# PowerWater® RNA Isolation Kit

**(For isolation of total RNA from membrane filtered water samples)**

| Catalog No. | Quantity | Filters |
|---|---|---|
| 14700-50-NF | 50 Preps | No filters |

## *Instruction Manual*

*Inhibitor Removal Technology® (IRT) is a registered trademark of MO BIO Laboratories, Inc. and is covered by the following patents USA US 7,459,548 B2, Australia 2005323451, Japan 5112064 and India 246946.*

**Please recycle**

Version: 05302013

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## Introduction

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

## Protocol Overview

The PowerWater® RNA Isolation Kit starts with the filtration of a water sample onto a filter membrane. Filter membranes can be purchased separately from MO BIO or can be user supplied. MO BIO filter membranes are sterile, disposable, and easy to use. The membrane is then added to our 5 ml bead beating tube containing a unique bead mix. Rapid and thorough lysis occurs through vortex mixing 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 the MO BIO Laboratories silica 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.

**This kit is for research purposes only. Not for diagnostic use.**

| Other Related Products | Catalog No. | Quantity |
|---|---|---|
| Vortex Adapter for Vortex Genie® 2 | 13000-V1-15<br>13000-V1-5 | Holds 4 (15 ml or 5 ml) Tubes<br>Holds 6 (5 ml only) Tubes |
| Water Filter Adapter | 14800-10-WFA | 1 |
| Water Filter (0.45 µm) | 14800-10-WF<br>14800-25-WF<br>14800-50-WF<br>14800-100-WF | 10 units<br>25 units<br>50 units<br>100 units |
| Water Filter (0.22 µm) | 14880-10-WF<br>14880-25-WF<br>14880-50-WF<br>14880-100-WF | 10 units<br>25 units<br>50 units<br>100 units |
| Vortex Genie® 2 Vortex | 13111-V-220<br>13111-V | 1 unit (220V)<br>1 unit (120V) |
| UltraClean® Lab Cleaner | 12095-250<br>12095-500<br>12095-1000 | 250 ml squeeze bottle<br>500 ml spray bottle<br>1 liter bottle |
| RNase-Free Gloves | 1556-XS<br>1556-S<br>1556-M<br>1556-L | Bag of 150<br>Bag of 150<br>Bag of 150<br>Bag of 150 |
| PowerWater® DNA Isolation Kit | 14900-50-NF<br>14900-100-NF | 50 preps (No filters)<br>100 preps (No filters) |

# PowerWater® RNA Isolation Kit

**Prepare Sample**

- Filter the water sample
- Insert filter into PowerWater® Bead Tube

**Cell Lysis**

- Add Solution PWR1

or

- Attach to Vortex Adapter
- Vortex

*Centrifuge*

**Inhibitor Removal Technology®**

- Add Solution PWR2
- Vortex
- Incubate at 4°C

*Centrifuge*

**Bind RNA**

- Add Solutions PWR3 and PWR4
- Vortex
- Load into Spin Filter

*Centrifuge*

**DNase I Treatment**

- Wash with Solution PWR5
- Add DNase I and Solution PWR6
- Incubate at room temperature

**Wash**

- Wash with Solution PWR7
- Wash with Solution PWR5
- Wash with Solution PWR4

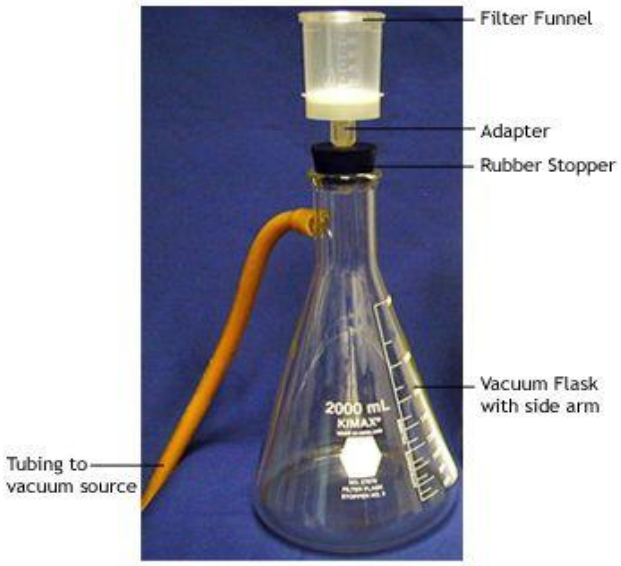
*Centrifuge*

**Elute**

- Elute with Solution PWR8

## Equipment Required

Centrifuge for 15 ml tubes (≤4000 x *g*)
Disposable/reusable filter funnels
Filter membranes (if using a reusable filter funnel)
Microcentrifuge (13,000 x *g*)
Pipettors
Vortex
Vortex Adapter
Vacuum Filtration System



