PowerPlant® RNA Isolation Kit

Catalog No.	Quantity
13500-50	50 Preps

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: 03262014

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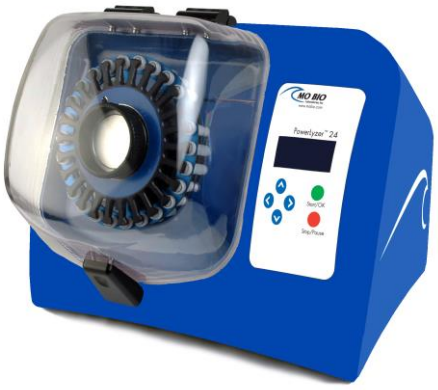
Introduction

The PowerPlant® RNA Isolation Kit is designed to isolate total RNA from a wide variety of plant materials including leaf, root and stem tissues. Sample sizes up to 50 mg can be processed. Contaminants that cause PCR inhibition, such as polysaccharides and polyphenolics, are removed from the lysate before purification of the RNA using patented Inhibitor Removal Technology® (IRT) and an optional Phenolic Separation Solution. Treatment of the RNA to remove residual genomic DNA may be performed post-elution using the RTS DNase™ Kit (cat# 15200-50) or on column using our On-Spin Column DNase I Kit, (cat# 15100-50). The protocol for using the On-Spin Column DNase I Kit is located on page 15 of this manual.

Protocol Overview

Fresh or frozen plant tissues are homogenized using standard methods such as a high powered bead beater (PowerLyzer), rotor-stator homogenizer, or a mortar and pestle with liquid nitrogen. After cell lysis and use of the optional Phenolic Separation Solution, the released RNA is treated with IRT and the inhibitor-free RNA is captured on a silica membrane spin filter. The RNA bound to the filter is washed to remove all contaminants. Finally, the RNA is recovered in certified RNase-Free water (provided). The eluted RNA is ready for most downstream applications.

Optimize for complete homogenization of any sample with the



PowerLyzer® 24
Bench Top Bead-Based Homogenizer
Catalog# 13155
(www.mobio.com/powerlyzer)

The PowerLyzer® 24 is a highly efficient bead beating system that allows for optimal RNA extraction from a variety of plant and animal tissues. 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. This kit provides Ceramic Bead Tubes prefilled with 2.8 mm beads for homogenizing plant tissue for optimal RNA isolation. Alternative pre-filled bead tube options are available for additional applications. Please contact technical service (technical@mobio.com) for details.

Using the PowerPlant® RNA Isolation Kit with other Homogenizers

For isolation of RNA using this kit with the FastPrep® or Precellys®, the following conversion chart will help you to adapt your current protocol. However, due to the highly efficient motion of beads in the PowerLyzer® 24, we have found that less cycle numbers are required to generate the same effect. You may want to perform extractions on the PowerLyzer® 24 at the equivalent speed and number of cycles as your current instrument and compare it to less time or lower speed to determine which settings give the best results.

As a starting point for the PowerLyzer® 24, we recommend that for RNA from plant tissues you begin with the settings specified in this manual of 1 cycle for 45 seconds at setting 4200 RPM.

PowerLyzer 24	Fastprep 24 m/s	Precellys 24
2000	-	-
2100	-	-
2200	-	-
2300	-	-
2400	-	-
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	-
4100	-	-
4200	-	-
4300	-	-
4400	-	-
4500	-	-
5000	-	-

Equivalent settings slower than 2500 RPM or higher than 4000 RPM on the PowerLyzer® 24 are not obtainable with the Fastprep® or Precellys®.

Fastprep® is a registered trademark of MP Biomedical. Precellys® is a registered trademark of Bertin Technologies.

