January 2020

DNeasy® PowerBiofilm® Kit Handbook

For the isolation of high-quality, pure DNA from biofilm samples

Sample to Insight

QIAGEN

Contents

Kit Contents

DNeasy PowerBiofilm Kit	**(50)**
Catalog no.	**24000-50**
Number of preps	**50**
PowerBiofilm Bead Tubes	50
MB Spin Columns	50
Solution MBL	25 ml
Solution FB	17 ml
Solution IRS	15 ml
Solution MR	50 ml
Solution PW	2 x 30 ml
Ethanol	2 x 30 ml
Solution EB	9 ml
Collection Tubes (2 ml)	5 x 50
Quick-Start Protocol	1

Storage

The DNeasy PowerBiofilm Kit 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.

QIAcube® Connect is designed to perform fully automated purification of nucleic acids and proteins in molecular biology applications. The system is intended for use by professional users trained in molecular biological techniques and the operation of QIAcube Connect.

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.**

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

Solution FB, 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 PowerBiofilm Kits is tested against predetermined specifications to ensure consistent product quality.

Introduction

The DNeasy PowerBiofilm Kit is the first of its kind and is designed for isolating high-quality DNA from all types of biofilm samples, including microbial mats. The novel bead tube mix and enhanced lysis buffers help dissolve polysaccharides and enable the lysis of organisms in even the most complex biofilm samples. The DNeasy PowerBiofilm Kit uses Inhibitor Removal Technology® (IRT) to yield inhibitor-free, purified DNA for use in downstream applications.

Principle and procedure

Up to 0.20 g of sample material is added to a PowerBiofilm Bead Tube, which is then heated to activate lysis components that help dissolve polysaccharides. Lysis continues through either vortex-mixing or bead-beating, depending on user preference. Proteins and inhibitors are removed followed by precipitation of humic substances, polyphenolics and polysaccharides. Total genomic DNA is captured using a silica spin filter. Finally, high-quality DNA is washed and eluted from the spin filter membrane and is ready for use in downstream applications, including PCR and qPCR.

Optimized for homogenization with the PowerLyzer® 24 Homogenizer

The DNeasy PowerBiofilm Kit may be used with a vortex or high velocity bead beater, such as the PowerLyzer 24 Homogenizer. The PowerLyzer 24 Homogenizer is suitable for fast homogenization of biofilm samples, including microbial mats.

The PowerLyzer 24 Homogenizer is a highly efficient bead beating system that allows for optimal DNA extraction from biofilms. The instrument's velocity and proprietary motion combine to provide the fastest homogenization time possible, minimizing the time spent processing samples. The programmable display allows for hands-free, walk-away extraction with up to ten cycles of bead beating for as long as 5 minutes per cycle. The DNeasy PowerBiofilm Kit provides PowerBiofilm Bead Tubes prefilled with glass and ceramic beads for homogenizing biofilm material for optimal DNA isolation.

Using the DNeasy PowerBiofilm Kit with other homogenizers

To isolate DNA using the DNeasy PowerBiofilm Kit with FastPrep® or Precellys® homogenizers, use the conversion chart shown in 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 using it 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 to use the DNeasy PowerBiofilm Kit with FastPrep or Precellys homogenizers

PowerLyzer 24 Homogenizer (rpm)	FastPrep 24 (m/s)	Precellys 24 (rpm)
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 of samples using the DNeasy PowerBiofilm Kit 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.