Vacuum Filtration System

## Reagents Required but not Included

β-Mercaptoethanol

## Kit Contents

| | **Kit Catalog# 14700-50-NF** |
|---|---|
| **Component** | **Amount** |
| PowerWater® Bead Tubes | 50 tubes |
| Solution PWR1 | 55 ml |
| Solution PWR2 | 11 ml |
| Solution PWR3 | 36 ml |
| Solution PWR4 | 3 x 23 ml |
| Solution PWR5 | 3 x 23 ml |
| Solution PWR6 | 2.5 ml |
| Solution PWR7 | 23 ml |
| Solution PWR8 | 5.5 ml |
| Spin Filters | 50 |
| DNase I (RNase-Free), 1500 units | 1 vial |
| 2 ml Collection Tubes | 250 |

## Kit Storage

Remove lyophilized DNase I and store at 4°C. Store all other reagents and kit components at room temperature (15-30°C). DNase I should be stored at 4°C when lyophilized and -20°C after resuspension (DNase is sensitive to physical denaturation. Do not vortex the resuspended DNase).

## Precautions

Please wear gloves when using this product. Avoid all skin contact with kit reagents. In case of contact, wash thoroughly with water. Do not ingest. See Material Safety Data Sheets for emergency procedures in case of accidental ingestion or contact. All MSDS information is available upon request (760-929-9911) or at www.mobio.com. Reagents labeled flammable should be kept away from open flames and sparks.

**WARNING:** Solutions PWR4, PWR5 and PWR7 contain alcohol. They are flammable.

## Important Notes Before Starting

Solution PWR1 must be warmed at 55°C for 5-10 minutes to dissolve precipitates prior to use. Solution PWR1 should be used while still warm. Shake to mix Solution PWR5 before use.

## Prepare Solution PWR1 by adding β- mercaptoethanol (βME)

Add 10 µl of β- mercaptoethanol (βME) for every 990 µl of the **Solution PWR1** for all samples to be processed. For each prep, 1 ml of PWR1/ β- mercaptoethanol (βME) will be needed.

**Alternatively:** Add 990 µl of PWR1 and 10 µl of βME directly to each tube.

**Note:** Prepare **Solution PWR1** 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. Use a fume hood when opening βME to avoid exposure to the chemical.

## DNase I Stock Enzyme and DNase I Solution Preparation and Storage

A. Prepare **DNase I stock enzyme** by adding **300 µl** of RNase-Free Water (**Solution PWR8**) to the DNase I (RNase-Free) lyophilized powder and mix gently. Aliquot the DNase I stock enzyme in 50 µl portions and store at -20°C for long term storage.

   **Note:** The DNase I stock enzyme can be freeze/thawed up to three times without loss of activity.

B. Prepare the **DNase I Solution**, by thawing the volume of DNase I stock enzyme needed according to the number of samples. Per prep, combine **5 µl of DNase I stock enzyme** with **45 µl of Solution PWR6**.

Example:

| Number of preps | DNase I stock enzyme | Solution PWR6 |
|---|---|---|
| 1 | 5 µl | 45 µl |
| 2 | 10 µl | 90 µl |
| 10 | 50 µl | 450 µl |

