October 2017

DNeasy® PowerFood® Microbial Kit Handbook

For the isolation of inhibitor-free DNA from a variety of cultured foods

Sample to Insight

QIAGEN

Contents

Kit Contents

DNeasy PowerFood Microbial Kit	**(100)**
Catalog no.	**21000-100**
Number of preps	**100**
PowerBead Tubes, Garnet (0.5 ml)	2 x 50
MB Spin Columns	2 x 50
Solution MBL	2 x 25 ml
Solution IRS	15 ml
Solution MR	2 x 50 ml
Solution PW	3 x 30 ml
Ethanol	3 x 30 ml
Solution EB	2 x 9 ml
Collection Tubes (2 ml)	10 x 50
Quick Start Protocol	1

Storage

The DNeasy PowerFood 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 PW and ethanol are flammable.**

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

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

Solution MBL and Solution MR 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 PowerFood Microbial Kits is tested against predetermined specifications to ensure consistent product quality.

Introduction

The DNeasy PowerFood Microbial Kit is designed to isolate high-quality genomic DNA from microorganisms cultured from food according to FDA guidelines (Bacteriological Analytical Manual, Edition 8, Revision A/1998). The kit combines Inhibitor Removal Technology® (IRT) with reformulated buffers to provide high-quality, inhibitor-free DNA for use in downstream applications, including PCR and qPCR.

DNA from a variety of known food pathogens has been isolated using the DNeasy PowerFood Microbial Kit and detected using real-time quantitative PCR (Table 1).

Table 1. DNA isolated from food pathogens using the DNeasy PowerFood Microbial Kit

Pathogen	Enriched food
Clostridium difficile	Raw ground beef, ready-to-eat salad
Clostridium perfringens	Raw ground beef, carrot juice
Enterococcus faecalis	Ready-to-eat salad
Escherichia coli	Strawberries, orange juice
Listeria monocytogenes	Brie cheese
Salmonella enterica	Strawberries, orange juice, Brie cheese
Shigella spp.	Shrimp
Vibrio spp.	Shrimp

Principle and procedure

Microbial food culture is pelleted by centrifugation and resuspended in lysis buffer. The lysed cells are transferred to a bead beating tube containing beads designed for small-cell (microbial) lysis and vortexed using a Vortex Adapter. After protein and inhibitor-removal steps, 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 Solution EB.

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.

