June 2017

DNeasy® UltraClean® 96 Microbial Kit Handbook

For the high-throughput isolation of DNA from microbial cultures

Sample to Insight

QIAGEN

Contents

Kit Contents

DNeasy UltraClean 96 Microbial Kit	**(384)**
Catalog no.	**10196-4**
Number of preps	**384**
PowerBead Solution	200 ml
Solution SL	2 x 15 ml
Solution IRS	44 ml
Solution SB	2 x 250 ml
Solution CB	11 x 30 ml
Solution EB	56 ml
PowerBead DNA Plates, Glass, 0.1 mm	4
Elution Sealing Mats	4
QIAamp® 96 Plates	4
Collection Plates (2 ml)	4
Collection Plates (1 ml)	4
S-Blocks	2
Airpore Tape Sheets	25
Elution Microtubes	4
Caps for Elution Microtubes	50 x 8
Sealing Tape	16
Quick Start Protocol	1

Storage

The DNeasy UltraClean 96 Microbial Kit reagents and components 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 alcohol and is flammable.

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 96 Microbial Kits is tested against predetermined specifications to ensure consistent product quality.

Introduction

The DNeasy UltraClean 96 Microbial Kit is designed to isolate high-quality genomic DNA from microorganisms. A variety of microorganisms, including bacterial spores and fungal types, have been tested successfully with this kit.

Using the DNeasy UltraClean 96 Microbial Kit, microbial cells are added to a bead beating plate containing beads, bead solution and lysis solution. 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 96-well plate shaker. The DNA released from the lysed cells is bound to a silica spin plate. The plate is washed, and the DNA is recovered in certified DNA-free Tris buffer.

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.

- Centrifuge that can spin two stacked 96-well blocks (13 x 8.5 x 7.5 cm) at 4500 x *g*
  Note: If you have a centrifuge with a maximum speed less than 4500 x *g*, please refer to the Troubleshooting Guide.
- Multi-channel pipettor (50–650 µl)
- Mechanical shaker for 96-well blocks and adapters
- TissueLyser II (cat. no. 85300)
- Plate Adapter Set (cat. no. 11990)
- Vortex-Genie® 2 vortex
- Reagent reservoirs

Protocol: Centrifuge

Important points before starting

- This protocol assumes you will be processing 192 samples (2 x 96-well preps). If you plan to process fewer than 192 samples, divide the samples between two plates such that they are balanced. See the Troubleshooting Guide for more information.
- If Solution SL has precipitated, heat at 60°C until precipitate dissolves.
- Shake to mix Solution SB before use.

Procedure

1. Dispense liquid culture into a clean 2 ml Collection Plate and cover with Sealing Tape.
2. Centrifuge at 4500 x *g* for 12 min. Discard tape and remove all supernatant.
3. Add 300 µl of PowerBead Solution and apply new Sealing Tape. Resuspend the cell pellet by vortexing.
4. Centrifuge a PowerBead DNA Plate at 4500 x *g* for 3 min to bring down all the beads.
5. Remove and discard the Elution Sealing Mat from the PowerBead DNA Plate and transfer the resuspended cells from step 3 to the PowerBead DNA Plate.
6. Add 60 µl of Solution SL and seal the PowerBead Plate securely with a new Sealing Mat.
7. Place the securely-sealed PowerBead Plate in a TissueLyser II (cat. no. 85300).
   Note: Final order of components: Adapter Plate (cat. no. 11990), Sealing Mat, PowerBead Plate, Adapter Plate.
8. Shake at speed 20 Hz for 5 min. Re-orient plates so that the side that was closest to the machine body is now furthest from it and shake again at speed 20 Hz for 5 min.
9. Centrifuge at 4500 x *g* for 6 min at room temperature.
10. Remove and discard Sealing Mat. Transfer supernatant to a clean 1 ml Collection Plate.
    Note: Supernatant may still contain some beads.
11. Add 100 µl of Solution IRS. Apply Sealing Tape and vortex for 5 s.
12. Incubate at 2–8°C for 10 min. Centrifuge the plate at 4500 x *g* for 9 min.
13. Avoiding the pellet, transfer supernatant to a clean 2 ml Collection Plate.

