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December 2018

# RNeasy<sup>®</sup> PowerWater<sup>®</sup> Kit Handbook

For the isolation of total RNA from filtered  
water samples, including turbid water

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# Kit Contents

<b>RNeasy PowerWater Kit</b>	<b>(50)</b>
<b>Catalog no.</b>	<b>14700-50-NF</b>
<b>Number of preps</b>	<b>50</b>
MB RNA Spin Columns	50
PowerWater DNA Bead Tubes	50
Solution PM1	55 ml
Solution IRS	15 ml
Solution PM3	36 ml
Solution PM4	3 x 24 ml
Solution PM5	3 x 30 ml
DNase Digestion Solution	2 x 1.5 ml
Solution PM7	23 ml
RNase-Free Water	10 ml
DNase, RNase-Free	1
Collection Tubes (2 ml)	5 x 50
Quick Start Protocol	1

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## Storage

Lyophilized RNase-Free DNase should be stored at 2 – 8°C. After resuspension, DNase should be stored at –30°C to –15°C. All other reagents and components of the RNeasy PowerWater Kit can be stored at room temperature (15° – 25°C) until the expiration date printed on the box label.

## 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 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](http://www.qiagen.com/safety) where you can find, view, and print the SDS for each QIAGEN kit and kit component.

**WARNING**



Solutions PM4, PM5 and PM7 contain alcohol and are flammable.

**CAUTION**



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

Solution PM1, Solution PM3, and Solution PM7 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 RNeasy PowerWater Kits is tested against predetermined specifications to ensure consistent product quality.

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# Introduction

The RNeasy PowerWater Kit can isolate total RNA from a variety of filtered water samples. Using our Inhibitor Removal Technology® (IRT), even water containing heavy amounts of contaminants that could inhibit downstream applications can be processed to provide high-quality RNA. The RNeasy PowerWater Kit can isolate RNA equally well from any commonly used type of filter membrane. RNase-Free DNase I is provided for on-column removal of genomic DNA during the protocol, which saves time and post-processing steps.

## Principle and procedure

The RNeasy PowerWater Kit starts with the filtration of a water sample onto a filter membrane. Filter membranes may be user supplied (see section on Equipment and Reagents to Be Supplied by User for recommendations). The membrane is then added to our special 5 ml bead beating tube containing a unique bead mix. Rapid and thorough lysis occurs through vortexing in a novel lysis buffer that enhances the isolation of RNA from microorganisms trapped on filter membranes. After the protein and inhibitor removal steps, total RNA is captured on an MB RNA Spin Column, where an on-column DNase step is incorporated to remove genomic DNA. The column is then washed and the RNA eluted. The purified RNA is ready to use in downstream applications including RT-PCR, qRT-PCR, cDNA synthesis or RNA amplification.