Tissue Preparation

Before placing samples into the bead tube you may want to cut the plant material into smaller pieces. This will increase the homogenization efficiency. Young and fresh tissues are ideal for isolating the maximal amount of high quality RNA. Because the polyphenolic content of the plant material increases with age, the quality of RNA is affected by compounds extracted along with nucleic acids, particularly polyphenols and their quinone oxidation products, and polymeric carbohydrates from the cell wall and middle lamella. During the homogenization process, cells are disrupted leading to the oxidation of phenolic compounds which can then interact irreversibly with proteins and nucleic acids (Dimick and Hoskin, 1981). These substances which bind to nucleic acids inhibit translational activity of RNA (Callahan et al. 1989) and interfere with the quantitation of total DNA and RNA by UV absorption (Newbury and Possingham, 1977). Thus, it is essential to consider the age and type of plant tissues processed before starting with the isolation of RNA.

Phenolic Separation Solution (PSS)

For plant samples high in polyphenolic compounds, the addition of the Phenolic Separation Solution is recommended. PSS prevents loss of nucleic acids by preventing irreversible binding to phenolics which are removed by IRT. Not all plant samples require the addition of PSS. Examples of plant tissues that require the addition of PSS are pine needle and grape leaf. If you are unsure of the phenolic content of your samples, evaluate the RNA recovery with and without PSS to determine the optimal protocol for your work. If in doubt, try using PSS and if yields are acceptable, continue to use it for your plant type.

Amount of Starting Material

We recommend the use of up to 50 mg of fresh or frozen tissue samples as the starting materials in the bead tubes. Exceeding 50 mg may reduce the efficiency of the kit and may result in problems such as increased genomic DNA contamination or clogging of the spin filters. If working with dehydrated or freeze dried samples, begin with 25 mg of tissue.

Processing Frozen Tissue

If working with frozen samples and using a mortar and pestle, the tissue should never be allowed to thaw during maceration and should be completely crushed to a fine powder in a mortar and pestle that is compatible with liquid nitrogen before adding to the bead tube. Resuspend the powdered material in lysis buffer to assure full lysis of the cells, denature proteins, and free the nucleic acids for isolation and purification.

Average Yields of RNA from Various Plant Samples

Plant RNA yields will vary considerably depending on the age of the plant sample and how it was stored. The following table lists estimated yields of RNA achieved using the PowerPlant® RNA Isolation Kit with fresh samples either with or without PSS added.

Table 1.

Plant	RNA yield 50 mg	PSS
Strawberry leaf	8-14 µg	+
Tomato stem	12-20 µg	+
Tomato leaf	12-15 µg	-
Cotton leaf	8-13 µg	-
Cotton seed	40-50 µg	-
Grass leaf	20-30 µg	-
Pine needle	20-25 µg	+
Coffee leaf	3-7 µg	+/-
Mint leaf	5-7 µg	-

+, yields were improved with the addition of PSS, **-,** yields were reduced with the addition of PSS, **+/-,** yields were the same with or without PSS.

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

Other Related Products	Catalog No.	Quantity
PowerPlant® RNA Isolation Kit with DNase	13550-50	50 preps
RTS DNase™ Kit	15200-50	50 preps
PowerPlant® Pro DNA Isolation Kit	13400-50	50 preps
On-Spin Column DNase I Kit (RNase-Free)	15100-50	50 preps
UltraClean® Lab Cleaner	12095-250 12095-500 12095-1000	250 ml squeeze bottle 500 ml spray bottle 1 liter bottle
RNase-Free Gloves	1556-XS 1556-S 1556-M 1556-L	Bag of 150 Bag of 150 Bag of 150 Bag of 150
DEPC Treated Water	17011-200 17011-5200	200 ml 5 x 200 ml

PowerPlant® RNA Isolation Kit

Prepare Sample

- Add up to 50 mg of plant tissue to the 2 ml ceramic bead tube
- Add Solution PR1 containing βME