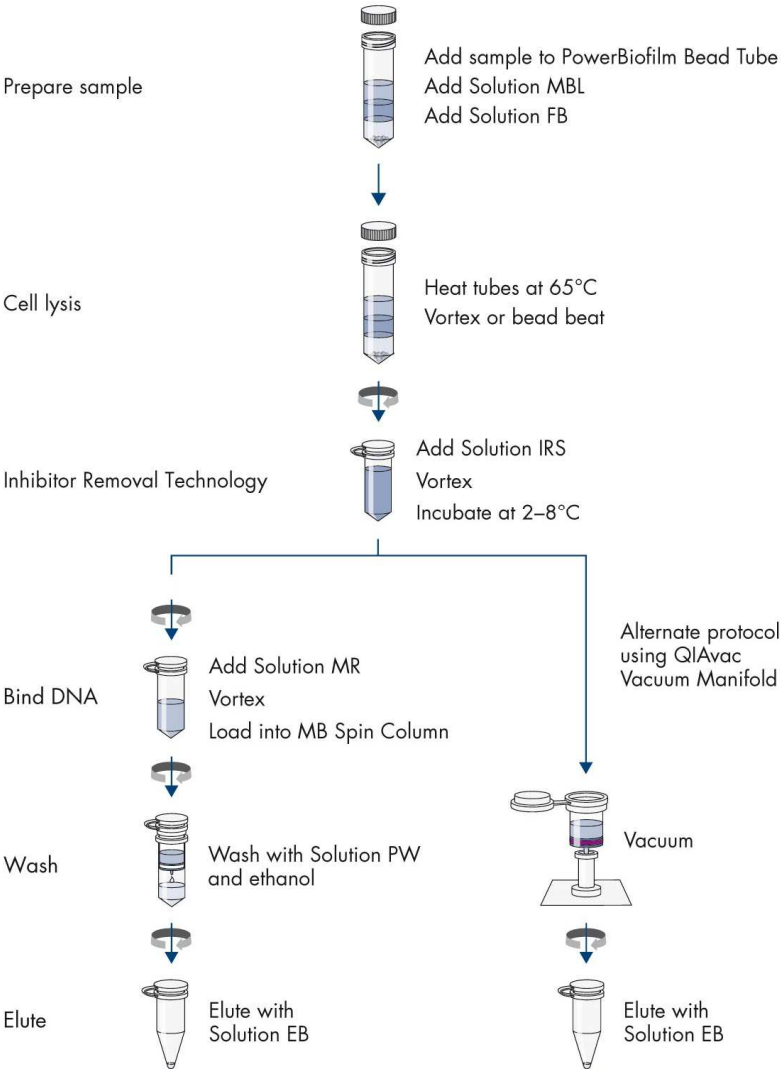
Automated purification of DNA on QIAcube Instruments

Purification of DNA can be fully automated on QIAcube Connect or the classic QIAcube. The innovative QIAcube instruments use advanced technology to process QIAGEN spin columns, enabling seamless integration of automated, low-throughput sample prep into your laboratory workflow. Sample preparation using QIAcube instruments follows the same steps as the manual procedure (i.e., lyse, bind, wash and elute), enabling you to continue using the DNeasy PowerBiofilm Kit for purification of high-quality DNA.

QIAcube instruments are preinstalled with protocols for purification of plasmid DNA, genomic DNA, RNA, viral nucleic acids and proteins, plus DNA and RNA cleanup. The range of protocols available is continually expanding, and additional QIAGEN protocols can be downloaded free of charge at **www.qiagen.com/qiacubeprotocols**.

QIAcube Connect.

DNeasy PowerBiofilm 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 (up to13,000 x *g*)
- PowerLyzer 24 or another bead homogenizer
- Pipettor (100–1000 µl)
- Vortex-Genie® 2
- Vortex Adapter for vortexing 1.7 or 2 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

- Warm Solution MBL at 55°C for 5–10 min to dissolve precipitates prior to each use. Solution MBL should be used while still warm.
- If Solution MR has precipitated warm at 55°C for 5–10 minutes.
- Shake to mix Solution PW before use.
- Use only PowerBiofilm Bead Tubes with this kit.
- Perform all centrifugation steps at room temperature (15–25°C).

Procedure

1. Weigh out 0.05–0.20 g of biofilm material and place into a 2 ml Collection Tube (provided). Centrifuge at 13,000 x *g* for 1min. Remove excess liquid using a pipette tip.

