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DNeasy® PowerLyzer® Microbial Kit Handbook

For the isolation of high-quality DNA from microbial cultures using a bead-based homogenizer

Sample to Insight

QIAGEN

Contents

Kit Contents

DNeasy PowerLyzer Microbial Kit	**(50)**
Catalog no.	**12255-50**
Number of preps	**50**
PowerBead Tubes, Glass 0.1 mm	50
PowerBead Solution	16.5 ml
MB Spin Columns	50
Solution SL	1.5 ml
Solution IRS	15 ml
Solution SB	50 ml
Solution CB	30 ml
Solution EB	9 ml
Collection Tubes (2 ml)	4 x 50
Quick Start Protocol	1

Storage

The DNeasy PowerLyzer 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 PowerLyzer Microbial Kits is tested against predetermined specifications to ensure consistent product quality.

Introduction

The DNeasy PowerLyzer Microbial Kit is designed to isolate high-quality genomic DNA from microorganisms in about half the time required by traditional homogenization and vortex methods and enables up to 24 samples to be homogenized simultaneously. A variety of microorganisms in pure cultures and from plates, including bacterial and fungal spores, have been tested successfully with this kit.

Principle and procedure

Microbial cell are resuspended in a bead solution and added to a bead beating tube containing 0.1 mm glass beads. Then, lysis solution is added. The microorganisms are lysed by a combination of heat, detergent and mechanical force using a PowerLyzer 24 Homogenizer or a specially designed Vortex Adapter on a standard vortex. The DNA released from the lysed cells is captured on a silica membrane in a spin column format. DNA is then washed and eluted. The isolated DNA is ready for PCR analysis and other downstream applications.

Optimized for homogenization with the PowerLyzer 24 Homogenizer

The DNeasy PowerLyzer Microbial Kit contains PowerBead Tubes with 0.1 mm glass beads, which allows for more options in choosing homogenization methods, including the use of the PowerLyzer 24 Homogenizer. The PowerLyzer's velocity and proprietary motion combine to provide the fastest homogenization possible, minimizing time spent processing samples. For species that are difficult to lyse, such as some fungi or spores, faster settings (up to 2800 RPM for DNA isolation) and heating to 65°C before homogenization may be employed to enhance lysis.

Using the DNeasy PowerLyzer Microbial Kit with other homogenizers

To isolate DNA using the DNeasy PowerLyzer Microbial Kit with FastPrep® or Precellys® homogenizers, use the conversion chart (see Table 1 below) to adapt your current protocol. However, due to the highly efficient motion of beads in the PowerLyzer 24 Homogenizer, fewer cycles are required to generate the same effect compared to other homogenizers.

You may want to perform extractions using the PowerLyzer 24 Homogenizer at the equivalent speed and number of cycles as your current instrument and then compare the results to those obtained using less time or lower speeds to determine which settings give the best results.

Table 1. Conversion chart for using other homogenizers with the DNeasy PowerLyzer Microbial Kit

PowerLyzer 24	FastPrep 24 (m/s)	Precellys 24
2500	4	5000
2600	–	5200
2700	–	5400
2800	4.5	5600
2900	–	5800
3000	–	6000
3100	5	6200
3200	–	6400
3300	–	6600
3400	5.5	6800
3500	–	–
3600	–	–
3700	6	–
3800	–	–
3900	–	–
4000	6.5	–

Note: Settings equivalent to slower than 2500 RPM or faster than 4000 RPM on the PowerLyzer 24 Homogenizer are not obtainable with FastPrep or Precellys homogenizers.

High-throughput options

We offer a vacuum-based protocol for faster processing without centrifugation for the DNA-binding and column-washing steps using MB Spin Columns. 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, we offer the DNeasy UltraClean 96 Microbial Kit (cat. no. 10196-4) for processing up to 2 x 96 samples using a centrifuge capable of spinning two stacked 96-well blocks (13 cm x 8 cm x 5.5 cm) at 2500 x *g*. For 96-well homogenization of bacteria, we offer the TissueLyser II and Plate Adapter Set (cat. no. 85300 and 11990, respectively).