**Optional: Add Phenolic Separation Sol'n*

Cell Lysis

- Attach to Vortex Adapter or place into the PowerLyzer™ 24
- Homogenize

Centrifuge

Inhibitor Removal Technology®

- Add Solution PR2
- Incubate at 4°C

Centrifuge

Bind RNA

- Add Solution PR3 and PR4
- Load into Spin Filter

Wash

- Wash with Solution PR5
- Wash with Solution PR4

Centrifuge

Elute

- Elute with Solution PR6

Equipment Required

PowerLyzer® 24 Homogenizer or similar instrument
Microcentrifuge (up to 16,000 x *g*)
Pipettor (volumes required 5 µl - 1000 µl)

Reagents Required but not Included

βME - Beta mercaptoethanol

Kit Contents

	Kit Catalog # 13500-50	
Component	**Catalog#**	**Amount**
Solution PR1	13500-50-1	33 ml
Solution PR2	13500-50-2	11 ml
Solution PR3	13500-50-3	36 ml
Solution PR4	13500-50-4	3 x 23 ml
Solution PR5	13500-50-5	30 ml
Solution PR6	13500-50-6	5.5 ml
Phenolic Separation Solution	13500-50-7	2.8 ml
PowerPlant® RNA Bead Tubes	13500-50-BT	50
Spin Filters	13500-50-SF	50
2 ml Collection Tubes	13500-50-T	150

Kit Storage

Store all reagents and kit components at room temperature (15-30°C).

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: Solution PR4 and PR5 are flammable. To avoid injury or fire, keep away from open flames and sparks.

Important Notes Before Starting

1. **Solution PR1 must be warmed at 55°C for 5-10 minutes to dissolve precipitates prior to use**. Solution PR1 should be used while still warm.

2. **Prepare Solution PR1/βME by adding β-mercaptoethanol (βME) to Solution PR1**
   Add the appropriate amount of β-mercaptoethanol to Solution PR1 to produce a final concentration of 10 µl/ml. For each prep 600 µl of **PR1/βME** will be needed. Alternatively, add 594 µl of **PR1** and 6 µl of βME directly to each bead tube.

   NOTE: You can prepare larger amounts of Solution **PR1/βME** with fresh βME according to the number of samples you need to process that day instead of adding βME to the whole bottle of Solution PR1. Use a fume hood when opening βME to avoid exposure to the chemical.

Removal of Genomic DNA
This protocol will result in the co-isolation of RNA and DNA. To remove the genomic DNA, the On-Spin Column DNase I Kit may be purchased separately and used during the procedure (see protocol page 15). The genomic DNA may also be removed after elution using the RTS DNase™ Kit (cat# 15200-50). The RTS DNase™ Kit uses a high velocity, room temperature stable DNase followed by a gentle and efficient resin based DNase removal system. RNA is purified away from genomic DNA without the use of heat or EDTA to inactivate the enzyme. Samples of the RTS DNase™ Kit are available on the website. To order use MO BIO catalog #15200-S.

For added convenience, we offer the PowerPlant® RNA Kit with DNase, catalog number 13550-50. The On-Spin Column DNase I Kit reagents are included in this kit.

Experienced User Protocol

Please wear gloves at all times.

Warm Solution PR1 prior to use at 55°C for 5-10 minutes. Use Solution PR1 while still warm.

1. Place up to 50 mg of plant sample into the 2 ml PowerPlant® RNA Bead Tube provided.
   Note: It is recommended to cut the material into smaller pieces prior to weighing and loading.

2. Add **600 µl of Solution PR1/βME** to the PowerPlant® RNA Bead Tube (See **Important Notes Before Starting** section for how to prepare **Solution PR1/βME)**. You may prepare the **Solution PR1/βME** in advance or if you prefer, you can add PR1 and βME individually to each RNA Bead Tube. Each sample requires 594 µl PR1 and 6 µl βME.
   Note: If your sample is high in phenolics and you are using the **Phenolic Separation Solution**, reduce **Solution PR1/βME to 550 µl and add 50 µl of the Phenolic Separation Solution.**

3. For the highest yields of RNA, a high powered bead beater is recommended.

   A. On the PowerLyzer® 24 Instrument, we recommend a starting setting of 1 cycle for 45 seconds at 4200 rpm for leaf tissue and seeds. See the table on page 4 to determine comparable settings for other homogenizers.