## Experienced User Protocol

**Please wear gloves at all times**

**Warm Solution PWR1 prior to use at 55°C for 5-10 minutes. Use Solution PWR1 while still warm.**

1. Filter water samples using a reusable or disposable filter funnel attached to a vacuum source. Disposable filter funnels, containing 0.22 µm or 0.45 µm filter membranes, can be ordered from MO BIO Laboratories (see "Other Related Products" on page 3). The volume of water filtered will depend on the microbial load and turbidity of the water sample. **(Please see Types of Water Samples in the "Hints and Troubleshooting Guide" section of this Instruction Manual).**
2. If using a reusable filter funnel, remove the upper portion of the apparatus. If using MO BIO Laboratories filter funnels, remove the 100 ml upper portion of the filter cup from the catch reservoir by snapping it off.
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, please visit the PowerWater® RNA Isolation Kit product page on www.mobio.com.
4. Insert the filter into the 5 ml PowerWater® Bead Tube.
5. Add **1 ml of Solution PWR1** containing βME (see **Important Notes before Starting** section) to the PowerWater® Bead Tube. **Alternatively:** Add 990 µl of PWR1 and 10 µl of βME directly to each tube.
   **Note:** Solution PWR1 must be warmed to dissolve precipitates prior to use. Solution PWR1 should be used while still warm.
6. Make sure cap is securely tightened on PowerWater® Bead Tube.
   **Note:** For samples containing organisms that are difficult to lyse (fungi, algae) an additional heating step can be included. See **Alternate Lysis Method in the "Hints and Troubleshooting Guide".**
7. Secure the PowerWater® Bead Tube horizontally to a MO BIO Vortex Adapter, catalog number 13000-V1-15 or 13000-V1-5. The tube caps should be oriented pointing toward the center of the Vortex Adapter.
8. Vortex at maximum speed for 5 minutes.
9. Centrifuge the tubes ≤ 4000 x *g* for 1 minute at room temperature. The speed will depend on the capability of your centrifuge. **(This step is optional if a centrifuge with a 15 ml tube rotor is not available, but will 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. Pipette more than once to ensure removal of all supernatant. Any carryover of beads will not affect subsequent steps. Expect to recover between 600-650 µl of supernatant depending on the type of filter membrane used.
11. Centrifuge at 13,000 x *g* for 1 minute.
12. Avoiding the pellet, transfer the supernatant to a clean 2 ml Collection Tube (provided).
13. Add **200 µl of Solution PWR2** and vortex briefly to mix. Incubate at 4°C for 5 minutes.
    **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 15.
14. Centrifuge the tubes at 13,000 x *g* for 1 minute.
15. Avoiding the pellet, transfer the supernatant to a clean 2 ml Collection Tube (provided).
16. Add **650 µl of Solution PWR3** and **650 µl of Solution PWR4.** Then vortex briefly to mix.

17. Load 650 µl of supernatant onto a Spin Filter and centrifuge at 13,000 x *g* for 1 minute. Discard the flow through and repeat until all the supernatant has been loaded onto the Spin Filter.
    **Note:** A total of three loads for each sample processed are required.
18. Shake to mix Solution PWR5. Add **650 µl of Solution PWR5** and centrifuge at 13,000 x *g* for 1 minute. Discard the flow through.
    **Note:** Skip steps **19-23** if you want to isolate both RNA and DNA.
19. Centrifuge again at 13,000 x *g* for 1 minute to remove residual wash.
20. Place the Spin Filter basket into a clean 2 ml Collection Tube (provided).
21. To the center of the Spin Filter, add **50 µl of DNase I Solution** (prepared by mixing **45 µl of Solution PWR6** and **DNase I stock solution.** See **Important Notes Before Starting** section).
22. Incubate at room temperature for 15 minutes.
    **Note**: Do not centrifuge the Spin Filter before the addition of Solution PWR7.
23. Add **400 µl Solution PWR7** and centrifuge the column at 13,000 x *g* for 1 minute.
24. Discard the flow through and add **650 µl of Solution PWR5** and centrifuge at 13,000 x *g* for 1 minute.
25. Discard the flow through and add **650 µl of Solution PWR4** and centrifuge at 13,000 x *g* for 1 minute.
26. Discard the flow through and centrifuge again at 13,000 x *g* for 2 minutes to remove residual wash.
27. Place the Spin Filter basket into a clean 2 ml Collection Tube (provided).
28. Add **100 µl of Solution PWR8** to the center of the white filter membrane.
    **Note:** Eluting with 100 µl of Solution PWR8 will maximize RNA yield. For more concentrated RNA, a minimum of 50 µl of Solution PWR8 can be used. Do not use less than 50 µl of Solution PWR8.
29. Centrifuge at 13,000 x *g* for 1 minute.
30. Discard the Spin Filter basket. The RNA is now ready for any downstream application. No further steps are required. The RNA in the tube can be stored at -80°C until ready for use.