## RNeasy PowerWater Kit Procedure

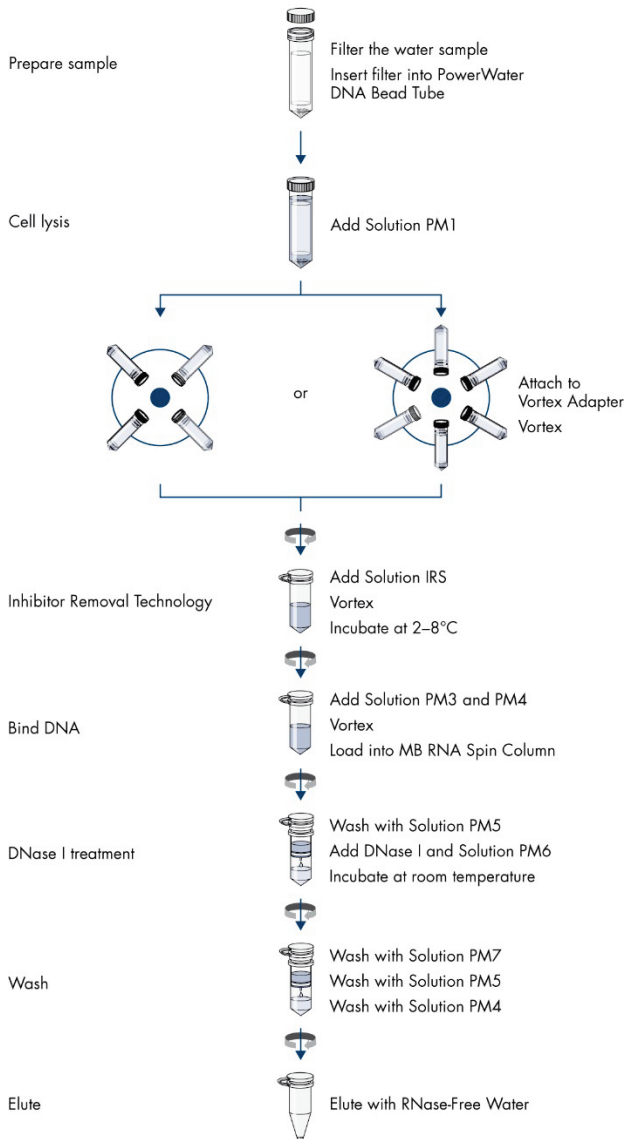


Figure 1. RNeasy PowerWater Kit procedure

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## Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs) available from the product supplier.

- Centrifuge for 15 ml tubes ( $\leq 4000 \times g$ )
- Disposable/reusable filter funnels
- Filter membranes (if using a reusable filter funnel)
- Microcentrifuge ( $13,000 \times g$ )
- Pipettors
- $\beta$ -Mercaptoethanol ( $\beta$ -ME)
- Vortex-Genie® 2 Vortex
- Vortex adapter
- **Recommended:** Pall Laboratory MicroFunnel Disposable Filter Funnels ( $0.22 \mu\text{m}$  or  $0.45 \mu\text{m}$ ) (VWR cat. no. 28143-542 or 55095-060, respectively)



# Protocol: Experienced User

## Important points before starting

- Solution PM1 must be warmed at 55°C for 5–10 minutes prior to use.
- Shake to mix Solution PM5 before use.
- Prepare Solution PM1 by adding 10 µl β-mercaptoethanol (β-ME) for every 990 µl of Solution PM1 (a total of 1 ml for each prep).
- Prepare DNase I stock enzyme by adding 550 µl of RNase-Free water to the DNase I (RNase-Free) lyophilized powder and mixing gently. Aliquot the DNase I stock enzyme in 50 µl portions and store at –20°C for long term storage (but do not freeze/thaw more than 3 times). To prepare DNase I Solution, thaw and combine 5 µl of DNase I stock enzyme with 45 µl of DNase Digestion Solution per prep.

## Procedure

1. Filter water samples using a reusable or disposable filter funnel (0.22 µm or 0.45 µm filter membranes) attached to a vacuum source. The volume of water filtered will depend on the microbial load and turbidity of the water sample.
2. If using a reusable filter funnel, remove the upper portion of the apparatus.
3. Using two sets of sterile forceps, pick up the white filter membrane at opposite edges and roll the filter into a cylinder with the top side facing inward.  
**Note:** Do not tightly roll or fold the filter membrane. To see a video of this technique, visit the RNeasy PowerWater Kit product page at <https://mobio.com/powerwater-filter>.
4. Insert the filter into a 5 ml PowerWater DNA Bead Tube.
5. Add 1 ml of Solution PM1/ β-ME to the PowerWater Bead Tube. Alternatively, you can add 990 µl of PM1 and 10 µl of β-ME directly to the tube.
6. Make sure the PowerWater DNA Bead Tube cap is securely tightened.  
**Note:** For samples containing difficult-to-lyse organisms (fungi, algae) an additional heating step can be included. Refer to the Troubleshooting Guide.