   B. Homogenization may also be performed on a Vortex Genie® 2 using the MO BIO Vortex Adapter (MO BIO Catalog# 13000-V1-24) for soft leaf tissue only. Set the vortex on full speed for ten minutes.

4. Centrifuge at 13,000 x *g* for 2 minutes at room temperature.

5. Transfer all the supernatant to a clean 2 ml Collection Tube (provided). Expect between 500 to 600 µl of lysate.

6. Add **150 µl of Solution PR2** and vortex briefly to mix. Incubate at 4°C for 5 minutes.
   Note: For plant samples still containing PCR inhibitors after RNA purification, try adding up to 200 µl of Solution PR2.

7. Centrifuge the tubes at 13,000 x *g* for 2 minutes.

8. Avoiding the pellet, transfer the supernatant to a clean 2 ml Collection Tube (provided). Transfer no more than 650 µl at this step.

9. Add **650 µl of Solution PR3** and **650 µl of Solution PR4.** Vortex briefly to mix.
   Note: For the purification of small RNAs, such as microRNA and siRNA, transfer the lysate to a larger tube to accommodate the higher volume (2.6 ml) and add an additional 650 µl of 100% ethanol. You will need to supply additional 100% ethanol for this step.

10. Load 650 µl of supernatant onto the Spin Filter and centrifuge at 13,000 x *g* for 1 minute. Discard the flow through and place the Spin Filter back into the 2 ml Collection Tube. Repeat until all the supernatant has been loaded onto the Spin Filter.
    Note: A total of three loads for each sample processed are required and four loads if an additional volume of 100% ethanol is added for the miRNA and siRNA protocol.

11. Shake to mix Solution PR5. Add **600 µl of Solution PR5** to the Spin Filter and centrifuge at 13,000 x *g* for 1 minute.

OPTIONAL: If the **On-Spin Column DNase I Kit** (MO BIO Catalog# 15100-50) was purchased separately, it should be incorporated after step 11. Perform steps A-E on page 15 of this manual.

12. Discard the flow through, place the Spin Filter back into the 2 ml Collection Tube, and add **600 µl of Solution PR4.** Centrifuge at 13,000 x *g* for 1 minute.

13. Discard the flow through and centrifuge again at 16,000 x *g* for 2 minutes to remove residual wash solution.

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

15. Add **50-100 µl of Solution PR6** (RNase-Free Water) to the center of the white Spin Filter membrane. Allow the water to incubate at room temperature for one minute prior to centrifugation.

    Note: Eluting with 100 µl of Solution PR6 will maximize RNA yield. For more concentrated RNA, a minimum of 50 µl of Solution PR6 can be used. Do not use less than 50 µl of Solution PR6.

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

17. Discard the Spin Filter. The RNA is now ready for downstream applications. The RNA in the tube can be stored at -80°C until ready for use.

If post extraction DNase treatment is desired, we recommend the RTS DNase™ Kit, cat# 15200-50. The RTS DNase™ Kit allows for gentle and inhibitor free removal of genomic DNA and DNase without the use of heat or EDTA.

Thank you for choosing the PowerPlant® RNA Isolation Kit.

Detailed Protocol (Describes what is happening at each step)

Please wear gloves at all times.

Warm Solution PR1 prior to use at 55°C for 5-10 minutes. Use Solution PR1 while still warm.

1. Place up to 50 mg of plant sample into the 2 ml PowerPlant® RNA Bead Tube provided.
   Note: It is recommended to cut the material into smaller pieces prior to weighing and loading.