**Thank you for choosing the PowerWater® RNA Isolation Kit!**

## Detailed Protocol (Describes what is happening at each step)

**Please wear gloves at all times**

**Warm Solution PWR1 prior to use at 55°C for 5-10 minutes. Use Solution PWR1 while still warm.**

1. Filter water samples using a reusable or disposable filter funnel attached to a vacuum source. Disposable filter funnels, containing 0.22 µm or 0.45 µm filter membranes, can be ordered from MO BIO Laboratories (see "Other Related Products" on page 3). The volume of water filtered will depend on the microbial load and turbidity of the water sample. **(Please see Types of Water Samples in the "Hints and Troubleshooting Guide" section of this Instruction Manual).**

   *What's happening: Reusable or disposable filter funnels are attached to a vacuum filtration system. Microorganisms are trapped on top of and within the filter.*

2. If using a reusable filter funnel, remove the upper portion of the apparatus. If using MO BIO Laboratories filter funnels, remove the 100 ml upper portion of the filter cup from the catch reservoir by snapping it off.

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, please visit the PowerWater® RNA Isolation Kit product page on www.mobio.com.

4. Insert the filter into the 5 ml PowerWater® Bead Tube.

   *What's happening: 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 PWR1** containing βME (see **Important Notes before Starting** section) to the PowerWater® Bead Tube. **Alternatively:** Add 990 µl of PWR1 and 10 µl of βME directly to each tube.

   **Note:** Solution PWR1 must be warmed to dissolve precipitates prior to use. Solution PWR1 should be used while still warm.

   *What's happening: Solution PWR1 is patented 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 PWR1 can be used while it is still warm.*

6. Make sure cap is securely tightened on PowerWater® Bead Tube.

   **Note:** For samples containing organisms that are difficult to lyse (fungi, algae) an additional heating step can be included. See **Alternate Lysis Method in the "Hints and Troubleshooting Guide".**

7. Secure the PowerWater® Bead Tube horizontally to a MO BIO Vortex Adapter, catalog number 13000-V1-15 or 13000-V1-5. The tube caps should be oriented pointing toward the center of the Vortex Adapter.

8. Vortex at maximum speed for 5 minutes

   *What's happening: 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 equal distance and angle from the center of rotation. Avoid using tape, which can become loose and result in reduced homogenization efficiency.*

9. Centrifuge the tubes ≤ 4000 x *g* for 1 minute at room temperature. The speed will depend on the capability of your centrifuge. **(This step is optional if a centrifuge with a 15 ml tube rotor is not available, but will result in minor loss of supernatant).**

10. Transfer 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. Pipette more than once to ensure removal of all supernatant. Any carryover of beads will not affect subsequent steps. Expect to recover between 600-650 µl of supernatant depending on the type of filter membrane used.

    *What's happening: The filter membrane and beads are separated and removed at this step.*

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

    *What's happening: 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. Avoiding the pellet, transfer the supernatant to a clean 2 ml Collection Tube (provided).

13. Add **200 µl of Solution PWR2** and vortex briefly to mix. Incubate at 4°C for 5 minutes.

    **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 15.

    *What's happening: Solution PWR2 is patented Inhibitor Removal Technology® (IRT) and is a second reagent to remove additional organic and inorganic material including humic acid, cell debris, and proteins. It is important to remove contaminating organic and inorganic matter that may reduce RNA purity and inhibit downstream RNA applications.*

14. Centrifuge the tubes at 13,000 x *g* for 1 minute.

15. Avoiding the pellet, transfer the supernatant to a clean 2 ml Collection Tube (provided).

    *What's happening: The pellet at this point contains additional organic and inorganic material. For best RNA yields and quality, avoid transferring any of the pellet.*

16. Add **650 µl of Solution PWR3** and **650 µl of Solution PWR4.** Then vortex briefly to mix.

    *What's happening: Solution PWR3 is a high concentration salt solution and solution PWR4 is ethanol. Both components are necessary to create the conditions required for efficient binding of the RNA to the spin column while allowing proteins and cellular debris to pass through.*

17. Load 650 µl of supernatant onto a Spin Filter and centrifuge at 13,000 x *g* for 1 minute. Discard the flow through and repeat until all the supernatant has been loaded onto the Spin Filter.

    **Note:** A total of three loads for each sample processed are required.

    *What's happening: RNA is selectively bound to the silica membrane in the Spin Filter basket and the flow through containing non-RNA components is discarded.*

18. Shake to mix Solution PWR5. Add **650 µl of Solution PWR5** and centrifuge at 13,000 x *g* for 1 minute. Discard the flow through.
    **Note:** Skip steps **19-23** if you want to isolate both RNA and DNA.