7. Secure the PowerWater DNA Bead Tube horizontally to a Vortex Adapter (cat. nos. 13000-V1-15 or 13000-V1-5). The tube caps should point toward the center of the Vortex Adapter.
8. Vortex at maximum speed for 5 min.
9. Centrifuge the tubes  $\leq 4000 \times g$  for 1 min.  
**Note:** This step is optional if a centrifuge with a 15 ml tube rotor is not available but may result in minor loss of supernatant.
10. Transfer all the supernatant to a clean 2 ml Collection Tube (provided). Draw up the supernatant using a 1 ml pipette tip by placing it down into the beads.  
**Note:** Placing the pipette tip down into the beads is required. Pipet more than once to ensure removal of all supernatant. Expect to recover 600 – 650  $\mu$ l of supernatant.
11. Centrifuge at  $13,000 \times g$  for 1 min. Avoiding the pellet, transfer the supernatant to a clean 2 ml Collection Tube (provided).
12. Add 200  $\mu$ l of Solution IRS and vortex briefly to mix. Incubate at 2–8°C for 5 min.  
**Note:** This step can be omitted for non-turbid water samples that are known to be free of PCR inhibitors. Continue the protocol at step 13.
13. Repeat Step 11. Then proceed to Step 14.
14. Add 650  $\mu$ l each of Solution PM3 and Solution PM4. Vortex briefly to mix.
15. Load 650  $\mu$ l of supernatant onto an MB RNA Spin Column. Centrifuge at  $13,000 \times g$  for 1 min. Discard flow-through and repeat until all the supernatant has been loaded.
16. Add 650  $\mu$ l of Solution PM5. Centrifuge at  $13,000 \times g$  for 1 min. Discard flow-through.  
**Note:** Skip steps 16–18 if you want to isolate both RNA and DNA.
17. Centrifuge again at  $13,000 \times g$  for 1 min and place the MB RNA Spin Column into a clean 2 ml Collection Tube (provided).
18. Add 50  $\mu$ l of DNase I Solution to the center of the column membrane and incubate at room temperature for 15 min.
19. Add 400  $\mu$ l Solution PM7 and centrifuge the column at  $13,000 \times g$  for 1 min.
20. Discard flow-through. Add 650  $\mu$ l of Solution PM5. Centrifuge at  $13,000 \times g$  for 1 min.

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21. Discard flow-through. Add 650  $\mu$ l of Solution PM4. Centrifuge at 13,000  $\times g$  for 1 min.
  22. Discard flow-through and centrifuge again at 13,000  $\times g$  for 2 min.
  23. Place the MB RNA Spin Column into a clean 2 ml Collection Tube (provided).
  24. Add 100  $\mu$ l of RNase-Free Water (provided) to the center of the white filter membrane.
  25. Centrifuge at 13,000  $\times g$  for 1 min. Discard the MB RNA Spin Column. The RNA is now ready for downstream applications and can be stored at  $-65^{\circ}\text{C}$  to  $-90^{\circ}\text{C}$ .

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# Protocol: Detailed

## Important points before starting

- Solution PM1 must be warmed at 55°C for 5–10 minutes prior to use.
- Shake to mix Solution PM5 before use.
- Prepare Solution PM1 by adding 10 µl β-mercaptoethanol (β-ME) for every 990 µl of Solution PM1 (a total of 1 ml for each prep).
- Prepare DNase I stock enzyme by adding 550 µl of RNase-Free water to the DNase I (RNase-Free) lyophilized powder and mixing gently. Aliquot the DNase I stock enzyme in 50 µl portions and store at –20°C for long term storage (but do not freeze/thaw more than 3 times). To prepare DNase I Solution, thaw and combine 5 µl of DNase I stock enzyme with 45 µl of DNase Digestion Solution per prep.

## Procedure

1. Filter water samples using a filter funnel attached to a vacuum source. The volume of water filtered will depend on the microbial load and turbidity of the water sample.  
**Note:** Please see Appendix A: Types of water samples. A reusable or disposable filter funnel is attached to a vacuum filtration system. Microorganisms are trapped on top of and within the filter membrane.
2. If using a reusable filter funnel, remove the upper portion of the apparatus.
3. Using two sets of sterile forceps, pick up the white filter membrane at opposite edges and roll the filter into a cylinder with the top side facing inward.  
**Note:** Do not tightly roll or fold the filter membrane. To see a video of this technique, visit the RNeasy PowerWater Kit product page at <https://mobio.com/powerwater-filter>.
4. Insert the filter into a 5 ml PowerWater DNA Bead Tube.  
**Note:** Loosely rolling and inserting the filter membrane into the PowerWater Bead Tube allows for efficient bead beating and homogenization in proceeding steps.

