October 2017

RNeasy® PowerBiofilm® Kit Handbook

For the isolation of high-quality total RNA from biofilm samples

Sample to Insight

QIAGEN

Contents

Kit Contents

RNeasy PowerBiofilm Kit	**(50)**
Catalog no.	**25000-50**
Number of preps	**50**
PowerBiofilm Bead Tubes	50
Solution MBL	25 ml
Solution FB	17 ml
Solution IRS	15 ml
Solution PB	37 ml
Solution PW	3 x 30 ml
Ethanol	2 x 30 ml
Solution WB	28 ml
RNase-free water	7 ml
DNase Digestion Solution	2 x 1.5 ml
DNase I (RNase-free)	1 vial (1500 units)
PowerBiofilm Bead Tubes	50
MB RNA Spin columns	50
Collection Tubes (2 ml)	5 x 50
Quick Start Protocol	1

Storage

The RNeasy PowerMicrobiome Kit contains lysophilized DNase I. Remove and store at 4°C immediately upon receipt. All other reagents and components should be stored at room temperature (15–25°C). After resuspension, DNase I must be stored at –20°C. DNase is sensitive to physical denaturation. Do not vortex the resuspended DNase.

Intended Use

All RNeasy 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 RNA 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 	**Flammable reagents** **[W1]** Ethanol and Solution PW (which contains alcohol) are flammable.

CAUTION 	**Potentially reactive compound** **[C1]** Do not add bleach or acidic solutions directly to the sample preparation waste. The sample preparation waste contains guanidine hydrochloride from Solution WB, Solution FB and Solution MBL (guanidine thiocyanate) and Solution PB (guanidine hydrochloride), which can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilt, clean with 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 RNeasy PowerBiofilm Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

The RNeasy PowerBiofilm Kit is designed for isolating high-quality RNA from all types of biofilm samples, including microbial mats. The novel bead tube mix and enhanced lysis buffers help to dissolve polysaccharides to enable lysis of organisms in even the most complex biofilm samples. Inhibitor Removal Technology® (IRT) allows for inhibitor free, purified RNA ready for a host of downstream applications. RNase-free DNase I is provided for on-column genomic DNA removal during the protocol, saving time and post-processing steps.

Principle and Procedure

The protocol starts by adding 0.5 to 0.20 g of sample material to the PowerBiofilm Bead Tube. The tube is then heated to activate lysis components that help to dissolve polysaccharides. As the material is vortexed, lysis continues. Proteins and inhibitors are removed and precipitated along with polyhenolics, polysaccharides and other humic materials.

Total RNA is captured on the novel flat bottom silica-spin column where an on-column DNase step is incorporated to remove genomic DNA. The column is then washed and the RNA and the RNA eluted. The purified RNA is ready to use in downstream applications including RT-PCR, qRT-PCR, cDNA synthesis or RNA amplification.