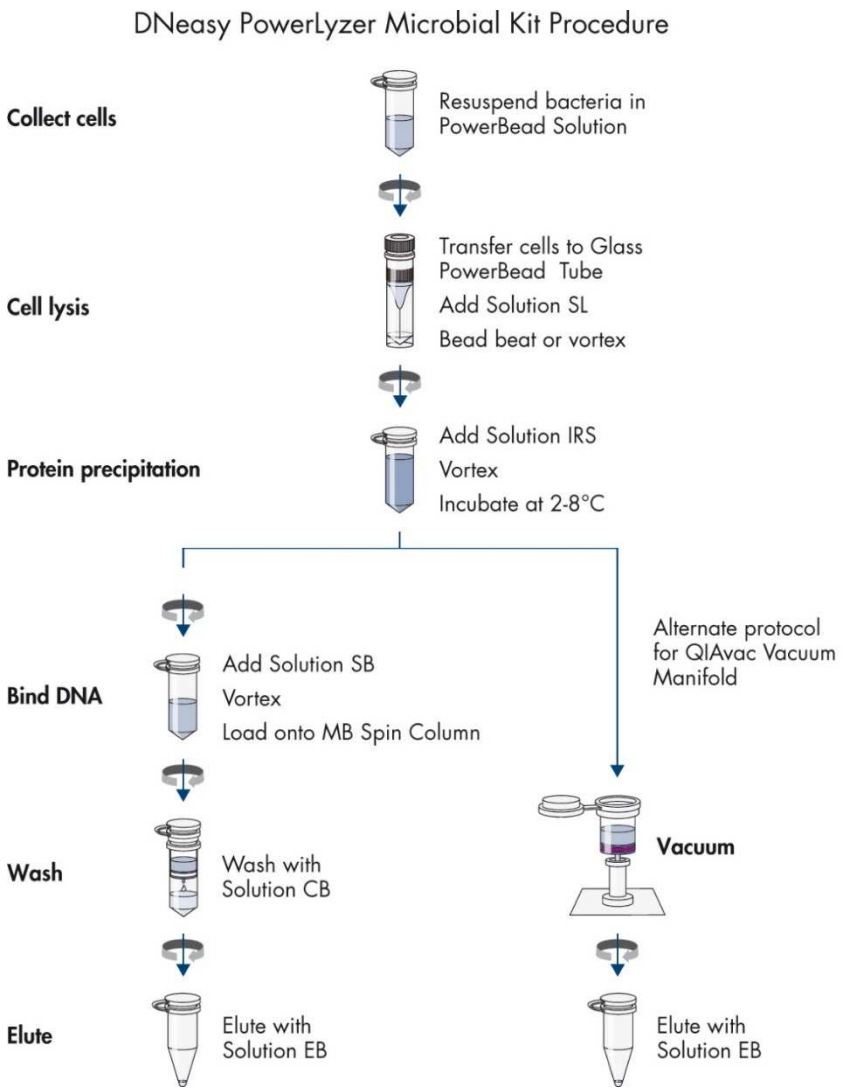

Figure 1. DNeasy PowerLyzer Microbial Kit procedure.

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*)
- PowerLyzer 24 or another bead homogenizer
- Pipettor (50–200 µl; 100–1000 µl)
- Vortex-Genie® 2
- Vortex Adapter (cat. no. 13000-V1-24)
- QIAvac 24 Plus Vacuum Manifold
- 100% ethanol (for QIAvac Vacuum Manifold protocol)

Protocol: Experienced User

Important points before starting

- The PowerLyzer 24 Homogenizer may cause marring of labels on the tops of the PowerBead Tubes. To ensure proper sample identification, label sides and tops of the tubes.
- If Solution SL has precipitated, heat at 60°C until the precipitate has dissolved.
- 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 at 10,000 x *g* for 30 s at room temperature and completely remove the media supernatant with a pipet tip.
   Note: Based on the type of microbial culture, it may be necessary to centrifuge longer than 30 seconds.
2. Resuspend the cell pellet in 300 µl of PowerBead Solution and gently vortex to mix. Transfer resuspended cells to a PowerBead Tube Glass, 0.1 mm.
3. Add 50 µl of Solution SL to the PowerBead Tube.
   Note: To increase yields, to minimize DNA shearing or for cells that are difficult to lyse, refer to the Troubleshooting Guide.
4. Homogenization options:

   a) PowerLyzer 24 Homogenizer: Balance PowerBead Tubes in the tube holder for the PowerLyzer 24. Homogenize for 5 min at 2000 RPM.
   Note: Depending on the sample, you can homogenize at a higher speed for less time.
   b) Vortex: Secure PowerBead Tube horizontally using the Vortex Adapter tube holder (cat. no. 13000-V1-24). Vortex at maximum speed for 10 min.
   Note: To minimize DNA shearing, refer to the Troubleshooting Guide.