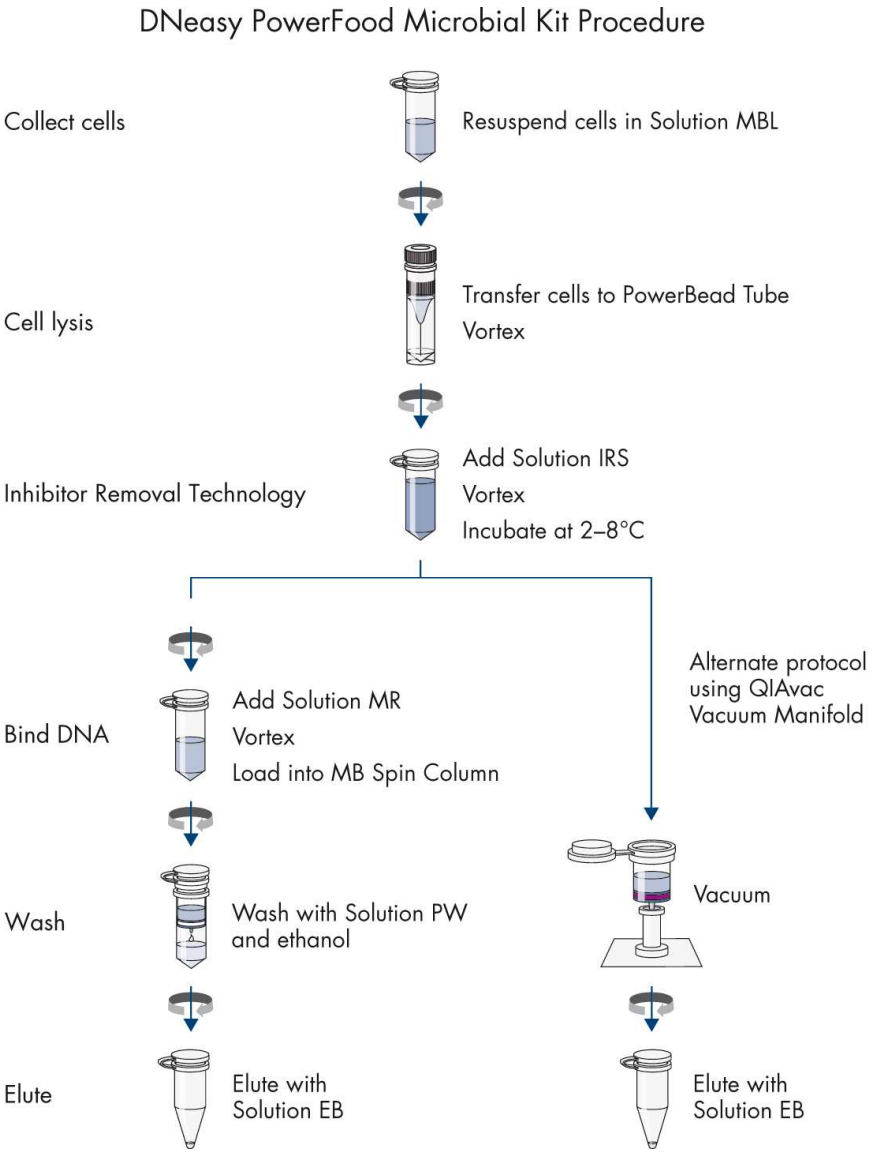

Figure 1. DNeasy PowerFood Microbial Kit Procedure.

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 (13,000 x *g*)
- BagMixer® 400 VW or other lab blender
- BagMixer 400 Bags or other lab blender bags
- Pipettors
- 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

- Solution MBL must be warmed at 55°C for 5–10 min to dissolve precipitates prior to use. Solution MBL should be used while still warm.
- If Solution MR precipitates, warm at 55°C for 5–10 min. Solution MR can be used while still warm.
- Shake to mix Solution PW before use.

Procedure

1. Homogenize the food sample using a lab blender, such as a BagMixer 400 VW, and incubate homogenates according to FDA guidelines (Bacteriological Analytical Manual, Edition 8, Revision A/1998).
2. Add 1.8 ml of microbial food culture to a 2 ml Collection Tube (provided) and centrifuge at 13,000 x *g* for 1 min at room temperature. Decant the supernatant and spin the tubes at 13,000 x *g* for 1 min. Remove remaining supernatant completely with a pipette tip.
3. Resuspend the cell pellet in 450 µl of Solution MBL.
4. Transfer the resuspended cells to a PowerBead Tube.
   Note: To increase yields or for difficult cells, please refer to the Alternative Lysis Methods section in the Troubleshooting Guide.
5. Secure PowerBead Tubes horizontally to a Vortex Adapter (cat. no. 13000-V1-24).
6. Vortex at maximum speed for 10 min.
   Note: To reduce DNA shearing, please refer to the Alternative Lysis Methods section in the Troubleshooting Guide.
7. Centrifuge the tubes at a **maximum** of 13,000 x *g* for 1 min at room temperature.
8. Transfer the supernatant to a clean 2 ml Collection Tube (provided).
   Note: Expect approximately 400 µl of supernatant.
9. Add 100 µl of Solution IRS and vortex briefly to mix. Incubate at 2–8°C for 5 min.
10. Centrifuge the tubes at 13,000 x *g* for 1 min at room temperature.

11. Avoiding the pellet, transfer the entire volume of supernatant to a clean 2 ml Collection Tube (provided).
    Note: Expect approximately 450 µl of supernatant.
12. Add 900 µl of Solution MR and vortex to mix.
13. Load 650 µl of supernatant onto an MB Spin Column and centrifuge at 13,000 x *g* for 1 min. Discard the flow-through and repeat until all the supernatant has been loaded onto the MB Spin Column.
    Note: A total of two loads are required for each sample processed.
14. Place the MB Spin Column into a clean 2 ml Collection Tube (provided).
15. Add 650 µl of Solution PW. Centrifuge at 13,000 x *g* for 1 min at room temperature.
16. Discard the flow-through and add 650 µl of ethanol (provided) and centrifuge at 13,000 x *g* for 1 min at room temperature.
17. Discard the flow-through and centrifuge at 13,000 x *g* for 2 min.
18. Place the MB Spin Column into a clean 2 ml Collection Tube (provided).
19. Add 100 µl of Solution EB to the center of the white filter membrane and centrifuge at 13,000 x *g* for 1 min.
20. 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

- Solution MBL must be warmed at 55°C for 5–10 min to dissolve precipitates prior to use. Solution MBL should be used while still warm.
- If Solution MR precipitates, warm at 55°C for 5–10 min. Solution MR can be used while still warm.
- Shake to mix Solution PW before use.