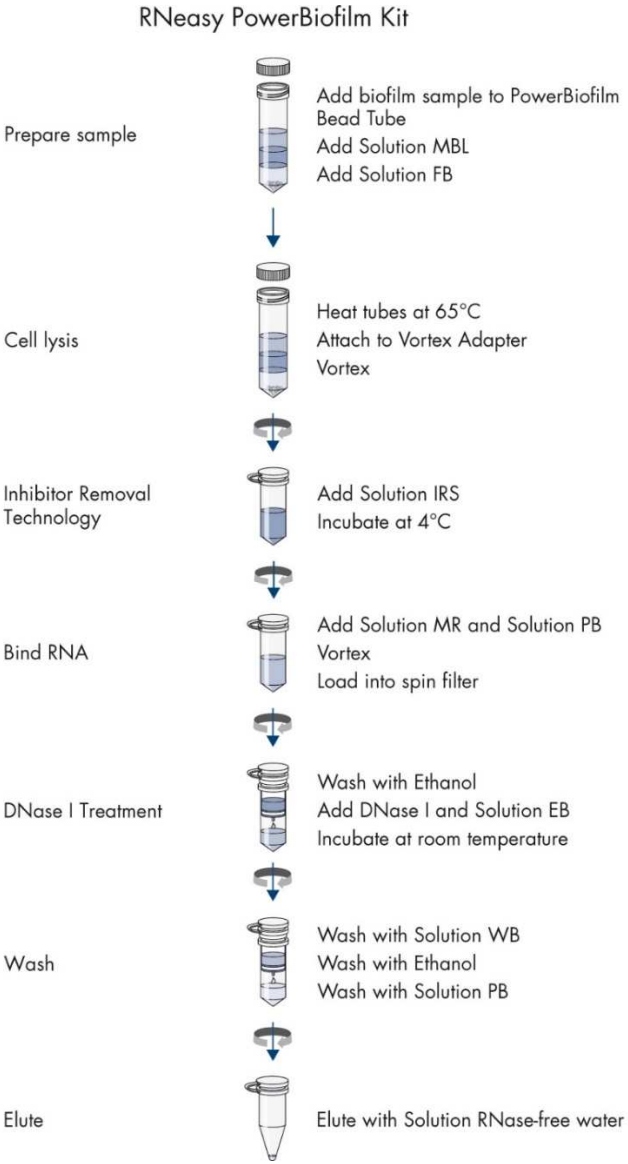

Figure 1. RNeasy PowerBiofilm Kit procedure.

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 (13,000 x *g*)
- Pipettor (1.5–1000 µl)
- Vortex-Genie® 2 Vortex
- Vortex Adapter for 24 (1.5–2.0 ml) tubes (cat. no. 13000-V1-24)
- β–mercaptoethanol (βME)

Protocol: Experienced User

Notes before starting

- Warm Solution MBL at 55°C for 5–10 min to dissolve precipitates prior to each use.
- Shake to mix Solution PW before use.
- Use only PowerBiofilm Bead Tubes with this kit.
- Add 5 µl of β-mercaptoethanol (βME) to 345 µl of Solution MBL (i.e., a total of 350 µl) for each sample to be processed. Prepare just enough fresh Solution MBL/βME for samples to be processed that day instead of adding βME to the entire bottle of Solution MBL. Use a fume hood when using βME to avoid exposure.
- Prepare DNase I stock solution by adding 300 µl RNase-free water to the lyophilized DNase I and mixing gently. Aliquot the enzyme in 50 µl portions and store at –20°C. **Note:** The enzyme can be freeze/thawed up to three times without loss of activity. To prepare DNase I Solution, combine 5 µl of DNase I stock solution with 45 µl of DNase Digestion Solution (i.e., a total of 50 µl) for each sample to be processed.

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 1 min. 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/βME and transfer to the PowerBiofilm Bead Tube.

   Note: For less-saturated samples, add 350 µl of Solution MBL/βME directly to the PowerBiofilm Bead Tube 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. Secure the PowerBiofilm Bead Tube horizontally to a Vortex Adapter.
6. Vortex at maximum speed for 10 min.

   Note: If using the 24 place Vortex Adapter for >12 preps, increase time by 5–10 min.
7. Centrifuge the tubes at a maximum speed 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 375–450 µl in volume depending on sample material. If the volume falls below this range, use less starting material.
9. Add 100 µl of Solution IRS and vortex briefly to mix. Incubate at 4°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.
10. Centrifuge the tubes at 13,000 x *g* for 1 min at room temperature.
11. Avoiding the pellet, transfer all the supernatant to a 2 ml Collection Tube (provided).
    Note: Expect approximately 375–450 µl in volume depending on sample material.
12. Add 450 µl of Solution PB and 450 µl of ethanol (provided) and vortex briefly to mix.
13. Load 650 µl of supernatant onto a MB RNA 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 Spin Column.
14. Add 650 µl of Solution PW and centrifuge at 13,000 x *g* for 1 min. Discard the flow-through and centrifuge again at 13,000 x *g* for 1 min to remove residual wash.
15. Place the MB RNA Spin Column into a clean 2 ml Collection Tube (provided).
16. Add 50 µl of DNase I Solution to the center of the MB Spin Column. Incubate at room temperature for 15 min.
17. Add 400 µl of Solution WB and centrifuge the column at 13,000 x *g* for 1 min.
18. Discard flow-through. Add 650 µl of Solution PW. Centrifuge at 13,000 x *g* for 1 min.
19. Discard flow-through. Add 650 µl of ethanol and centrifuge at 13,000 x *g* for 1 min.
20. Discard flow-through. Centrifuge at 13,000 x *g* for 2 min to remove residual wash.