5. Make sure the 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 to 350 µl of supernatant.
7. Add 100 µl of Solution IRS and vortex for 5 s. Incubate at 4°C for 5 min.
8. Centrifuge 10,000 x *g* for 1 min at room temperature.
9. Avoiding the pellet, transfer all of the supernatant to a 2 ml Collection Tube (provided).
   Note: Expect approximately 450 µl of supernatant. A small carryover of glass beads is possible. This will not affect the results.
10. Add 900 µl of Solution SB to the supernatant and vortex for 5 s.
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 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 and centrifuge at 10,000 x *g* for 1min at room temperature.
14. Being careful not to splash liquid on the spin filter basket, place MB Spin Column in a new 2 ml Collection Tube (provided).
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 point before starting

- The PowerLyzer 24 Homogenizer may cause marring of labels on the tops of the PowerBead Tubes. To ensure proper sample identification, label sides and tops of the tubes.
- If Solution SL has precipitated, heat at 60°C until the precipitate has dissolved.
- 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 pipet tip.
   Note: Based 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, Glass 0.1 mm.
   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 cells that are difficult to lyse, 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 60°C will dissolve the SDS. Solution SL can be used while it is still warm.

4. Homogenization options:

   a) PowerLyzer 24 Homogenizer: Balance PowerBead Tubes in the tube holder for the PowerLyzer 24. Homogenize for 5 min at 2000 RPM.
   Note: Depending on the sample, you can homogenize at a higher speed for less time.
   b) Vortex: Secure PowerBead Tube horizontally using the Vortex Adapter tube holder (cat. no. 13000-V1-24). Vortex at maximum speed for 10 min.
   Note: To minimize DNA shearing, refer to the Troubleshooting Guide. 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 FastPrep machines.

5. Make sure the 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 and vortex for 5 s. Incubate at 4°C for 5 min.
8. Centrifuge 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 all of the supernatant to a 2 ml Collection Tube (provided).
   Note: Expect 450 µl of supernatant. A small carryover of glass beads is possible. This will not affect the results. 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. Being careful not to splash liquid on the spin filter basket, place the MB Spin Column in a new 2 ml Collection Tube (provided).
15. Add 50 µl of Solution EB to the center of the white filter membrane.
    Note: Placing Solution EB (elution buffer) in the center of the small white membrane will ensure that 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 point before starting

- If Solution SL has precipitated, heat at 60°C until the precipitate dissolves.
- 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 8 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 (after step 10 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 VacValve to keep the spin filter steady. Allow the lysate to pass through the MB Spin Column.
7. 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 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.

8. 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 VacValve.
9. Add 300 µl of Solution CB to each MB Spin Column. Open the VacValve 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.
10. Turn off the vacuum source and open an unused port to vent the manifold. If all the 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. Place into the centrifuge and spin 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 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.
13. Centrifuge at 13,000 x *g* for 1 min at room temperature.
14. 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 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. 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).
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 sterile, DNA-free PCR-grade water (cat. no. 17000-10).
Alternative lysis methods		
a)	To increase yields	Incubate at 65°C for 10 minutes after adding Solution SL (Step 3). Then, continue with Step 4.
b)	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. Homogenization using a PowerLyzer 24 may be performed at higher speeds (up to 2800 RPM) and for up to 5 minutes to increase the lysis of tough organisms, but higher speeds may result in significant DNA shearing

		Comments and suggestions
c)	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 PowerLyzer Microbial Kit (50)	For 50 preps: Isolation of high-quality DNA from microbial cultures using a bead-based homogenizer	12255-50
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 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

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Limited License Agreement for DNeasy PowerLyzer Microbial Kit

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