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DNeasy® UltraClean® Microbial Kit Handbook

For the isolation of high-quality DNA from microbial cultures

Sample to Insight

QIAGEN

Contents

Kit Contents

DNeasy UltraClean Microbial Kit	**(50)**	**(250)**
Catalog no.	**12224-50**	**12224-250**
Number of preps	**50**	**250**
PowerBead Tubes, Garnet 100	50	5 x 50
PowerBead Solution	16.5 ml	2 x 42 ml
MB Spin Columns	50	5 x 50
Solution SL	2 x 1.5 ml	15 ml
Solution IRS	15 ml	44 ml
Solution SB	50 ml	250 ml
Solution CB	30 ml	3 x 30 ml
Solution EB	9 ml	2 x 9 ml
Collection Tubes (2 ml)	4 x 50	20 x 50
Quick Start Protocol	1	1

Storage

The DNeasy UltraClean Microbial Kit reagents and components can be stored at room temperature (15–25°C) until the expiration date printed on the box 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 alcohol and is flammable.

WARNING: Do not use bleach to clean the inside of the QIAvac® 24 Plus Manifold.

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

PowerBead Solution 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 UltraClean Microbial Kits is tested against predetermined specifications to ensure consistent product quality.

Introduction

The DNeasy UltraClean Microbial Kit is designed to isolate high-quality genomic DNA from microorganisms. A variety of microorganisms, including bacterial and fungal spores, have been tested successfully with this kit.

Principle and procedure

Microbial cells are resuspended in a bead solution and added to a bead beating tube containing beads. Then, lysis solution is added. The microorganisms are lysed by a combination of heat, detergent and mechanical force against specialized beads. The cellular components are lysed by mechanical action using a specially designed Vortex Adapter on a standard vortex. The DNA released from the lysed cells is bound to a silica spin filter. The spin filter is washed, and the DNA is recovered in DNA-free Tris buffer.

High-throughput options

We offer a vacuum-based protocol for faster processing without centrifugation for the DNA-binding and column-washing steps. The QIAvac 24 Plus Manifold (cat. no. 19413) allows for processing of up to 24 MB Spin Column preps at a time.

For additional high-throughput options, the DNeasy Ultraclean 96 Microbial Kit (cat. no. 10196-4) is available for processing up to 4 x 96 samples using a centrifuge capable of spinning two 96 Well Blocks stacked (13 cm x 8 cm x 5.5 cm) at 2500 x *g*. For 96-well homogenization of bacteria, we offer the TissueLyzer II and Plate Adapter Set (cat. no. 85300 and 11990, respectively).