2. Add **600 µl of Solution PR1/βME** to the PowerPlant® RNA Bead Tube (See **Important Notes Before Starting** section for how to prepare **Solution PR1/βME)**. You may prepare the **Solution PR1/βME** in advance or if you prefer, you can add PR1 and βME individually to each RNA Bead Tube. Each sample requires 594 µl PR1 and 6 µl βME.
   Note: If your sample is high in phenolics and you are using the **Phenolic Separation Solution**, reduce **Solution PR1/βME to 550 µl and add 50 µl of the Phenolic Separation Solution.**

   What's happening: Solution PR1 is an RNA lysis buffer that protects RNA during the homogenization and provides the buffer conditions required for IRT. βME is used as an additional RNase inhibitor to fully protect the RNA during the initial steps.

   Phenolic Separation Solution (PSS) prevents cross-linking of nucleic acids to oxidized phenolic compounds which are removed by IRT. Not all samples require PSS. A partial list in Table 1 on page 6 will give you an indication if PSS might increase your yield of RNA.

3. For the highest yields of RNA, a high powered bead beater is recommended.

   A. On the PowerLyzer® 24 Instrument, we recommend a starting setting of 1 cycle for 45 seconds at 4200 rpm for leaf tissue and seeds. See the table on page 4 to determine comparable settings for other homogenizers.

   B. Homogenization may also be performed on a Vortex Genie® 2 using the MO BIO Vortex Adapter (MO BIO Catalog# 13000-V1-24) for soft leaf tissue only. Set the vortex on full speed for ten minutes.

   What's happening: Rapid and efficient homogenization is critical to high quality RNA purification. The vortex is suitable for soft leaf tissue. For the highest yields, a method such as the PowerLyzer® 24 that quickly pulverizes the sample and breaks genomic DNA is recommended.

4. Centrifuge at 13,000 x *g* for 2 minutes at room temperature.

   What's happening: Centrifugation is used to clear the lysate of any remaining particulate before the next step.

5. Transfer all the supernatant to a clean 2 ml Collection Tube (provided). Expect between 500 to 600 µl of lysate.

6. Add **150 µl of Solution PR2** and vortex briefly to mix. Incubate at 4°C for 5 minutes.
   Note: For plant samples still containing PCR inhibitors after RNA purification, try adding up to 200 µl of Solution PR2.

What's happening: Solution PR2 is patented IRT which removes the inhibiting compounds such as the polyphenolics and carbohydrates from the lysate.

7. Centrifuge the tubes at 13,000 x *g* for 2 minutes.

   What's happening: The pellet contains the inhibitors from the plant lysate. It is now ready for binding to the Spin Filter.

8. Avoiding the pellet, transfer the supernatant to a clean 2 ml Collection Tube (provided). Transfer no more than 650 µl at this step.

9. Add **650 µl of Solution PR3** and **650 µl of Solution PR4.** Vortex briefly to mix.
   Note: For the purification of small RNAs, such as microRNA and siRNA, transfer the lysate to a larger tube to accommodate the higher volume (2.6 ml) and add an additional 650 µl of 100% ethanol. You will need to supply additional 100% ethanol for this step.

   What's happening: Solution PR3 contains the binding salts necessary to capture RNA on the Spin Filter membrane. Solution PR4 is 100% ethanol. Solutions PR3 and PR4 are added in equal volume to the amount of lysate for optimal binding of the rRNA and mRNA. For binding of small RNAs less than 200 bases, the ethanol content needs to be increased by adding an additional 650 µl volume of user supplied 100% ethanol. You will need a larger tube to hold the total volume of sample.

10. Load 650 µl of supernatant onto the Spin Filter and centrifuge at 13,000 x *g* for 1 minute. Discard the flow through and place the Spin Filter back into the 2 ml Collection Tube. Repeat until all the supernatant has been loaded onto the Spin Filter.
    Note: A total of three loads for each sample processed are required and four loads if an additional volume of 100% ethanol is added for the miRNA and siRNA protocol.

    What's happening: The RNA is bound to the Spin Filter membrane. Salts and any remaining impurities from the plant material flow through.

11. Shake to mix Solution PR5. Add **600 µl of Solution PR5** to the Spin Filter and centrifuge at 13,000 x *g* for 1 minute.