5. Add 1 ml of Solution PM1/  $\beta$ -ME to the PowerWater Bead Tube. Alternatively, you can add 990  $\mu$ l of PM1 and 10  $\mu$ l of  $\beta$ -ME directly to the tube.  
**Note:** Solution PM1 must be warmed to dissolve precipitates prior to use. Solution PM1 should be used while still warm.
6. Make sure the PowerWater DNA Bead Tube cap is securely tightened.  
**Note:** For samples containing difficult-to-lyse organisms (fungi, algae) an additional heating step can be included. Refer to the Troubleshooting Guide.
7. Secure the PowerWater DNA Bead Tube horizontally to a Vortex Adapter (cat. no. 13000-V1-15 or 13000-V1-5). The tube caps should point toward the center of the Vortex Adapter.
8. Vortex at maximum speed for 5 min.  
**Note:** The mechanical action of bead beating will break apart the surface of the filter membrane that contains trapped cells and aids in cell lysis. Use of the vortex adapter will maximize homogenization by holding the tubes at equal distances and angles from the center of rotation. Avoid using tape, which can become loose and result in reduced homogenization efficiency.
9. Centrifuge the tubes  $\leq 4000 \times g$  for 1 min.  
**Note:** This step is optional if a centrifuge with a 15 ml tube rotor is not available but may result in minor loss of supernatant.
10. Transfer all the supernatant to a clean 2 ml Collection Tube (provided). Draw up the supernatant using a 1 ml pipette tip by placing it down into the beads.  
**Note:** Placing the pipette tip down into the beads is required. Pipet more than once to ensure removal of all supernatant. Expect to recover 600–650  $\mu$ l of supernatant. The supernatant is separated and removed from the filter membrane and beads at this step.
11. Centrifuge at 13,000  $\times g$  for 1 min. Avoiding the pellet, transfer the supernatant to a clean 2 ml Collection Tube (provided).  
**Note:** Any remaining beads, proteins and cell debris are removed at this step. This step is important for removal of any remaining contaminating organic and inorganic matter that may reduce RNA purity and inhibit downstream RNA applications.

12. Add 200  $\mu$ l of Solution IRS and vortex briefly to mix. Incubate at 2–8°C for 5 min.  
**Note:** This step can be omitted for non-turbid water samples that are known to be free of PCR inhibitors. Continue the protocol at step 13. Solution IRS is a part of the IRT and is the second reagent to remove additional organic and inorganic material including humic acid, cell debris, and proteins. It is important to remove organic and inorganic contaminants that may reduce RNA purity and inhibit downstream RNA applications
13. Repeat Step 11. Then proceed to Step 14.  
**Note:** The pellet at this point contains additional non-RNA organic and inorganic material. For best RNA yields and quality, avoid transferring any of the pellet.
14. Add 650  $\mu$ l each of Solution PM3 and Solution PM4. Vortex briefly to mix.  
**Note:** Solution PM3 is a high concentration salt solution and solution PM4 is ethanol. Both components are necessary to create the conditions required for efficient binding of the RNA to the MB RNA Spin Column while allowing proteins and cellular debris to pass through.
15. Load 650  $\mu$ l of supernatant onto an MB RNA Spin Column. Centrifuge at 13,000  $\times g$  for 1 min. Discard flow-through and repeat until all the supernatant has been loaded.  
**Note:** RNA is selectively bound to the silica membrane in the MB RNA Spin Column basket and the flow-through containing non-RNA components is discarded.
16. Add 650  $\mu$ l of Solution PM5. Centrifuge at 13,000  $\times g$  for 1 min. Discard flow-through.  
**Note:** Skip steps 16–18 if you want to isolate both RNA and DNA. Solution PM5 is an ethanol-based wash solution used to wash the MB RNA Spin Column in preparation for the on-column DNase I digestion. Solution PM5 removes residual salt and other contaminants while allowing the RNA to stay bound to the silica membrane
17. Centrifuge again at 13,000  $\times g$  for 1 min and place the MB RNA Spin Column into a clean 2 ml Collection Tube (provided).  
**Note:** Complete removal of Solution PM5 is required for efficient and complete DNase I digestion.
18. Add 50  $\mu$ l of DNase I Solution to the center of the column membrane and incubate at room temperature for 15 min.

