tube (provided). Avoid splashing any Solution CB onto the MB Spin Column.
16. 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 is possible to obtain more concentrated DNA.
17. Centrifuge at 10,000 x *g* for 30 s at room temperature.
18. Discard the MB Spin Column. The DNA is now ready for downstream applications.
    Note: We recommend storing DNA frozen (–15 to –30°C or –65 to –90°C) as Solution EB does not contain EDTA.

Protocol: Detailed

Notes before starting

- Shake to mix Solution SB.
- If Solution SL has precipitated, heat at 60°C, gently inverting the tube periodically, until the precipitate has dissolved. Solution SL may be used while still warm.
- Wear gloves at all times.

Procedure

1. Add up to 150 µl of DNA sample to a clean 2 ml Collection Tube (provided). If there is less than 150 µl of sample, adjust volume with distilled or deionized water.
   Note: After the sample has been added to the 2 ml Collection Tube, it is disassociated through the use of the DNeasy PowerClean Cleanup Kit solutions. The 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 Solution CU to DNA. Gently invert 3–5 times to mix.
   Note: Gentle inversion of the tube mixes the components in the tube and begins to disassociate DNA from PCR-inhibiting substances.
3. Add 20 µl of Solution SL and invert 3–5 times to mix.
   Note: Solution SL contains detergents and other agents required for complete dissociation. The chemicals in Solution SL will precipitate under cold storage conditions. Heating to 60°C will dissolve the reagent or the other disassociation agents. Solution SL can be used while warm.
4. Add 85 µl of Solution AA and invert 3–5 times to mix. Incubate at 2–8°C for 5 min.
   Note: Solution AA has 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.
5. Centrifuge tubes at 10,000 x *g* for 1 min at room temperature.

6. Taking care to avoid the pellet, transfer the entire volume of the supernatant to a clean 2 ml Collection Tube (provided).
   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.
7. Add 70 µl of Solution IRS and invert 3–5 times to mix. Incubate at 2–8°C for 5 min.
   Note: Solution IRS is the second part of the IRT process and 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.
8. Centrifuge Tubes at 10000 x *g* for 1 min at room temperature.
9. Taking care to avoid the pellet, transfer the entire volume of supernatant into a clean 2 ml Collection Tube (provided).
   Note: 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.
10. Add 800 µl of Solution SB and vortex for 5 s.
    Note: Solution SB has a high salt concentration. Since DNA binds tightly to silica at high salt concentrations, this solution will adjust the salt concentration to allow binding of DNA, but not non-organic and inorganic material that may still be present at low levels, to the MB Spin Column filter membrane. DNA is selectively bound to the silica membrane in the MB Spin Column in the high-salt solution. Contaminants pass through the filter membrane, leaving only the DNA bound to the membrane.
11. Load approximately 600 µl into an MB Spin Column and centrifuge at 10,000 x *g* for 1 min at room temperature. Discard flow-through.
12. Add the remaining 600 µl supernatant to the MB Spin Column and centrifuge at 10,000 x *g* for 1 min at room temperature.
    Note: A total of two loads may be required for each sample processed.

13. 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 flow-through.
    Note: An additional wash of the MB Spin Column with 100% ethanol may be performed prior to the final dry spin. This may enhance the purity of the DNA obtained from some sample types. After discarding the flow-through in step 13, add 650 µl of 100% ethanol to the spin column and centrifuge at 10,000 x *g* for 30 s. To completely remove residual ethanol, discard the flow-through and increase the dry spin (step 14) to 2 min at 13,000 x *g* or max speed. Then continue with step 15.
    Note: Solution CB is an ethanol-based wash solution that further cleans the DNA bound to the silica filter membrane in the MB Spin Column. This solution removes residues of salt, humic substances and other contaminants while allowing DNA to stay bound to the silica membrane. The flow-through fraction is non-DNA organic and inorganic waste removed from the MB Spin Column filter membrane by the ethanol wash.
14. Centrifuge the MB Spin Column at 13,000 x *g* for 1 min at room temperature.
15. Carefully place the MB Spin Column in new 2 ml Collection Tube (provided). Avoid splashing any Solution CB onto the MB Spin Column.
16. 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 is possible to obtain more concentrated DNA.
17. Centrifuge at 10,000 x *g* for 30 s at room temperature.
18. Discard the MB Spin Column. The DNA is now ready for downstream applications.
    Note: We recommend storing DNA frozen (–15 to –30°C or –65 to –90°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
DNA		
a)	Amount of DNA to process is large	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.
b)	DNA does not amplify	Make sure the check DNA yields by gel electrophoresis or spectrophotometer reading. Template DNA concentration could influence the outcome of PCR along with other 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.
c)	DNA floats out of a well when loading a gel	Residual Solution CB remains in the final sample. Prevent this by being careful not to transfer liquid onto the bottom of the MB Spin Column filter basket. Ethanol precipitation is the best way to remove residual Solution CB. (See concentrating DNA).
d)	Concentrating eluted 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.
e)	Eluted DNA sample is brown	We have not observed any coloration in DNA isolated using the DNeasy PowerClean Cleanup Kit. If you observe coloration in your samples, please contact technical support for suggestions.

		Comments and suggestions
f)	DNA storage	DNA is eluted in Solution EB (10 mM Tris) and must be stored at –15 to –30°C or –65 to –90°C to prevent degradation. DNA can be eluted in TE but EDTA may inhibit reactions such as PCR and automated sequencing. DNA may also be eluted with DNase-Free PCR Grade Water (cat. no. 17000-10).
g)	DNA has low A260/280 ratio	The ratio for pure DNA should be 1.7–1.9. Any $A_{260/280}$ reading below 1.6 may indicate significant protein contamination. A low $A_{260/280}$ ratio may also occur when the sample is measured by UV spectrophotometry in water. The low pH of water can influence the 280 reading and cause reduced sensitivity to protein contamination. Remeasure the 260/280 by diluting the DNA in 10 mM Tris pH 7.5. If using a Nanodrop™, blank the instrument with Solution EB.

Ordering Information

Product	Contents	Cat. no.
DNeasy PowerClean Cleanup Kit (50)	For 50 preps: For secondary DNA cleanup and removal of inhibitors from heparin	12877-50
Related products		
DNeasy PowerSoil® Kit (50/100)	For 50 or 100 preps: For the isolation of bacterial DNA from soil samples	12888-50 12888-100
DNeasy PowerWater® Kit (50/100)	For 50 or 100 preps: For the isolation of bacterial DNA from water samples	14900-50 14900-100
DNeasy PowerMax® PowerSoil Kit (10)	For 10 preps: For high-throughput isolation of bacterial DNA from soil samples	12988-10
DNeasy PowerPlant® Pro Kit (50)	For 50 preps: For the isolation of genomic DNA from plant and seed samples	13400-50
DNeasy PowerLyzer® PowerSoil Kit (50/100)	For 50 or 100 preps: For the isolation of DNA from tough soil samples	12855-50 12855-100

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.

Revision History

Document	Description of changes
HB-2260-001	Initial release.
HB-2260-002	Kit Contents volumes updated: • Solution IRS (15 ml instead of 11 ml) • Solution EB (9 ml instead of 8 ml) DNA storage temperatures updated to reflect appropriate temperature ranges. Editorial changes.

Notes

Notes

Limited License Agreement for DNeasy PowerClean Cleanup Kit

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

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