21. Place the MB RNA Spin Column into a clean 2 ml Collection Tube (provided). Add 100 µl of RNase-free water (provided) to the center of the white filter membrane.
    Note: Eluting with 100 µl of RNase-free water will maximize RNA yield. For more concentrated RNA, a **minimum** of 50 µl of RNase-free water can be used.
22. Centrifuge at 13,000 x *g* for 1 min.
23. Discard the MB Spin Column. The RNA is now ready for downstream applications.

Protocol: Detailed

Notes before starting

- Warm Solution MBL at 55°C for 5–10 min to dissolve precipitates prior to each use.
- Shake to mix Solution PW before use.
- Use only PowerBiofilm Bead Tubes with this kit.
- Add 5 µl of β-mercaptoethanol (βME) to 345 µl of Solution MBL (i.e., a total of 350 µl) for each sample to be processed. Prepare just enough fresh Solution MBL/βME for samples to be processed that day instead of adding βME to the entire bottle of Solution MBL. Use a fume hood when using βME to avoid exposure.
- Prepare DNase I stock solution by adding 300 µl RNase-free water to the lyophilized DNase I and mixing gently. Aliquot the enzyme in 50 µl portions and store at –20°C. **Note:** The enzyme can be freeze/thawed up to three times without loss of activity. To prepare DNase I Solution, combine 5 µl of DNase I stock solution with 45 µl of DNase Digestion Solution (i.e., a total of 50 µl) for each sample to be processed.

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 1 min. 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 the in their moisture content. It is important to remove residual liquid to prevent dilution of the lysis components which could result in reduced RNA yield. This step is not needed for some biofilm samples, such as microbial mats.

2. Resuspend the biofilm material in 350 µl of Solution MBL/βME and transfer to the PowerBiofilm Bead Tube.

   Note: For less-saturated samples, add 350 µl of Solution MBL/βME directly to the PowerBiofilm Bead Tube containing the biofilm material.

   Note: Solution MBL is a component of Inhibitor Removal Technology (IRT). It is a strong lysing reagent that includes a detergent to help break cell walls and stabilizes and protects RNA from degradation. When cold, this solution will form a white precipitate in the bottle. Heating to 55°C will dissolve the components without harm. Solution MBL can be used while 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 RNA 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.

5. Secure the PowerBiofilm Bead Tube horizontally to a Vortex Adapter.

6. Vortex at maximum speed for 10 minutes.

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

   Note: Dissolution of the biofilm matrix and lysis of microbial cells occurs using a combination of chemical (lysis buffers) and mechanical (bead beating) lysis conditions. Use of the Vortex Adapter will maximize homogenization by holding the tubes equal distance and angle from the center of rotation. Avoid using tape, which can become loose and result in reduced homogenization efficiency.

7. Centrifuge the tubes at a maximum speed of 13,000 x *g* for 1 min at room temperature.

   Note: Cell debris is pelleted along the side of the tube while the RNA remains in the supernatant. This step is important for the removal of contaminating non-RNA organic and inorganic matter that may reduce the RNA purity and inhibit downstream applications.

8. Transfer the supernatant to a clean 2 ml Collection Tube (provided).

   Note: Expect approximately 375–450 µl in volume depending on sample material. If the volume falls below this range, use less starting material.

9. Add 100 µl of Solution IRS and vortex briefly to mix. Incubate at 4°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 is the second reagent in the IRT. It removes additional organic and inorganic material including humic acid, cell debris, polyphenolics, polysaccharides and proteins. The system works by using changes in pH to precipitate the insoluble large macromolecules. The nucleic acids do not precipitate and are cleaned of inhibitors. It is important to remove contaminating organic and inorganic matter that may reduce the RNA purity and inhibit downstream RNA applications.

10. Centrifuge the tubes at 13,000 x *g* for 1 min at room temperature.

11. Avoiding the pellet, transfer all 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-RNA organic and inorganic material. For best RNA yields and quality, avoiding transferring any of the pellet.

12. Add 450 µl of Solution PB and 450 µl of ethanol (provided) and vortex briefly to mix.

    Note: Solution PB is a high concentration salt solution. It and ethanol are both necessary to create the conditions required for efficient blinding of the RNA to the spin column while allowing proteins and cellular debris to pass through.