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**Note:** DNase I is mixed with high-activity digestion buffer and is used to completely remove genomic DNA from the MB RNA Spin Column membrane. If the RNA is to be used for reverse transcription and or RT PCR, we highly recommend removal of all genomic DNA with a DNase I digestion.

19. Add 400  $\mu$ l Solution PM7 and centrifuge the column at 13,000  $\times g$  for 1 min.

**Note:** Solution PM7 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 MB RNA Spin Column.

20. Discard flow-through. Add 650  $\mu$ l of Solution PM5. Centrifuge at 13,000  $\times g$  for 1 min.

**Note:** Solution PM5 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 Solution PM5 is critical.

21. Discard flow-through. Add 650  $\mu$ l of Solution PM4. Centrifuge at 13,000  $\times g$  for 1 min.

**Note:** Solution PM4 ensures complete removal of Solution PM5, which will result in higher RNA purity and yield.

22. Discard flow-through and centrifuge again at 13,000  $\times g$  for 2 min.

**Note:** The second spin removes residual Solution PM4. It is critical to remove all traces of Solution PM4 because the ethanol in it can interfere with downstream RNA applications.

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

24. Add 100  $\mu$ l of RNase-Free Water (provided) to the center of the white filter membrane.

**Note:** Eluting with 100  $\mu$ l of RNase-Free Water will maximize RNA yield. For more concentrated RNA, a **minimum** of 50  $\mu$ l of water can be used. Placing the water 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 RNA from the silica MB RNA Spin Column membrane. As the water passes through the silica membrane, the RNA that was bound in the presence of high salt is selectively released.

25. Centrifuge at 13,000  $\times g$  for 1 min. Discard the MB RNA Spin Column. The RNA is now ready for downstream applications and can be stored at  $-65^{\circ}\text{C}$  to  $-90^{\circ}\text{C}$ .

# 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](http://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](http://www.qiagen.com).

## Comments and suggestions

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### Sample processing

- |    |   |  |
|----|---|--|
| a) | Filter membrane selection                                       | We recommend Pall Laboratory MicroFunnel Disposable Filter Funnels (0.22 $\mu\text{m}$ or 0.45 $\mu\text{m}$ ) (VWR cat. no. 28143-542 or 55095-060, respectively). The 0.22 $\mu\text{m}$ filter membrane consists of polyethersulfone, while the 0.45 $\mu\text{m}$ filter membrane consists of cellulose acetate. Some filter membranes may bind and concentrate inhibitors. To reduce the likelihood of this occurring, filter membrane types may need to be evaluated prior to use. |
| b) | Solution PW1 is not warmed prior to use                         | Continue with the protocol. You will still obtain RNA, but the yields may not be optimal.  |
| c) | A centrifuge with a 15 ml tube rotor is not available at Step 9 | Centrifugation at this step helps separate the supernatant from the filter membrane, which aids in the recovery of as much of the supernatant as possible. If a centrifuge is not available, this step can be skipped with some minor loss of supernatant.   |

### RNA

- |    |                                  |  |
|----|----------------------------------|--|
| a) | RNA has low $A_{260/280}$ ratios | <p><math>A_{260/280}</math> readings are one measure of RNA purity. The ratio for pure RNA should be 1.9–2.1. <math>A_{260/280}</math> readings below 1.6 may have significant protein contamination.</p> <ul style="list-style-type: none"><li>• Make sure that the PM7 wash was performed after the DNase I treatment.</li><li>• 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. Re-measure <math>A_{260/280}</math> after diluting the RNA for measurement in 10 mM Tris (pH 7.5).</li></ul> |
| b) | Storing RNA                      | RNA is eluted in RNase-Free water and must be stored at $-65^{\circ}\text{C}$ to $-90^{\circ}\text{C}$ to prevent degradation.   |



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### Comments and suggestions