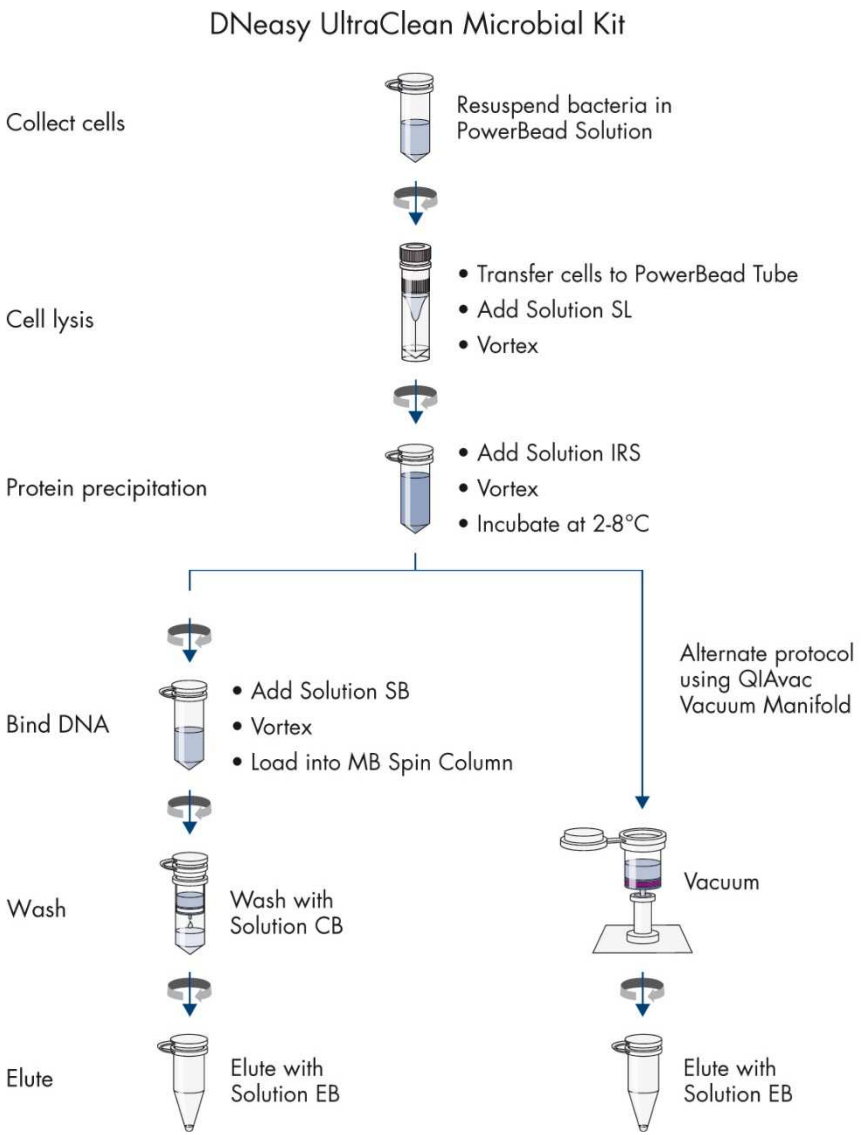

Figure 1. DNeasy UltraClean Microbial Kit procedure.

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 (10,000 x *g*)
- Pipettor (50–200 µl; 100–1000 µl)
- Vortex-Genie® 2
- Vortex Adapter for 24 (1.5–2.0 ml) tubes (cat. no. 13000-V1-24)
- QIAvac 24 Plus Vacuum Manifold
- 100% ethanol (for QIAvac 24 Plus Vacuum Manifold protocol)

Protocol: Experienced User

Important points before starting

- If Solution SL has precipitated, heat at 55°C for 5–10 min.
- Shake to mix Solution SB before use.

Procedure

1. Add 1.8 ml of microbial (bacteria, yeast) culture to a 2 ml Collection Tube (provided) and centrifuge at 10,000 x *g* for 30 s at room temperature. Decant the supernatant and spin the tubes again at 10,000 x *g* for 30 s at room temperature. Completely remove the supernatant with a pipette tip.
   Note: Depending on the type of microbial culture, it may be necessary to centrifuge longer than 30 s.
2. Resuspend the cell pellet in 300 µl of PowerBead Solution and gently vortex to mix. Transfer resuspended cells to a PowerBead Tube.
3. Add 50 µl of Solution SL to the PowerBead Tube.
   Note: To increase yields, to minimize DNA shearing or for difficult cells, refer to the Troubleshooting Guide.
4. Secure PowerBead Tubes horizontally using the Vortex Adapter (cat. no. 13000-V1-24). Vortex at maximum speed for 10 min.
5. Make sure the 2 ml PowerBead Tubes rotate freely in the centrifuge without rubbing. Centrifuge the tubes at a **maximum** of 10,000 x *g* for 30 s at room temperature.
6. Transfer the supernatant to a clean 2 ml Collection Tube (provided).
   Note: Expect 300–350 µl of supernatant.
7. Add 100 µl of Solution IRS to the supernatant and vortex for 5 s. Incubate at 4°C for 5 min.
8. Centrifuge the tubes at 10,000 x *g* for 1 min at room temperature.