    *What's happening: Solution PWR5 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.*

19. Centrifuge again at 13,000 x *g* for 1 minute to remove residual wash.

    *What's happening: Complete removal of Solution PWR5 is required for efficient and complete DNase I digestion.*

20. Place the Spin Filter basket into a clean 2 ml Collection Tube (provided).

21. To the center of the Spin Filter, add **50 µl of DNase I Solution** (prepared by mixing **45 ul of Solution PWR6** and **DNase I stock solution.** See **Important Notes before Starting** section).

22. Incubate at room temperature for 15 minutes.

    **Note**: Do not centrifuge the Spin Filter before the addition of Solution PWR7.

    *What's happening: 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 a DNase I digestion.*

23. Add **400 µl Solution PWR7** and centrifuge the column at 13,000 x *g* for 1 minute.

    *What's happening: Solution PWR7 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.*

24. Discard the flow through and add **650 µl of Solution PWR5** and centrifuge at 13,000 x *g* for 1 minute.

    *What's happening: Solution PWR5 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.*

25. Discard the flow through and add **650 µl of Solution PWR4** and centrifuge at 13,000 x *g* for 1 minute.

    *What's happening: Solution PWR4 ensures complete removal of Solution PWR5 which will result in higher RNA purity and yield.*

26. Discard the flow through and centrifuge again at 13,000 x *g* for 2 minutes to remove residual wash.

*What's happening: The second spin removes residual Solution PWR4. It is critical to remove all traces of wash solution because the ethanol in Solution PWR4 can interfere with downstream RNA applications.*

27. Place the Spin Filter basket into a clean 2 ml Collection Tube (provided).

28. Add **100 µl of Solution PWR8** to the center of the white filter membrane.

    **Note:** Eluting with 100 µl of Solution PWR8 will maximize RNA yield. For more concentrated RNA, a minimum of 50 µl of Solution PWR8 can be used. Do not use less than 50 µl of Solution PWR8.

    *What's happening: Solution PWR8 is highly pure water used to elute the RNA from the silica membrane of the spin column. Placing Solution PWR8 (sterile elution buffer) in the center of the small white membrane will make sure the entire membrane is wetted. This will result in a more efficient and complete release of the RNA from the silica Spin Filter membrane. As Solution PWR8 passes through the silica membrane, RNA that was bound in the presence of high salt is selectively released by Solution PWR8 which lacks salt.*

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

30. Discard the Spin Filter basket. The RNA is now ready for any downstream application. No further steps are required. The RNA in the tube can be stored at -80°C until ready for use.

**Thank you for choosing the PowerWater® RNA Isolation Kit!**

# Hints and Troubleshooting Guide

### *Types of Water Samples*

**A. Clear Water Samples:** Water samples may vary from clear to highly turbid. 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. Some users report processing even higher volumes. For clear water samples, known to be free of PCR inhibitors, Steps 12–14 of the protocol can be omitted.

**B. Turbid Water Samples:** Turbid samples with high levels of suspended solids or sediments will tend to clog filters with a smaller pore size (0.22 micron). Use of 0.45 micron filters is recommended for theses types of samples. MO BIO Laboratories offers disposable filter funnels containing membranes of either 0.22 micron or 0.45 micron pore sizes (See page 3 for ordering information). 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 micron 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 100% efficient, it will increase the overall yield of microbial RNA

### *Filter Membrane Selection*

MO BIO Laboratories offers disposable filter funnels containing filter membranes commonly used for water research and testing. The 0.22 micron filter membrane consists of polyethersulfone (Pall Supor®), while the 0.45 micron 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.

### *Forgetting to Warm Solution PWR1*

If PWR1 is not warmed prior to use, continue with the protocol. You will still obtain RNA, but the yields may not be optimal.

### *Alternate Lysis Method*

Heating can aid in lysis of some organisms (fungi and algae) and lead to increased yields. At Step 6, heat the PowerWater® Bead Tube at 65°C for 10 minutes then continue with the protocol at Step 7.

### *If a Centrifuge for 15 ml Tubes is not Available for use with the 5 ml Tubes in Step 9*

Centrifugation at this step helps to separate the supernatant from the filter membrane so that as much of the solution as possible is recovered. If a centrifuge is not available, this step can be skipped with some minor loss of supernatant.