   Note: Add less saturated samples (e.g., microbial mats) directly to the PowerBiofilm Bead Tube (For information on selecting the right amount of starting material, refer to the Troubleshooting Guide).
2. Resuspend the biofilm material in 350 µl of Solution MBL and transfer to the PowerBiofilm Bead Tube. For less saturated samples, add 350 µl of Solution MBL to the PowerBiofilm Bead Tube already containing the biofilm material.
3. Add 100 µl of Solution FB. Vortex briefly to mix.
4. Incubate the PowerBiofilm Bead Tube at 65°C for 5 min.

5. Bead beat the sample following one of the methods described below:

   A. PowerLyzer 24 Homogenizer

   1. Identify each PowerBiofilm Bead Tube on **both** the cap and on the side.

   2. Properly balance the Bead Tubes in the tube holder of the PowerLyzer 24 and homogenize for 1 cycle at 3200 rpm for 30 s.

   3. Centrifuge the tube at 13,000 x *g* for 1 min. Transfer the supernatant to a new 2 ml Collection Tube (provided).

   Note: Expect approximately 325–400 µl of supernatant depending on sample material. If the volume falls below this range, use less starting material.

   B. Vortex Adapter

   1. Secure the PowerBiofilm Bead Tube horizontally to a Vortex Adapter and vortex at maximum speed for 10 min.

   Note: If using the 24-place Vortex Adapter for ≥12 preps, increase time by 5–10 min.

   2. Centrifuge the tube at 13,000 x *g* for 1 min. Transfer the supernatant to a clean 2 ml Collection Tube (provided).

   Note: Expect approximately 400–450 µl of supernatant depending on sample material. If the volume falls below this range, use less starting material.

6. Add 100 µl of Solution IRS and vortex briefly to mix. Incubate at 2–8°C for 5 min.

   Note: Use 200 µl of Solution IRS if the sample is known to contain excessive amounts of inhibitors or the supernatant is very darkly colored. Refer to the Troubleshooting Guide.

7. Centrifuge the tube at 13,000 x *g* for 1 min.

8. Avoiding the pellet, transfer all of the supernatant to a 2 ml Collection Tube (provided).

   Note: Expect approximately 375–450 µl in volume depending on sample material.

9. Add 900 µl of Solution MR and vortex briefly to mix.

10. Load 650 µl of supernatant onto a 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 processed.

11. Place the MB Spin Column into a clean 2 ml Collection Tube (provided).

12. Add 650 µl of Solution PW and centrifuge at 13,000 x *g* for 1 min.

13. Discard the flow-through and add 650 µl of ethanol (provided) and centrifuge at 13,000 x *g* for 1 min.
14. Discard the flow-through and centrifuge again at 13,000 x *g* for 2 min.
15. Place the MB Spin Column into a clean 2 ml Collection Tube (provided).
16. Add 100 µl of Solution EB to the center of the white filter membrane.
17. Centrifuge at 13,000 x *g* for 1 min.
18. Discard the MB Spin Column. The DNA is now ready for downstream applications.

Protocol: Detailed

Important points before starting

- Warm Solution MBL at 55°C for 5–10 min to dissolve precipitates prior to each use. Solution MBL should be used while still warm.
- If Solution MR has precipitated warm at 55°C for 5–10 minutes.
- Shake to mix Solution PW before use.
- Use only PowerBiofilm Bead Tubes with this kit.
- Perform all centrifugation steps at room temperature (15–25°C).

Procedure

1. Weigh out 0.05–0.20 g of biofilm material and place into a 2 ml Collection Tube (provided). Centrifuge at 13,000 x *g* for 1min. Remove excess liquid using a pipette tip.

   Note: Add less saturated samples (e.g., microbial mats) directly to the PowerBiofilm Bead Tube (For information on selecting the right amount of starting material, refer to the Troubleshooting Guide).

   Note: Biofilm samples will vary in their moisture content. It is important to remove residual liquid to prevent dilution of the lysis components, which could result in reduced DNA yield. Some biofilm samples, such as microbial mats, may be added directly to the PowerBiofilm Bead Tube without an initial centrifugation step.