13. Load 650 µl of supernatant onto a MB RNA 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 Spin Filter.

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

14. Add 650 µl of Solution PW and centrifuge at 13,000 x *g* for 1 min. Discard the flow-through and centrifuge again at 13,000 x *g* for 1 min to remove residual wash.

Note: Solution PW is an alcohol based wash solution used to wash the spin filter column in preparation for the on-column DNase I digestion. This wash solution removes residual salt and other contaminants while allowing the RNA to stay bound to the silica membrane. Complete removal of Solution PQ is required for efficient and complete DNase I digestion.

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

16. Add 50 µl of DNase I Solution to the center of the MB Spin Column. Incubate at room temperature for 15 min.

Note: DNase I is mixed with high activity digestion buffer and is used to completely remove genomic DNA from the Spin Filter membrane. If the RNA is to be used for reverse transcription and/or RT PCR, it is highly recommended to remove all genomic DNA with DNase I digestion.

17. Add 400 µl of Solution WB and centrifuge the column at 13,000 x *g* for 1 min.

Note: Solution WB is a wash buffer used to inactivate DNase I and wash away residual enzyme and digested DNA while allowing RNA to remain tightly bound to the spin column.

18. Discard flow-through. Add 650 µl of Solution PW. Centrifuge at 13,000 x *g* for 1 min.

Note: Solution PW is an ethanol based wash buffer used to remove residual salt and contaminants on the column in preparation for the release and elution of the bound RNA. Complete removal of all traces of the wash solution is critical.

19. Discard flow-through. Add 650 µl of ethanol and centrifuge at 13,000 x *g* for 1 min.

Note: Ethanol ensures complete removal of Solution PW which will result in higher RNA purity and yield.

20. Discard flow-through. Centrifuge at 13,000 x *g* for 2 min to remove residual wash.

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

21. Place the MB RNA Spin Column into a clean 2 ml Collection Tube (provided). Add 100 µl of RNase-free water (provided) to the center of the white filter membrane.

    Note: Eluting with 100 µl of RNase-free water will maximize RNA yield. For more concentrated RNA, a minimum of 50 µl of RNase-free water can be used.

    Note: RNase-free water used to elute the RNA from the silica membrane of the spin column. Placing the sterile elution buffer 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 the RNA from the silica-spin filter membrane. As the RNase-free water passes through the silica membrane, RNA that was bound in the presence of high salt is selectively released, as the sterile water buffer lacks salt.

22. Centrifuge at 13,000 x *g* for 1 minute.

23. Discard the MB Spin Column. The RNA 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		
a)	Amount of starting material	This kit is designed to process 0.05 to 0.2 g of biofilm or microbial mat material. The actual amount to use will depend on the type of biofilm and microbial density. If supernatant amounts fall under the range provided in step 8 of the protocol then RNA yields will not be optimal and less sample material should be used for processing. A recommended starting amount is 0.1–0.15 g. For examples of expected yields, see "Expected RNA Yields."
Solution		
a)	Forgetting to warm Solution MBL	If Solution MBL is not warmed prior to use, continue with the protocol. You will still obtain RNA, but the yields may not be optimal.
RNA		
a)	Expected RNA yields	RNA yields will vary depending on the type of biofilm. Yields may also vary between samples of the same biofilm due to their structure. Examples of expected yields are provided as a reference. Due to diversity of biofilm types, yields may fall outside of the examples provided.

Biofilm type	Sample amount (g)	RNA yield (ng/µl)
Lagoon rocks	0.20–0.23	37–60
Phototrophic Biofilm (microbial mat)	0.10–0.15	10–100
Button thrombolites (microbial mat)	0.25	1–1.70
Deep sea microbial mat	0.20–0.30	11–35