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- c) Concentrating eluted RNA  
The final volume of eluted RNA will be 50–100  $\mu$ l. The RNA may be concentrated by adding 5  $\mu$ l of 3 M NaCl and inverting 3–5 times to mix. Next, add 2 volumes of cold 100% ethanol and invert 3–5 times to mix. Incubate at  $-70^{\circ}\text{C}$  for 15 minutes or  $-20^{\circ}\text{C}$  for 2 hours to overnight. Centrifuge at  $10,000 \times g$  for 10–15 min at  $2-8^{\circ}\text{C}$ . 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.
- d) RNA floats out of a well when loading a gel  
This usually occurs because residual Solution PM4 remains in the final sample. To ensure complete drying of the membrane after adding Solution PM4, centrifuge the MB RNA Spin Column in a clean 2 ml Collection Tube for an additional minute.  
Ethanol precipitation (described in “Concentrating eluted RNA”) is the best way to remove residual ethanol.  
If you live in a humid climate, you may experience increased difficulty drying the membrane in the centrifuge. Increase the centrifugation time at step 21 by another minute.
- e) RNA is contaminated with genomic DNA  
The RNeasy PowerWater Kit is provided with high-quality RNase-Free DNase I for on-column digestion. When used with the DNase Digestion Solution included in the kit, the activity of the DNase I will be optimal for on-column digestion.
  - Use only the buffer provided with the DNase I for on-column digest.
  - Make sure to perform the digest for 15 minutes, as recommended. Shortening the digest time may result in incomplete genomic DNA removal. RNA will not be degraded during this incubation. You may extend the DNase I digest up to 30 minutes.
- f) RNA appears degraded on agarose gels  
The use of  $\beta$ -ME will destroy RNases and it should be added fresh to Solution PM1 before each use. If RNA still appears degraded, make sure the following steps are being followed:
  - Make sure that water samples are fresh and stored at  $2-8^{\circ}\text{C}$  if not processed immediately. Storage at either room temperature or  $-20^{\circ}\text{C}$  will cause considerable RNA degradation and loss.
  - Prepare Solution PM1 in smaller aliquots with fresh  $\beta$ -ME according to the number of samples you need to process that day instead of adding  $\beta$ -ME to the whole bottle.
  - RNA will not always run correctly on non-denaturing gels and may appear smeared due to secondary structure. Run RNA on a denaturing gel according to the protocol for formaldehyde gel electrophoresis in Appendix C.
  - The  $A_{260}/_{280}$  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.

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## Comments and suggestions

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### Alternative lysis methods

- |  |  |
|--|--|
| a) Sample contains organisms that are difficult to lyse (e.g., fungi, algae) | Heating can aid the lysis of some organisms (fungi, algae). After adding Solution PM1 (Step 5 of the protocol), heat the sample at 65°C for 10 min. Resume protocol from step 6. |
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## Appendix A: Types of Water Samples

This appendix describes types of water sample and how to effectively process them.

### Clear water samples

Larger volumes of clear water can be processed because there is less chance of filter clogging. Potable drinking water will generally allow for very high volumes depending on the quality and particulate count. In most cases, 100 ml to 10 liters can be processed, although some users report processing even higher volumes.

### Turbid water samples

Turbid samples with high levels of suspended solids or sediments will tend to clog filters with smaller pore sizes (0.22  $\mu\text{m}$ ). Use of 0.45  $\mu\text{m}$  filters is recommended for these types of samples. (See section on Equipment and Reagents to Be Supplied by User).

Prior to filtering, samples can be stored in a container to allow suspended solids to settle out. For samples where settling does not occur or is not desired, a method involving stacking filters with larger pore sizes on top of the filter membrane of the desired pore size is recommended. A common set-up is to stack a sterile 1  $\mu\text{m}$  filter. This layering will filter out large debris and allow the smaller micron filter to trap microorganisms. The layered filter system can be washed with sterile water or sterile phosphate buffer to knock down some of the trapped microorganisms on the larger pore size filters. Although this is not completely efficient, it will increase the overall yield of microbial RNA.

## Appendix B: Expected RNA Yields

RNA yields will vary depending on the type of water, sample location and time of year. Examples of expected yields are provided as a reference. Due to diversity of water sample types, yields may fall outside of the examples provided.