    What's happening: Solution PR5 is a wash buffer to remove the salts in Solution PR3 from the Spin Filter membrane in preparation for the DNase digest in the next step.

OPTIONAL: If the **On-Spin Column DNase I Kit** (MO BIO Catalog# 15100-50) was purchased separately, it should be incorporated after step 11. Perform steps A-E on page 15 of this manual.

12. Discard the flow through, place the Spin Filter back into the 2 ml Collection Tube, and add **600 µl of Solution PR4.** Centrifuge at 13,000 x *g* for 1 minute.

    What's happening: Solution PR4 is 100% ethanol and is used to completely flush the spin filter membrane of any remaining salts. The ethanol evaporates quickly and prepares the membrane for elution.

13. Discard the flow through and centrifuge again at 16,000 x *g* for 2 minutes to remove residual wash solution.

    What's happening: The final spin is to dry the Spin Filter membrane to remove all traces of ethanol before the elution step.

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

15. Add **50-100 µl of Solution PR6** (RNase-Free Water) to the center of the white Spin Filter membrane. Allow the water to incubate at room temperature for one minute prior to centrifugation.

    Note: Eluting with 100 µl of Solution PR6 will maximize RNA yield. For more concentrated RNA, a minimum of 50 µl of Solution PR6 can be used. Do not use less than 50 µl of Solution PR6.

    What's happening: Solution PR6 is RNase-Free Water and will elute the purified RNA from the Spin Filter membrane into solution.

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

17. Discard the Spin Filter. The RNA is now ready for downstream applications. The RNA in the tube can be stored at -80°C until ready for use.

If post extraction DNase treatment is desired, we recommend the RTS DNase™ Kit, cat# 15200-50. The RTS DNase™ Kit allows for gentle and inhibitor free removal of genomic DNA and DNase without the use of heat or EDTA.

Thank you for choosing the PowerPlant® RNA Isolation Kit.

Appendix: Additional Protocols

A. Protocol for On-Spin Column DNase I Kit (Catalog #15100-50)

The On-Spin Column DNase I Kit is for removal of genomic DNA during the RNA extraction procedure.

Kit Contents

Components	Amount
Solution D1 (DNase I Buffer)	2.5 ml
Solution D2 (DNase Wash Buffer)	25 ml
Solution D3 (Wash Buffer II)	2 x 28 ml
DNase I (RNase-Free)	1500 units
Sterile Water (DNase I Resuspension Water)	1 ml
2 ml Collection Tubes	50

DNase I Solution Preparation and Storage:

Prepare **DNase I stock enzyme** by adding **300 µl** of Sterile Water to the lyophilized DNase I vial and mix gently. Do not vortex DNase I. Aliquot the enzyme in 50 µl portions and store at -20° C for long term storage. Note**:** The enzyme can be freeze/thawed up to three times without loss of activity.

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 D1**.

Protocol:

DNase I Treatment:

A. After the Solution PR5 wash step (step 11), centrifuge for 2 minutes at 13,000 x *g* to thoroughly remove all traces of wash buffer from the Spin Filter membrane.

B. Transfer the Spin Filter to a new 2 ml Collection Tube (provided). To the center of the Spin Filter, add **50 µl of DNase I Solution** (a mixture of **5 µl of DNase I stock enzyme and 45 µl of Solution D1).** Incubate at room temperature for 15 minutes.

C. To the Spin Filter add **400 µl Solution D2** and centrifuge at 13,000 x *g* for 1 minute. Discard the flow through and place the Spin Filter back into the 2 ml Collection Tube.

D. Add **500 µl of Solution D3** to the Spin Filter. Centrifuge at 13,000 x *g* for 1 minute. Discard the flow-through and place the Spin Filter back into the 2 ml Collection Tube.

E. Repeat the wash by adding another **500 µl of Solution D3** to the Spin Filter. Centrifuge at 13,000 x *g* for 1 minute. Discard the flow-through and place the Spin Filter back into the 2 ml Collection Tube. Centrifuge the Spin Filter for 2 minutes at 16,000 x *g* to completely dry the membrane. Continue with step 14 of the protocol.