Procedure

1. Homogenize the food sample using a lab blender, such as a BagMixer 400 VW, and incubate homogenates according to FDA guidelines (Bacteriological Analytical Manual, Edition 8, Revision A/1998).
2. Add 1.8 ml of microbial food culture to a 2 ml Collection Tube (provided) and centrifuge at 13,000 x *g* for 1 min at room temperature. Decant the supernatant and spin the tubes at 13,000 x *g* for 1 min. Remove remaining supernatant completely with a pipette tip.
   Note: 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.
3. Resuspend the cell pellet in 450 µl of Solution MBL.
   Note: Solution MBL is a strong lysing reagent that includes a detergent to break cell walls and help remove non-DNA organic and inorganic material. Solution MBL is also an important part of our IRT technology. When cold, this solution will form a white precipitate in the bottle. Heating to 55°C for 5–10 min will dissolve the components without harm. Solution MBL should be used while it is still warm.
4. Transfer the resuspended cells to a PowerBead Tube.
   Note: To increase yields or for difficult cells, please refer to the Alternative Lysis Methods section in the Troubleshooting Guide.
5. Secure PowerBead Tubes horizontally to a Vortex Adapter (cat. no. 13000-V1-24).
6. Vortex at maximum speed for 10 min.

Note: To reduce DNA shearing, please refer to the Alternative Lysis Methods section in the Troubleshooting Guide.

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.

7. Centrifuge the tubes at a **maximum** of 13,000 x *g* for 1 min at room temperature.
   Note: Cell debris is sent to the bottom of the tube while DNA remains in the supernatant. This step is important for the removal of any remaining non-DNA organic and inorganic contaminants that may reduce DNA purity and inhibit downstream applications.
8. Transfer the supernatant to a clean 2 ml Collection Tube (provided).
   Note: Expect approximately 400 µl of supernatant.
9. Add 100 µl of Solution IRS and vortex briefly to mix. Incubate at 2–8°C for 5 min.
   Note: Solution IRS is the second step of our IRT and further removes additional non-DNA organic and inorganic material, including cell debris and proteins.
10. Centrifuge the tubes at 13,000 x *g* for 1 min at room temperature.
11. Avoiding the pellet, transfer the entire volume of supernatant to a clean 2 ml Collection Tube (provided).
    Note: Expect approximately 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.
12. Add 900 µl of Solution MR and vortex to mix.
    Note: Solution MR is a highly concentrated salt solution. DNA binds tightly to silica at high salt concentrations, and Solution MR will adjust the DNA solution salt concentration to allow binding of DNA, but not non-DNA organic and inorganic material that may still be present at low levels, to the MB Spin Column filter.

13. Load 650 µl of supernatant onto an MB Spin Column and centrifuge at 13,000 x *g* for 1 min. Discard the flow-through and repeat until all the supernatant has been loaded onto the MB Spin Column.
    Note: A total of two loads are required for each sample processed. DNA is selectively bound to the MB Spin Column silica membrane. Contaminants pass through the filter membrane, leaving only the DNA bound.
14. Place the MB Spin Column in a new 2 ml Collection Tube (provided).
    Note: Due to the high concentration of salt in Solution MR, it is important to place the MB Spin Column in a clean 2 ml Collection Tube to aid in the subsequent wash steps and improve DNA purity and yield.
15. Add 650 µl of Solution PW. Centrifuge at 13,000 x *g* for 1 min at room temperature.
    Note: Solution PW is an alcohol-based wash solution used to further clean the DNA that is bound to the MB Spin Column silica filter membrane. Solution PW removes residual salt and other contaminants while allowing DNA to stay bound to the membrane.
16. Discard the flow-through and add 650 µl of ethanol (provided) and centrifuge at 13,000 x *g* for 1 min at room temperature.
    Note: Ethanol ensures complete removal of Solution PW, which will result in higher DNA purity and yield.
17. Discard the flow-through and centrifuge at 13,000 x *g* for 2 min.
    Note: This second spin removes residual ethanol. It is critical to remove all traces of ethanol because it can interfere with many downstream DNA applications, such as PCR, restriction digests and gel electrophoresis.
18. Place the MB Spin Column into a clean 2 ml Collection Tube (provided).
19. Add 100 µl of Solution EB to the center of the white filter membrane and centrifuge at 13,000 x *g* for 1 min.
    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.
    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. Alternatively, sterile DNA-Free PCR-Grade Water (cat. no. 17000-10) or sterile TE may be used for DNA elution from the MB Spin Column membrane.