2. Resuspend the biofilm material in 350 µl of Solution MBL and transfer to the PowerBiofilm Bead Tube. For less saturated samples, add 350 µl of Solution MBL to the PowerBiofilm Bead Tube already containing the biofilm material.

   Note: Solution MBL contains Inhibitor Removal Technology (IRT). It is a strong lysing reagent that includes a detergent to help break cell walls and stabilize and protect DNA from degradation. When cold, Solution MBL will form a white precipitate in the bottle. Heating to 55°C will dissolve the components without harm. Solution MBL should be used while it is still warm.

3. Add 100 µl of Solution FB. Vortex briefly to mix.

   Note: Solution FB contains a chaotropic agent that aids in lysis. Solution FB also stabilizes and protects DNA integrity.

4. Incubate the PowerBiofilm Bead Tube at 65°C for 5 min.

   Note: Lysis components are heat-activated to aid in the breakdown of extracellular polymeric substances (EPS).

5. Bead beat the sample following one of the methods described below:

   Note: Dissolution of the biofilm matrix and lysis of microbial cells occurs through a combination of chemical (lysis buffers) and mechanical (bead beating) lysis conditions. The resulting cell debris is pelleted along the side of the tube while DNA remains in the supernatant. This step is important for the removal of contaminating non-DNA organic and inorganic matter that may reduce DNA purity and inhibit downstream applications.

   A. PowerLyzer 24 Homogenizer

   1. Identify each PowerBiofilm Bead Tube on **both** the cap and on the side.
   Note: The high energies of the PowerLyzer 24 Homogenizer may lead to marring of labels on the tops of the PowerBiofilm Tube caps. Therefore, we recommend marking the sides of the PowerBiofilm Bead Tubes, as well as the caps, to ensure proper sample identification.

   2. Properly balance the Bead Tubes in the tube holder of the PowerLyzer 24 and homogenize for 1 cycle at 3200 rpm for 30 s.

   3. Centrifuge the tube at 13,000 x *g* for 1 min. Transfer the supernatant to a new 2 ml Collection Tube (provided).
   Note: Expect approximately 325–400 µl of supernatant depending on sample material. If the volume falls below this range, use less starting material.

B. Vortex Adapter

1. Secure the PowerBiofilm Bead Tube horizontally to a Vortex Adapter and vortex at maximum speed for 10 min.

Note: If using the 24 place Vortex Adapter for ≥12 preps, increase time by 5–10 min.

2. Centrifuge the tube at 13,000 x *g* for 1 min. Transfer the supernatant to a clean 2 ml Collection Tube (provided).

Note: Expect approximately 400–450 µl of supernatant depending on sample material. If the volume falls below this range, use less starting material.

6. Add 100 µl of Solution IRS and vortex briefly to mix. Incubate at 2–8°C for 5 min.

Note: Use 200 µl of Solution IRS if the sample is known to contain excessive amounts of inhibitors or the supernatant is very darkly colored. Refer to the Troubleshooting Guide.

Note: Solution IRS contains IRT and is a second reagent that removes non-DNA organic and inorganic material, including humic acid, cell debris, polyphenolics, polysaccharides and proteins. The system works by using changes in pH to precipitate large insoluble macromolecules. Nucleic acids do not precipitate and are cleared of inhibitors. It is important to remove contaminating organic and inorganic matter that may reduce DNA purity and inhibit downstream applications.

7. Centrifuge the tube at 13,000 x *g* for 1 min.

8. Avoiding the pellet, transfer all of the supernatant to a 2 ml Collection Tube (provided).

Note: Expect approximately 375–450 µl in volume depending on sample material.
Note: The pellet at this point contains additional non-DNA organic and inorganic material. For best DNA yields and quality, avoid transferring any of the pellet.