		Comments and suggestions
b)	Low or no RNA yield	Yields may be significantly reduced if too much starting material is used or the PowerBiofilm Bead Tubes are not used. To avoid sample loss, do not use more sample than the specified range (0.05–0.20 g). Do not use any other bead tube except the ones designed for this kit. The tubes have been specially designed for use in this kit.
c)	cDNA does not amplify or has reduced amplification efficiency	Biofilms with high concentrations of humic substances and other contaminants may yield RNA with some inhibitor carryover, which can prevent target sequences from amplifying in RT-PCR. Under such circumstances, the template cDNA can be diluted one to several fold for successful PCR. For additional preps of the same or similar sample type, use 200 µl of Solution IRS at step 9 to eliminate inhibitor carry-over.
d)	RNA appears degraded on agarose gels	The use of Beta-mercaptoethanol (βME) will destroy RNases and should be added fresh to Solution MBL. If RNA still appears degraded make sure that biofilm samples are fresh and stored at 4°C or –20°C if not processed immediately. Storage at room temperature will cause considerable RNA degradation and loss. Prepare Solution MBL in smaller aliquots with fresh βME according to the number of samples you need to process that day instead of adding βME to the whole bottle. RNA will not always run correctly on non-denaturing gels and may appear smeared due to RNA secondary structure. Run RNA on a denaturing gel according to the "Protocol for Formaldehyde Gel Electrophoresis". The 260/280 ratio is a good indicator of RNA quality as the absorbance at 260 will increase as RNA is digested into smaller fragments and single nucleotides. A ratio above 2.3 may indicate RNA degradation.
e)	RNA floats out of well when loaded on a gel	Residual ethanol may be in the final sample. To ensure complete drying of the spin filter membrane, centrifuge the spin filter in a clean 2 ml Collection Tube for an additional minute. Precipitation is the best method to remove residual ethanol (see "Concentrating the RNA"). If you live in a humid climate, you may experience increased difficulty drying the membrane in the centrifuge. Increase centrifuge time in step 20 by another minute.
f)	DNA has low $A_{260/280}$ ratio	The ratio for pure RNA should be 1.9–2.1. Any $A_{260/280}$ reading below 1.6 may indicate significant protein contamination. Make sure that the BFR8 was performed after the DNase I treatment. A low ratio may also occur when the sample is measured by UV spectrophotometry in water. The low pH of water can influence the 280 reading and cause reduced sensitivity to protein contamination.* Remeasure the 260/280 diluting the RNA for measurement in the 10 mM Tris, pH 7.5.

		Comments and suggestions
g)	Genomic DNA contamination in the RNA	The RNeasy PowerBiofilm Kit contains high-quality RNase-free DNase I for on-column digest. When used with the DNase Digestion Solution included in the kit, activity of the DNase I will be optimal for on-column digestion. Only use the buffer provided with the DNase I for on-column digest. Digest must be performed for 15 minutes minimum for intended effect. Shortening digest time may result in incomplete genomic DNA removal. RNA will not be degraded during this incubation. Digest may be extended up to a maximum of 30 minutes.
h)	Concentrating the RNA	Your final volume will be 50–100 µl. if this is too dilute for your purposes, add 5 µl of 3 M Sodium Acetate and mix. Then add 2 volumes of 100% cold ethanol. Mix and incubate at –70°C for 15 minutes or –20°C for 2 hours to overnight. Centrifuge at 10,000 x *g* for 10–15 minutes at 4°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 RNA in desired volume of RNase-Free Water (provided).
i)	Storing RNA	RNA is eluted in RNase-Free Water and should be used immediately or stored at –20°C or –80°C to avoid degradation. RNA can be precipitated in ethanol and stored at –20°C to ensure minimal degradation during long term storage.

*Wilfinger, W.W., Mackey, M, and Chomczynski, P. (1997) Effect of pH and ionic strength on the spectrophotometric assessment of nucleic acid purity. BioTechniques 22, 474

Ordering Information

Product	Contents	Cat. no.
RNeasy PowerBiofilm Kit (50)	For 50 preps: For the isolation of high-quality total RNA from biofilm samples	25000-50
Related Products		
DNeasy® PowerBiofilm Kit	For 50 preps: For the isolation of high-quality, pure DNA from biofilm samples	24000-50
DNeasy PowerPlant® Pro Kit	For 50 preps: For the isolation of genomic DNA from plant and seed samples	13400-50
DNeasy PowerPlant Pro HTP 96 Kit	For 4 preps: For the high-throughput isolation of genomic DNA from plant and seed samples	13496-4
RNeasy PowerPlant Kit	For 50 preps: For the isolation of total RNA from difficult plant and seed samples	13500-50
Vortex Adapter	For maximizing homogenization with the Vortex-Genie 2 Vortex	13000-V1-24

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

Notes

Limited License Agreement for RNeasy 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.
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