20. 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

- Solution MBL must be warmed at 55°C for 5–10 min to dissolve precipitates prior to use. Solution MBL should be used while still warm.
- If Solution MR precipitates, warm at 55°C for 5–10 min. Solution MR can be used while still warm.
- Shake to mix Solution PW 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 12 of centrifugation protocol) to the MB Spin Column.
6. Turn on the vacuum source and open the VacValve 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 VacValve 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 VacValve while holding the column steady. Allow the ethanol to pass through the column completely. Close the stopcock.
8. Add 650 µl of Solution PW to each MB Spin Column. Open the VacValve and apply a vacuum until Solution PW has passed through the column completely. Continue to pull a vacuum for another minute to dry the membrane. Close each port.
9. Add 650 µl of ethanol to each MB Spin Column. Open the VacValve and apply a vacuum until the ethanol has passed through the column completely. Continue to pull a vacuum for another minute to dry the membrane. Close each port.
10. 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.
11. 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.
12. Transfer the MB Spin Column into a new 2 ml Collection Tube and add 100 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.
13. Centrifuge at 13,000 x *g* for 1 min at room temperature.
14. 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.

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 well when loading a gel	This usually occurs because residual ethanol remains in the final sample. Avoid transferring any ethanol 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 100 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. DNA may also be eluted in DNA-Free PCR-Grade Water.
Alternative lysis methods		
a)	Sample contains cells that are difficult to lyse	Incubate at 70°C for 10 minutes after step 4. Then, continue with step 5.
b)	To reduce DNA shearing	Incubate at 65°C for 10 minutes after step 4. Then, skip steps 5 and 6 and proceed to Step 7. This will reduce DNA shearing and may increase DNA yields from some organisms.
c)	To increase yields	Heat at 65°C for 10 minutes during step 4 and continue with step 5.

Appendix A: Isolating DNA Directly from Food Without Culturing

DNA can be isolated directly from small quantities of both liquid and solid food. Microbial yields may be low but are still detectable by PCR. For both solid food homogenates and liquids, microbes will pellet underneath all other food components. To get to the pellet, remove as much of the fat and food homogenates as possible before adding Solution MBL. For further assistance, please contact QIAGEN Technical Services (**support.qiagen.com**).

- For solid foods: Homogenize 0.25 g of food in 0.75 ml of PBS (not provided) in a 2 ml Collection Tube (provided). Centrifuge for 1–3 min at 13,000 x *g*. Remove as much of the residual food and liquid as possible with a pipette tip without disturbing the microbial pellet at the bottom of the tube. Resuspend the pellet in 450 µl of Solution MBL and proceed with the protocol.
- For liquid foods: Centrifuge 1–1.8 ml of liquid for 1–3 min at 13,000 x *g*. Remove as much of the residual food, fat and liquid as possible with a pipette tip without disturbing the microbial pellet at the bottom of the tube. Resuspend the pellet in 450 µl of Solution MBL and proceed with the protocol.

Microbial DNA has been isolated directly and PCR-amplified from the following spiked foods: cream (12% and 25% fat), Brie cheese (37% fat), orange juice, strawberries, lettuce, asparagus and milk/dark chocolate.

Ordering Information

Product	Contents	Cat. no.
DNeasy PowerFood Microbial Kit (100)	For 100 preps: Isolation of inhibitor-free DNA from a variety of cultured foods	21000-100
Related products		
DNeasy PowerFood Kit, 2 ml Tubes (100)	For 100 preps: Isolation of inhibitor-free DNA from a variety of cultured and uncultured foods	21000-100-MON
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
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 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

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 PowerFood Microbial Kit

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

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