9. Avoiding the pellet, transfer the entire volume of supernatant to a clean 2 ml Collection Tube (provided).
   Note: Expect 450 µl of supernatant.
10. Add 900 µl of Solution SB to the supernatant and vortex for 5 s.
11. Load about 700 µl into an MB Spin Column and centrifuge at 10,000 x *g* for 30 s at room temperature. Discard the flow-through, add the remaining supernatant to the MB Spin Column, and centrifuge again at 10,000 x *g* for 30 s at room temperature.
    Note: Each sample processed will require 2–3 loads. Discard all flow-through.
12. Add 300 µl of Solution CB and centrifuge at 10,000 x *g* for 30 s at room temperature.
13. Discard the flow-through. Centrifuge at 10,000 x *g* for 1 min at room temperature.
14. Place the MB Spin Column in a new 2 ml Collection Tube (provided).
    Note: Be careful not to splash any of the liquid on the MB Spin Column.
15. Add 50 µl of Solution EB to the center of the white filter membrane.
16. Centrifuge at 10,000 x *g* for 30 s at room temperature.
17. 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. To concentrate DNA, see the Troubleshooting Guide.

Protocol: Detailed

Important points before starting

- If Solution SL has precipitated, heat at 55°C for 5–10 min.
- Shake to mix Solution SB before use.

Procedure

1. Add 1.8 ml of microbial (bacteria, yeast) culture to a 2 ml Collection Tube (provided) and centrifuge at 10,000 x *g* for 30 s at room temperature. Decant the supernatant and spin the tubes again at 10,000 x *g* for 30 s at room temperature. Completely remove the supernatant with a pipette tip.
   Note: Depending on the type of microbial culture, it may be necessary to centrifuge longer than 30 s. This step concentrates and pellets the microbial cells. It is important to pellet the cells completely and remove all the culture media in this step.
2. Resuspend the cell pellet in 300 µl of PowerBead Solution and gently vortex to mix. Transfer resuspended cells to a PowerBead Tube.
   Note: The PowerBead Solution contains salts and a buffer that stabilizes and homogeneously disperses the microbial cells prior to lysis.
3. Add 50 µl of Solution SL to the PowerBead Tube.
   Note: To increase yields, to minimize DNA shearing or for difficult cells, refer to the Troubleshooting Guide. Solution SL contains SDS and other disruption agents required for cell lysis. In addition to aiding in cell lysis, SDS also breaks down fatty acids and lipids associated with the cell membrane of several organisms. SDS may precipitate when cold but heating at 55°C will dissolve the SDS. Solution SL can be used while it is still warm.
4. Secure PowerBead Tubes horizontally using the Vortex Adapter (cat. no. 13000-V1-24). Vortex at maximum speed for 10 min.
   Note: This step creates the combined chemical/mechanical lysis conditions required to release desired nucleic acids from microbial cells. Many cell types will not lyse without this chemically enhanced bead beating process. The vortex action is typically all that is

required; however, more robust bead beaters may also be used. In most cases bead beating times may be shorter with other devices but you run the risk of increased DNA shearing. This process is compatible with fast prep machines.