9. Add 900 µl of Solution MR and vortex briefly to mix.

Note: Solution MR is a highly concentrated salt solution. DNA binds tightly to silica at high salt concentrations, and Solution MR adjusts the salt concentration of the DNA solution 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 membrane.

10. Load 650 µl of supernatant onto a 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 processed.

    Note: DNA is selectively bound to the silica membrane in the MB Spin Column while the flow-through containing non-DNA components is discarded.

11. Place the MB Spin Column into a clean 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 into a clean 2 ml Collection Tube to aid in the subsequent wash steps and improve DNA purity and yield.

12. Add 650 µl of Solution PW and centrifuge at 13,000 x *g* for 1 min.

    Note: Solution PW is an alcohol-based wash solution used to further clean the DNA that is bound to the MB Spin Column filter membrane. Solution PW removes residual salt and other contaminants while allowing the DNA to stay bound to the MB Spin Column.

13. Discard the flow-through and add 650 µl of ethanol (provided) and centrifuge at 13,000 x *g* for 1 min.

    Note: Ethanol ensures complete removal of Solution PW, which results in higher DNA purity and yield.

14. Discard the flow-through and centrifuge again at 13,000 x *g* for 2 min.

    Note: The second spin removes residual ethanol. It is critical to remove all traces of ethanol because it can interfere with downstream DNA applications.

15. Place the MB Spin Column into a clean 2 ml Collection Tube (provided).

16. Add 100 µl of Solution EB to the center of the white filter membrane.

    Note: Placing Solution EB in the center of the small white membrane will make sure the entire membrane is wet. This will result in a more efficient and complete release of DNA from the MB Spin Column filter membrane. As Solution EB passes through the MB Spin Column membrane, DNA that was bound in the presence of high salt is selectively released. Alternatively, sterile DNA-Free PCR-Grade Water (cat. no. 17000-10) may be used to elute DNA at this step. Solution EB contains no EDTA; if DNA degradation is a concern, sterile TE may also be used instead of Solution EB.

17. Centrifuge at 13,000 x *g* for 1 min.

18. Discard the MB Spin Column. The DNA is now ready for downstream applications.

Protocol: QIAvac 24 Plus Vacuum Manifold

Important points before starting

- Warm Solution MBL at 55°C for 5–10 min to dissolve precipitates prior to each use. Solution MBL should be used while still warm.
- If Solution MR has precipitated warm at 55°C for 5–10 minutes.
- Shake to mix Solution PW before use.
- Use only PowerBiofilm Bead Tubes with this kit.
- 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 an 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 9 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 port.
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 (15–25°C).
14. Discard the MB Spin Column. The DNA 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
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. If you live in a humid climate, you may experience increased difficulty drying the MB Spin Column membrane. Increase the centrifugation time at step 14 by another minute or until no visible moisture remains on the membrane.
b)	Concentrating eluted DNA	The final volume of eluted DNA will be 100 µl. The DNA may be concentrated by adding 5–10 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 –30°C to –15°C for 30 minutes and centrifuge at 10,000 x *g* for 5 minutes at room temperature (15–25°C). 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 Solution EB.

		Comments and suggestions
c)	Storing DNA	DNA is eluted in Solution EB (10 mM Tris) and must be stored at –30°C to –15°C or –90°C to –65°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).
d)	DNA does not amplify or it has reduced amplification efficiency	Biofilms with high concentrations of humic substances and other contaminants may yield DNA with some inhibitor carryover, which can prevent target sequences from amplifying during PCR. Under such circumstances, the template DNA can be diluted one or several fold for successful PCR. For additional preps of the same or similar sample type, use 200 µl of Solution IRS at step 6 to avoid inhibitor carryover.
e)	Low $A_{260/230}$ ratios	$A_{260/230}$ readings are one measure of DNA purity. For samples with low biomass, which could lead to low DNA yields (<20 ng/µl), this ratio may fall below 1.5. The $A_{260/230}$ ratio is not an indicator of DNA integrity or amplification potential. Ethanol precipitation with resuspension in a smaller volume to concentrate the DNA may help to improve the $A_{260/230}$ ratio.