Hints and Troubleshooting Guide

Concentrating the RNA
Your final volume will be 50 -100 µl. If this is too dilute for your purposes, add 5 M NaCl to a final concentration of 0.2 M, or 3 M Sodium Acetate (pH: 5.2) to a final concentration of 0.3 M and mix. Then add two volumes of 100% cold ethanol. Mix by inversion and incubate at -20°C for 10 minutes to overnight. Centrifuge at 13,000 x *g* for 20 minutes to pellet the RNA. Decant the ethanol. Wash the RNA pellet with 70% ethanol (0.5 ml) by inversion. Centrifuge 10 minutes at 13,000 *x g*. Decant the ethanol and invert the tube to completely drain. Dry the residual ethanol in a speed vac or ambient air. Resuspend the precipitated RNA in the desired volume.

RNA Floats Out of Well When Loaded on a Gel
You may have inadvertently transferred residual solution PR4 to the final sample. Prevent this by being careful in step 14 not to transfer liquid onto the bottom of the spin filter basket. Ethanol precipitation is the best way to remove residual solution PR4. (See "Concentrating the RNA" above)

Storing RNA
RNA is eluted in Solution PR6 (RNase-Free Water) and should be used immediately or stored at -80°C to minimize degradation.

Removal of Genomic DNA
This protocol will result in the co-isolation of RNA and DNA. To remove the genomic DNA, the On-Spin Column DNase I Kit may be purchased separately and used during the procedure (see protocol page 15). The genomic DNA may also be removed after elution using the RTS DNase™ Kit (cat# 15200-50). The RTS DNase™ Kit uses a high velocity, room temperature stable DNase followed by a gentle and efficient resin based DNase removal system. RNA is purified away from genomic DNA without the use of heat or EDTA to inactivate the enzyme. Samples of the RTS DNase™ Kit are available on the website. To order use MO BIO catalog #15200-S.

For added convenience, we offer the PowerPlant® RNA Kit with DNase, catalog number 13550-50. The On-Spin Column DNase I Kit reagents are included in this kit.

Suggested 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% (12.3M) Formaldehyde
880 ml	DEPC treated water

5x RNA Loading Dye

16 µl	Saturated aqueous Bromophenol blue solution
80 µl	0.5 M EDTA, pH 8.0
720 µl	37% (12.3M) Formaldehyde
2 ml	100% Glycerol
3084 µl	Formamide
4 ml	10x Formaldehyde Agarose gel buffer

Formaldehyde Agarose Gel preparation

To make a 1.2% formaldehyde agarose gel with 100 ml volume 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% (12.3M) 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

The eluted RNA samples must be denatured before running on a formaldehyde agarose gel. To the sample to be loaded, 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 well and use a picofuge 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

Callahan, A., Morgens, P., and Walton, E. 1989. Hortic. Sci. 24: 356 - 358.
Dimick, P.S., and Hoskin, J.M. 1981. Can. Inst. Food Sci. technol. J. 14: 269 - 282.
Newbury, H.J., and Possingham, J.V. 1977. Plant Physiol. 60: 543 – 547.

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For the distributor nearest you, visit our web site at www.mobio.com/distributors

Products recommended for you

For a complete list of products available from MO BIO Laboratories, Inc., visit www.mobio.com

Description	Catalog No.	Quantity
PowerPlant® Pro DNA Isolation Kit	13400-50	50 preps
PowerPlant® RNA Isolation Kit with DNase	13550-50	50 preps
PowerPlant® Pro-htp 96 Well DNA Isolation Kit	13496-4	4 x 96 preps
RTS DNase™ Kit	15200-50	50 preps
PowerClean® Pro DNA Clean-Up Kit	12997-50	50 preps
PowerLyzer® 24 Bench Top Bead-Based Homogenizer	13155	1 unit