14. Add 800 µl of Solution SB to wells containing supernatant. Pipet up and down to mix.
15. Place a QIAamp 96 Plate onto an S-Block.
16. Transfer approximately 650 µl of supernatant to the QIAamp 96 Plate.
17. Apply an Airpore Tape Sheet and centrifuge at 4500 x *g* for 3 min.
18. Discard flow-through and replace the S-Block beneath the QIAamp 96 Plate. Discard the Airpore Tape Sheet.
19. Repeat steps 16–18 until all the supernatant has been processed.
20. Add 400 µl of Solution CB to the QIAamp 96 Plate and apply an Airpore Tape Sheet.
21. Centrifuge at 4500 x *g* for 3 min.
22. Discard the Airpore Tape Sheet and flow-through. Place the QIAamp 96 Plate on the same S-Block and add another 400 µl of Solution CB. Apply a new Airpore Tape Sheet and centrifuge at 4500 x *g* for 3 min.
23. Discard flow-through and place the QIAamp 96 Plate on the same S-Block.
24. Centrifuge at 4500 x *g* for 6 min. Carefully place the QIAamp 96 Plate on Elution Microtubes being careful not to splash any Solution CB onto the QIAamp 96 Plate.
25. Discard the flow-through. The S-Block can be re-used.
26. Remove and discard the Airpore Tape Sheet. Air dry for 10 min at room temperature.
27. Add 100 µl of Solution EB to the centers of the QIAamp 96 Plate filter membranes.
28. Apply a new Airpore Tape Sheet to the QIAamp 96 Plate. Centrifuge at 4500 x *g* for 3 min.
29. Cover the Elution Microtubes with Caps (provided). The DNA in the Elution Microtubes is now ready for downstream applications.

Protocol: Centrifugation and Vacuum

Important points before starting

- This protocol assumes you will be processing 192 samples (2 x 96-well preps). If you plan to process fewer than 192 samples, divide the samples between two plates such that they are balanced. See the Troubleshooting Guide for more information.
- If Solution SL has precipitated, heat at 60°C until precipitate dissolves.
- Shake to mix Solution SB before use.

Procedure

1. Dispense liquid culture into a clean 2 ml Collection Plate and cover with Sealing Tape.
2. Centrifuge at 4500 x *g* for 12 min. Discard tape and remove all supernatant.
3. Add 300 µl of PowerBead Solution and apply new Sealing Tape. Resuspend the cell pellet by vortexing.
4. Centrifuge a PowerBead DNA Plate at 4500 x *g* for 3 min to bring down all the beads.
5. Remove and discard the Elution Sealing Mat from the PowerBead DNA Plate and transfer the resuspended cells from step 3 to the PowerBead DNA Plate.
6. Add 60 µl of Solution SL and seal the PowerBead Plate securely with a new Sealing Mat.
7. Place the securely-sealed PowerBead Plate in a TissueLyser II (cat. no. 85300).
   Note: Final order of components: Adapter Plate (cat. no. 11990), Sealing Mat, PowerBead Plate, Adapter Plate.
8. Shake at speed 20 Hz for 5 min. Re-orient plates so that the side that was closest to the machine body is now furthest from it and shake again at speed 20 Hz for 5 min.
9. Centrifuge at 4500 x *g* for 6 min at room temperature.
10. Remove and discard Sealing Mat. Transfer supernatant to a clean 1 ml Collection Plate.
    Note: Supernatant may still contain some beads.
11. Add 100 µl of Solution IRS. Apply Sealing Tape and vortex for 5 s.
12. Incubate at 2–8°C for 10 min. Centrifuge the plate at 4500 x *g* for 9 min.
13. Avoiding the pellet, transfer supernatant to a clean 2 ml Collection Plate.