		Comments and suggestions
f)	Low or no DNA yields	Yields may be significantly reduced if too much starting material is used, if samples are bead beaten for too long or if PowerBiofilm Bead Tubes are not used. To avoid sample loss: • Do not use more sample than the specified range (0.05–0.20 g). • Reduce bead beating time for homogenization. For most biofilms 30 seconds is optimal. Tougher samples, such as microbial mats, may require longer bead beating times. When using the Vortex Adapter, no time adjustment is necessary unless you are processing more than 12 preps using the 24-place Vortex Adapter. In this case run for an additional 5–10 minutes. • Do not use any other bead tube except the PowerBiofilm Bead Tubes provided with this kit.
Sample processing		
a)	Amount of starting material to process	The DNeasy PowerBiofilm Kit is designed to process 0.05 to 0.2 g of biofilm or microbial mat material. The actual amount will depend on the type of biofilm and microbial density. If supernatant amounts fall under the range provided in step 5 of the protocol, then DNA yields will not be optimal and less sample material should be used. A recommended starting amount is 0.1 to 0.15 g. For examples of expected yields, please see Appendix A.
b)	Forgetting to warm Solution MBL	If Solution MBL is not warmed prior to use, continue with the protocol. You will still obtain DNA, but yields may not be optimal.

		Comments and suggestions
c)	MB Spin Columns become clogged during Vacuum Manifold protocol	Some sample lysates may be too viscous to move through the MB Spin Column filter membrane under vacuum. If this occurs, switch to the centrifugation protocol.

Appendix A: Expected DNA Yields

DNA yields will vary depending on the type of biofilm being processed. Yields may also vary between samples of the same biofilm due to differences in their structures. Examples of expected yields are provided as a reference in Table 2 (below). Due to the diversity of biofilm sample types, yields may fall outside of the examples provided.

Table 2. Expected DNA yields using the DNeasy PowerBiofilm Kit

Biofilm type	Sample amount (g)	DNA yield (ng/µl)
Sink pipe	0.20	94–198
Lagoon rocks	0.15	100–150
Phototrophic biofilm (microbial mat)	0.15	54–130
	0.10	70–76
	0.05	37–50
Stream rocks	<0.05	4–11
Bioreactor	0.25	56–130
Button thrombolites (microbial mat)	0.25	1–15
Gypsum crust	0.20	15–28

Ordering Information

Product	Contents	Cat. no.
DNeasy PowerBiofilm Kit (50)	For isolation of high-quality, pure DNA from biofilm samples	24000-50
QIAcube Connect — for fully automated nucleic acid extraction with QIAGEN spin-column kits		
QIAcube Connect*	Instrument, connectivity package, 1-year warranty on parts and labor	Inquire
Starter Pack, QIAcube	Filter-tips, 200 µl (1024), 1000 µl filter-tips (1024), 30 ml reagent bottles (12), rotor adapters (240), elution tubes (240), rotor adapter holder	990395
Related products		
RNeasy® PowerBiofilm Kit (50)	For the isolation of high quality total RNA from biofilm samples	25000-50
Vortex Adapter for 24 (1.5–2.0 ml) tubes	For vortexing 1.7 ml or 2 ml tubes using the Vortex-Genie® 2 Vortex	13000-V1-24
PowerLyzer 24 Homogenizer	For complete lysis and homogenization of any biological sample	13155

* All QIAcube Connect instruments are provided with a region-specific connectivity package, including tablet and equipment necessary to connect to the local network. Further, QIAGEN offers comprehensive instrument service products, including service agreements, installation, introductory training and preventive subscription. Contact your local sales representative to learn about your options.

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.

Document Revision History

Date	Changes
January 2020	Updated text, ordering information and intended use for QIAcube Connect.

Notes

Notes

Limited License Agreement for DNeasy PowerBiofilm 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.

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HB-2274-002