5. Make sure the 2 ml PowerBead Tubes rotate freely in the centrifuge without rubbing. Centrifuge the tubes at a **maximum** of 10,000 x *g* for 30 s at room temperature.
   Note: The cell debris is sent to the bottom of the tube while DNA remains in the supernatant.
6. Transfer the supernatant to a clean 2 ml Collection Tube (provided).
   Note: Expect 300–350 µl of supernatant. Volume will vary depending on the size of the cell pellet in Step 1.
7. Add 100 µl of Solution IRS to the supernatant and vortex for 5 s. Incubate at 4°C for 5 min.
8. Centrifuge the tubes at 10,000 x *g* for 1 min at room temperature.
   Note: Solution IRS contains a reagent to precipitate non-DNA organic and inorganic material, including cell debris and proteins. It is important to remove contaminating organic and inorganic matter that may reduce DNA purity and inhibit downstream DNA applications.
9. Avoiding the pellet, transfer the entire volume of supernatant to a clean 2 ml Collection Tube (provided).
   Note: Expect 450 µl of supernatant. The pellet at this point contains non-DNA organic and inorganic materials, including cell debris and proteins. For the best DNA quality and yield, avoid transferring any of the pellet.
10. Add 900 µl of Solution SB to the supernatant and vortex for 5 s.
    Note: Solution SB is a highly concentrated salt solution. It sets up the high-salt condition necessary to bind DNA to the MB Spin Column membrane in the following step.
11. Load about 700 µl into a MB Spin Column and centrifuge at 10,000 x *g* for 30 s at room temperature. Discard the flow-through, add the remaining supernatant to the MB Spin Column, and centrifuge again at 10,000 x *g* for 30 s at room temperature.

Note: Each sample processed will require 2–3 loads. Discard all flow-through. DNA is selectively bound to the MB Spin Column silica membrane. Contaminants pass through the filter membrane, leaving only the DNA bound.

12. Add 300 µl of Solution CB and centrifuge at 10,000 x *g* for 30 s at room temperature.
    Note: Solution CB is an ethanol-based wash solution used to further clean the DNA bound to the MB Spin Column silica filter membrane. This wash solution removes residues of salt and other contaminants but allows the DNA to stay bound to the silica membrane.
13. Discard the flow-through. Centrifuge at 10,000 x *g* for 1 min at room temperature.
    Note: The flow-through is waste, containing ethanol wash solution and contaminants that did not bind to the MB Spin Column membrane. This step removes any residual Solution CB (ethanol wash solution). It is critical to remove all traces of Solution CB because it can interfere with downstream DNA applications.
14. Place the MB Spin Column in a new 2 ml Collection Tube (provided).
    Note: Be careful not to splash any of the liquid on the MB Spin Column.
15. Add 50 µl of Solution EB to the center of the white filter membrane.
    Note: Placing the Solution EB (elution buffer) in the center of the small white membrane will make sure the entire membrane is wet. This will result in more efficient release of bound DNA.
16. Centrifuge at 10,000 x *g* for 30 s at room temperature.
    Note: As Solution EB passes through the silica membrane, DNA is released and flows through the membrane and into the Collection Tube. The DNA is released because it can only bind to the MB Spin Column membrane in the presence of salt. Solution EB is 10 mM Tris pH 8 and does not contain salt.
17. 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. To concentrate DNA, see the Troubleshooting Guide.

Protocol: QIAvac 24 Plus Vacuum Manifold

Important points before starting

- If Solution SL has precipitated, heat at 55°C for 5–10 min.
- Shake to mix Solution SB before use.
- For each sample lysate, use one MB Spin Column. Keep the MB Spin Column in the attached 2 ml Collection Tube and continue using the Collection Tube as a MB Spin Column holder until needed for the Vacuum manifold protocol.
- Label each Collection Tube top and MB Spin Column to maintain sample identity. If the MB Spin Column becomes clogged during the vacuum procedure, switch to the centrifugation protocol.
- You will need to provide 100% ethanol for step 7 of this protocol.

Procedure

1. Connect the QIAvac 24 Plus to the vacuum source using the QIAvac Connecting System (for more details, refer to the QIAvac 24 Plus Handbook, Appendix A, page 16).
2. Insert a VacValve into each Luer slot of the QIAvac 24 Plus that is to be used. Close unused Luer slots with Luer plugs or close the inserted VacValve.
3. Insert a VacConnector into each VacValve. Perform this step directly before starting the purification to avoid exposure of VacConnectors to potential contaminants in the air.
4. Place an MB Spin Column into each VacConnector on the manifold.
5. Transfer 650 µl of lysate (from step 10 of centrifugation protocol) to the MB Spin Column.
6. Turn on the vacuum source and open the stopcock of the port. Hold the tube in place when opening the stopcock to keep the spin filter steady. Allow the lysate to pass through the MB Spin Column. After the lysate has passed through the column completely, load again with 650 µl of lysate. Continue until all of the lysate has been loaded onto the MB Spin column. Close the one-way Luer-Lok stopcock of that port.