### *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.

| Type of Water Sample | Sample Volume | RNA Yield (µg) |
|---|---|---|
| Freshwater Lake | 50 ml | 1.2 |
| Lagoon | 50-100 ml | 1.0 – 2.7 |

# Hints and Troubleshooting Guide cont.

### *RNA Appears Degraded on Agarose Gels*

The use of Beta mercaptoethanol (βME) will destroy RNases and should be added fresh to Solution PWR1. If RNA still appears degraded, the problem may be caused by the following:

- Make sure that water samples are fresh and stored at 4°C if not processed immediately. Storage at either room temperature or -20°C will cause considerable RNA degradation and loss.
- Prepare Solution PWR1 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.

### *RNA Floats Out of Well When Loaded on a Gel*

Residual Solution PWR4 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.

- Ethanol precipitation is the best way to remove residual Solution PWR4. (See "Concentrating the RNA" below.)
- If you live in a humid climate, you may experience increased difficulty with drying of the membrane in the centrifuge. Increase the centrifugation times at step 25 by another minute.

### *RNA has Low $A_{260/280}$ Ratio*

The ratio for pure RNA should be1.9-2.1. $A_{260/280}$ reading below 1.6 may have significant protein contamination.

- Make sure that the PWR7 wash 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*. Re-measure the 260/280 diluting the RNA for measurement in 10 mM Tris pH 7.5.

**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.*

### *Genomic DNA Contamination in the RNA*

The PowerWater® RNA Isolation Kit is provided with high quality RNase-Free DNase I for on-column digest. When used with the Solution PWR6 included in the kit, 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 the 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.

### *Concentrating the RNA*

Your final volume will be 50 µl - 100 µl. If this is too dilute for your purposes, add 5 µl of 3M 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 (Solution PWR8).

## Hints and Troubleshooting Guide cont.

***Storing RNA***

RNA is eluted in RNase-Free water (Solution PWR8) and should be used immediately or stored at -20°C or -80°C to avoid degradation. RNA can be precipitated in EtOH and stored at -20°C to ensure minimal degradation during long term storage.

# Technical Guide

***Protocol for 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
pH to 7.0 with Sodium Hydroxide.

**1x Formaldehyde agarose gel buffer (1L)**
100 ml 10x Formaldehyde Agarose gel buffer
20 ml 37% (12.3 M) Formaldehyde
880 ml DEPC treated water

**5x RNA Loading Dye**
16 µl Saturated aqueous Bromophenol blue solution
80 µl .5 M EDTA, pH 8.0
720 µl 37% (12.3 M) Formaldehyde
2 ml 100% Glycerol
3084 µl Formamide
4 ml 10x Formaldehyde agarose gel buffer

**Formaldehyde Agarose Gel preparation 1.2% in 100 ml**
Mix the following:
1.2 g Agarose
10 ml 10x Formaldehyde agarose gel buffer
90 ml DEPC treated water

Heat the mixture in a microwave oven to melt the agarose. Cool to 65°C in a waterbath. Add 1.8 ml 37% (12.3 M) Formaldehyde and 2 µl of 5 mg/ml Ethidium Bromide. Swirl to mix and pour into a gel box. The gel must be pre-ran for 30 minutes in 1x Formaldehyde Agarose gel buffer before loading the samples.

***RNA Sample Preparation for Formaldehyde Gels***
The eluted RNA samples must be denatured before running on a formaldehyde agarose gel. To the sample, add 1 volume of 5x RNA loading dye for each 4 volumes of RNA sample (i.e. 2 µl of 5x RNA loading dye for each 8 µl of RNA sample).

Mix the samples and briefly centrifuge to collect the sample 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). Biochim. Biophys. Acta 310: 148-160.
2. Kaplan, B.B., Bernstein, S.L., and Gioio, A.E. (1979). Biochem. J. 183: 181-184.

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## Products recommended for you

*For a complete list of products available from MO BIO Laboratories, Inc., visit [www.mobio.com](http://www.mobio.com)*

| Description | Catalog No. | Quantity |
|---|---|---|
| PowerWater® DNA Isolation Kit | 14900-50-NF<br>14900-100-NF | 50 preps<br>100 preps |
| PowerWater® Sterivex™ DNA Isolation Kit | 14600-50-NF | 50 preps |
| Vortex-Genie® 2 Vortex | 13111-V<br>13111-V-220 | 1 unit (120V)<br>1 unit (220V) |
| Vortex Adapter for Vortex Genie® 2 | 13000-V1-15<br>13000-V1-5 | Holds 4 (15 or 5 ml) Tubes<br>Holds 6 (5 ml) Tubes |
| RTS™ DNase Kit | 15200-50 | 50 preps |