**Table 1: Water sample types**

Type of water sample	Sample volume (ml)	RNA yield (µg)
Freshwater lake	50	1.2
Lagoon	50–100	1.0–2.7

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# Appendix C: Formaldehyde Agarose Gel Electrophoresis

## Solutions needed:

- 10x formaldehyde agarose gel buffer
  - 200 mM 3-[N-morpholino] propanesulfonic acid (MOPS) (free acid)
  - 50 mM sodium acetate
  - 10 mM EDTA
  - Sodium hydroxide to adjust pH to 7.0
- 1x formaldehyde agarose gel buffer (1L)
  - 100 ml 10x formaldehyde agarose gel buffer
  - 20 ml 37% formaldehyde
  - 880 ml DEPC-treated water
- 5x RNA loading dye
  - 16  $\mu$ l saturated aqueous Bromophenol Blue solution
  - 80  $\mu$ l 0.5 M EDTA (pH 8.0)
  - 720  $\mu$ l 37% formaldehyde
  - 2 ml 100% glycerol
  - 3084  $\mu$ l formamide
  - 4 ml 10x formaldehyde agarose gel buffer

## Preparing formaldehyde agarose gel

Prepare the formaldehyde agarose gel (1.2% in 100 ml) by mixing 1.2 g agarose, 10 ml of 10x formaldehyde agarose gel buffer, and 90 ml DEPC-treated water.

Heat the mixture in a microwave oven to melt the agarose. Cool to 65°C in a water bath. Add 1.8 ml of 37% formaldehyde and 2  $\mu$ l of 5 mg/ml ethidium bromide. Swirl to mix and pour

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into a gel box. The gel must be pre-run for 30 minutes in 1x formaldehyde agarose gel buffer before loading the samples.

### RNA sample preparation

The eluted RNA samples must be denatured before running on a formaldehyde agarose gel. Add 1 volume of 5x RNA loading dye for each 4 volumes of RNA sample (e.g. 2  $\mu$ l of 5x RNA loading dye for each 8  $\mu$ l of RNA sample).

Mix the samples and briefly centrifuge to collect them at the bottom of the tube.

Incubate at 65°C for 3–5 minutes, then chill on ice and load in the formaldehyde agarose gel. Run the gel at 5–7 V/cm in 1x formaldehyde agarose gel buffer.

### References

1. Beintema, J.J., Campagne, R.N. and Gruber, M. (1973) Rat pancreatic ribonuclease. I. Isolation and properties. *Biochimica et Biophysica Acta* **310**, 148–160.
2. Kaplan, B.B., Bernstein, S.L. and Gioio, A.E. (1979) An improved method for the rapid isolation of brain ribonucleic acid. *Biochemical Journal* **183**, 181–184.

# Ordering Information

<b>Product</b>	<b>Contents</b>	<b>Cat. no.</b>
RNeasy PowerWater Kit (50)	For 50 preps: Isolation of total RNA from filtered water samples, including turbid water	14700-50-NF
<b>Related Products</b>		
DNeasy® PowerWater Kit (50)	For 50 preps: Isolation of genomic DNA from filtered water samples, including turbid water	14900-50-NF
DNeasy PowerWater Kit (100)	For 100 preps: Isolation of genomic DNA from filtered water samples, including turbid water	14900-100-NF
DNeasy PowerWater Sterivex Kit (50)	For 50 preps: Isolation of genomic DNA from water samples collected with Sterivex filter units	14600-50-NF
Vortex Adapter, Genie for 6 (5 & 15 ml) tubes	For vortexing 5 ml and 15 ml tubes using the Vortex-Genie 2 Vortex	13000-V1-5

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](http://www.qiagen.com) or can be requested from QIAGEN Technical Services or your local distributor.

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## Notes



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## Notes

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## Notes

## Document Revision History

R2 12/2018	Revised the Protocol: Experienced User and Protocol: Detailed sections to update the preparation of DNase I stock enzyme using 550 µl from 300 µl RNase-Free water. Updated Ordering Information section. Layout updates.
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### Limited License Agreement for RNeasy PowerWater 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](http://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.
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