14. Remove the top portion of a vacuum manifold and place a new 2 ml Collection Plate in the bottom of the vacuum manifold.
15. Replace the top of the manifold and place a QIAamp 96 Plate on it. Turn the vacuum pump on.
    Note: Test that you have a good seal with the manifold and the spin plate. You should be able to gently lift the entire unit without the spin plate separating from the manifold.
16. Add 800 µl of Solution SB to wells containing supernatant. Pipet up and down to mix.
17. Load 650 µl of the samples into the wells of the QIAamp 96 Plate. Allow the samples to flow through. Repeat until all the samples have been processed and turn the vacuum off.
18. Remove the QIAamp 96 Plate and set aside. Discard the flow-through from the 2 ml Collection Plate and then place it back into the manifold.
19. Replace the QIAamp 96 Plate on top of the manifold. Turn the vacuum on.
20. Add 400 µl of Solution CB to each well of the QIAamp 96 Plate.
21. After the first volume of Solution CB has passed through, add another 400 µl of Solution CB to each well of the QIAamp 96 Plate.
22. Once the entire volume of Solution CB has passed through, turn the vacuum off.
23. Apply a new Airpore Tape Sheet to the QIAamp 96 Plate. Place an S-Block under the QIAamp 96 plate and centrifuge at 4500 x *g* for 6 min.
24. Carefully place the QIAamp 96 Plate on Elution Microtubes being careful not to splash any Solution CB onto the QIAamp 96 Plate.
25. Discard the flow-through. The S-Block can be reused.
26. Remove and discard the Airpore Tape Sheet. Air dry for 10 min at room temperature.
27. Add 100 µl of Solution EB to the centers of the QIAamp 96 Plate filter membranes.
28. Apply a new Airpore Tape Sheet to the QIAamp 96 Plate. Centrifuge at 4500 x *g* for 3 min.
29. Cover the Elution Microtubes with Caps (provided). The DNA in the Elution Microtubes is now ready for downstream applications.

.

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		
a)	Processing fewer than 192 samples (less than 2 full plates)	Balance the number of samples so that centrifugation steps do not damage your centrifuge. It is best to match the total number of samples per plate as well as the orientation. For example, if you use wells A1–A12 in one plate, use those same wells in the second plate.
b)	Using the unused wells of a previously processed plate	Mark all used wells to prevent reusing wells and cross-contamination. Tap the used plate several times on the lab bench to force any beads to the bottom of the deep well plate before re-using a plate.
c)	Vacuum step seems to be taking a long time	Turn off the vacuum source and lift the filter plate off the vacuum to release any back-pressure. Replace the filter plate and turn the vacuum source back on. Ensure that there are no air leaks around the plate. If the vacuum continues to be slow, centrifuge the filter plate as an alternative.
d)	Using a centrifuge with a maximum speed less than 4500 x *g*	Multiply the protocol time and speed to determine total x *g*. Divide the total by the maximum speed of your centrifuge (round up if necessary). This will be the number of minutes your centrifuge will need to run to achieve the appropriate overall force. **Example:** 10 minutes at 4500 x *g* = 45,000. If your centrifuge has a maximum speed of 2500 x *g*, divide 45,000 by 2500 = 18 minutes of centrifugation.
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.

		Comments and suggestions
b)	Concentrating eluted DNA	The final volume of eluted DNA will be 100 µl. The DNA may be concentrated by adding 5 µl of 3 M NaCl and inverting 3–5 times to mix. Next, add 200 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	You can incubate at 70°C for 10 minutes after adding Solution SL (Step 6). Then, continue with Step 7.

Ordering Information

Product	Contents	Cat. no.
DNeasy UltraClean 96 Microbial Kit (384)	For 384 preps: High-throughput isolation of DNA from microbial cultures	10196-4
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
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
Plate Adapter Set	Set of four adapters required to assemble two 96-well plates onto the 96-Well Plate Shaker	11990
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.

Limited License Agreement for DNeasy UltraClean 96 Microbial 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.
2. Other than expressly stated licenses, QIAGEN makes no warranty that this kit and/or its use(s) do not infringe the rights of third-parties.
3. This kit and its components are licensed for one-time use and may not be reused, refurbished, or resold.
4. QIAGEN specifically disclaims any other licenses, expressed or implied other than those expressly stated.
5. The purchaser and user of the kit agree not to take or permit anyone else to take any steps that could lead to or facilitate any acts prohibited above. QIAGEN may enforce the prohibitions of this Limited License Agreement in any Court, and shall recover all its investigative and Court costs, including attorney fees, in any action to enforce this Limited License Agreement or any of its intellectual property rights relating to the kit and/or its components.

For updated license terms, see **www.qiagen.com**.

1104550 HB-2247-001 06/2017