Note: If the MB Spin Columns are filtering slowly, close the ports to samples that have completed filtering to increase the pressure to the other columns.

7. Add 800 µl of 100% ethanol to completely fill the MB Spin Column. Open the stopcock while holding the column steady. Allow the ethanol to pass through the column completely. Close the stopcock.
8. Add 300 µl of Solution CB to each MB Spin Column. Open the Luer-Lok stopcock and apply a vacuum until Solution CB has passed through the column completely. Continue to pull a vacuum for another minute to dry the membrane. Close each port.
9. Turn off the vacuum source and open an unused port to vent the manifold. If all 20 ports are in use, break the vacuum at the source. Make certain that all vacuum pressure is released before performing the next step. It is important to turn off the vacuum at the source to prevent backflow into the columns.
10. Remove the MB Spin Column and place in the original labeled 2 ml Collection Tube. Centrifuge at 13,000 x *g* for 1 min to completely dry the membrane.
11. Transfer the MB Spin Column into a new 2 ml Collection Tube and add 50 µl of Solution EB to the center of the white filter membrane. Alternatively, sterile DNA-free PCR-grade water (cat. no. 17000-10) may be used for elution from the silica spin filter membrane at this step.
12. Centrifuge at 13,000 x *g* for 1 min at room temperature.
13. Discard the MB Spin Column. The DNA in the tube is now ready for downstream applications.

    Note: We recommend storing DNA frozen (–20°C to –80°C) as Solution EB does not contain EDTA. To concentrate DNA, see the Troubleshooting Guide.

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)	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.
b)	Concentrating eluted DNA	The final volume of eluted DNA will be 50 µl. The DNA may be concentrated by adding 5 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. 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).
c)	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.
Alternative lysis methods		
a)	Sample contains cells that are difficult to lyse	Incubate at 70°C for 10 minutes after adding Solution SL (Step 3). Then, continue with Step 4.
b)	Reduction of DNA shearing	Incubate at 65°C for 10 minutes after adding Solution SL (Step 3). Then, skip Step 4 and proceed to Step 5 This will reduce DNA shearing and may increase DNA yields from some organisms.

Ordering Information

Product	Contents	Cat. no.
DNeasy UltraClean Microbial Kit (50)	For 50 preps: Isolation of high-quality DNA from microbial cultures	12224-50
DNeasy UltraClean Microbial Kit (250)	For 250 preps: Isolation of high-quality DNA from microbial cultures	12224-250
DNeasy UltraClean 96 Microbial Kit (384)	For 384 preps: High-throughput isolation of DNA from microbial cultures	10196-4
Related Products		
MagAttract® Microbial DNA Kit (384)	For 384 preps: Automated isolation of DNA from microbial and food cultures using automated processing and liquid handling systems	27200-4
DNeasy PowerFood® Microbial Kit (100)	For 100 preps: Isolation of inhibitor-free DNA from a variety of cultured foods	21000-100
DNeasy PowerLyzer® Microbial Kit (50)	For 50 preps: Isolation of high-quality DNA from microbial cultures using a bead-based homogenizer	12255-50
Vortex Adapter	For vortexing 1.7 ml or 2 ml tubes using the Vortex-Genie 2 Vortex	13000-V1-24
QIAvac 24 Plus Manifold	Vacuum manifold for processing 1–24 spin columns; includes QIAvac 24 Plus Vacuum Manifold, Luer Plugs, and Quick Couplings	19413
TissueLyser II	For medium- to high-throughput sample disruption for molecular analysis	85300

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.

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