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RNeasy® PowerPlant® Kit Handbook

For the isolation of total RNA from plant and seed samples, including those high in polyphenols and polysaccharides

Sample to Insight

QIAGEN

Contents

Kit Contents

RNeasy PowerPlant Kit	**(50)**
Catalog no.	**13500-50**
Number of preps	**50**
PowerBead Tubes, Ceramic 2.8 mm	50
MB RNA Spin Columns	50
Solution MBL	2 x 25 ml
Solution IRS	15 ml
Solution PM3	36 ml
Solution PM4	3 x 24 ml
Solution PM5	30 ml
RNase-Free Water	10 ml
Phenolic Separation Solution	2 x 1.5 ml
Collection Tubes (2 ml)	3 x 50
Quick Start Protocol	1

Storage

All components of the RNeasy PowerPlant 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** where you can find, view and print the SDS for each QIAGEN kit and kit component.

WARNING 	**Solution PM4 and Solution PM5 are flammable.**

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

Solution MBL and Solution PM3 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 PowerPlant Kits is tested against predetermined specifications to ensure consistent product quality.

Introduction

The RNeasy PowerPlant Kit is designed to isolate total RNA from up to 50 mg of a wide variety of plant materials, including leaf, root and stem tissues. Contaminants that cause PCR inhibition, such as polysaccharides and polyphenolics, are removed from the lysate before purification of the RNA using our Inhibitor Removal Technology® (IRT) and an optional step using Phenolic Separation Solution. Residual genomic DNA may be removed from the isolated RNA post-elution using the DNase Max® Kit (cat. no. 15200-50).

Principle and procedure

Fresh or frozen plant tissues may be homogenized using standard methods, including a high-powered bead beater, such as the PowerLyzer® 24 Homogenizer, a 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. The inhibitor-free RNA is captured on a silica-membrane spin filter. The RNA bound to the filter is washed to remove contaminants. Finally, the RNA is recovered in certified RNase-Free Water (provided). The eluted RNA is ready for downstream applications..

Optimized for homogenization with the PowerLyzer 24 Homogenizer

The RNeasy PowerPlant Kit may be used with a vortex or high-velocity bead beater, such as the PowerLyzer 24 Homogenizer. The PowerLyzer 24 Homogenizer is suitable for fast homogenization of plant materials including stems, roots, seeds or difficult leaf tissue without the need of liquid nitrogen grinding.

The PowerLyzer 24 Homogenizer is a highly efficient bead beating system that allows for optimal RNA extraction from a variety of plant 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

PowerBead Tubes prefilled with 2.38 mm ceramic beads for homogenizing plant tissue for optimal RNA isolation. Alternative pre-filled bead tube options are available for additional applications. Please contact QIAGEN Technical Service at **support.qiagen.com** for details.

To isolate RNA from plant tissues using the RNeasy PowerPlant Kit and the PowerLyzer 24 Homogenizer, please refer to guidelines for getting started in Step 3 of the protocols on page12 or on page14.

Using the RNeasy PowerPlant Kit with other homogenizers

To isolate RNA using the RNeasy PowerPlant Kit with FastPrep® or Precellys® homogenizers, use the conversion chart shown in Table 1 (page 8) to adapt your current protocol. However, due to the highly efficient motion of beads in the PowerLyzer 24 Homogenizer, fewer cycles are required to generate the same effect using it compared to other homogenizers. You may want to perform extractions using the PowerLyzer 24 Homogenizer at the equivalent speed and number of cycles as your current instrument and then compare the results to those obtained using less time or lower speeds to determine which settings give the best results.

Phenolic Separation Solution

For plant samples high in polyphenolic compounds, we recommend adding the Phenolic Separation Solution. This solution prevents loss of nucleic acids by preventing their irreversible binding to phenolics, which are removed by IRT. Not all plant samples require the addition of the Phenolic Separation Solution. Examples of plant tissues that require the addition includes pine and grape leaf. If you are unsure of the phenolic content of your samples, evaluate the RNA recovery with and without using the Phenolic Separation Solution to determine the optimal protocol.

Table 1. Conversion chart to use the RNeasy PowerPlant Kit with FastPrep or Precellys homogenizers

PowerLyzer 24 Homogenizer (rpm)	FastPrep 24 (m/s)	Precellys 24 (rpm)
2500	4	5000
2600	–	5200
2700	–	5400
2800	4.5	5600
2900	–	5800
3000	–	6000
3100	5	6200
3200	–	6400
3300	–	6600
3400	5.5	6800
3500	–	–
3600	–	–
3700	6	–
3800	–	–
3900	–	–
4000	6.5	–

Note: Settings equivalent to slower than 2500 rpm or faster than 4000 rpm on the PowerLyzer 24 Homogenizer are not obtainable with FastPrep or Precellys homogenizers.

Average RNA yields from various samples

RNA yields from plant tissues can vary considerably based on the age and type of tissue and how the samples were stored. Table 2 (page 9) lists estimated RNA yields from a variety of fresh plant samples evaluated using the RNeasy PowerPlant Kit.

Table 2. Estimated RNA yields from plant samples using the RNeasy PowerPlant Kit

Plant sample	RNA yield (from 50 mg of sample)	Phenolic Separation Solution (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	+
Mint leaf	5–7 µg	–
Coffee leaf	3–7 µg	+/–

Note: Positive (+) yields were improved with the addition of PSS; negative (–) yields were reduced with the addition of PSS; **+/–** yields were the same with or without PSS.

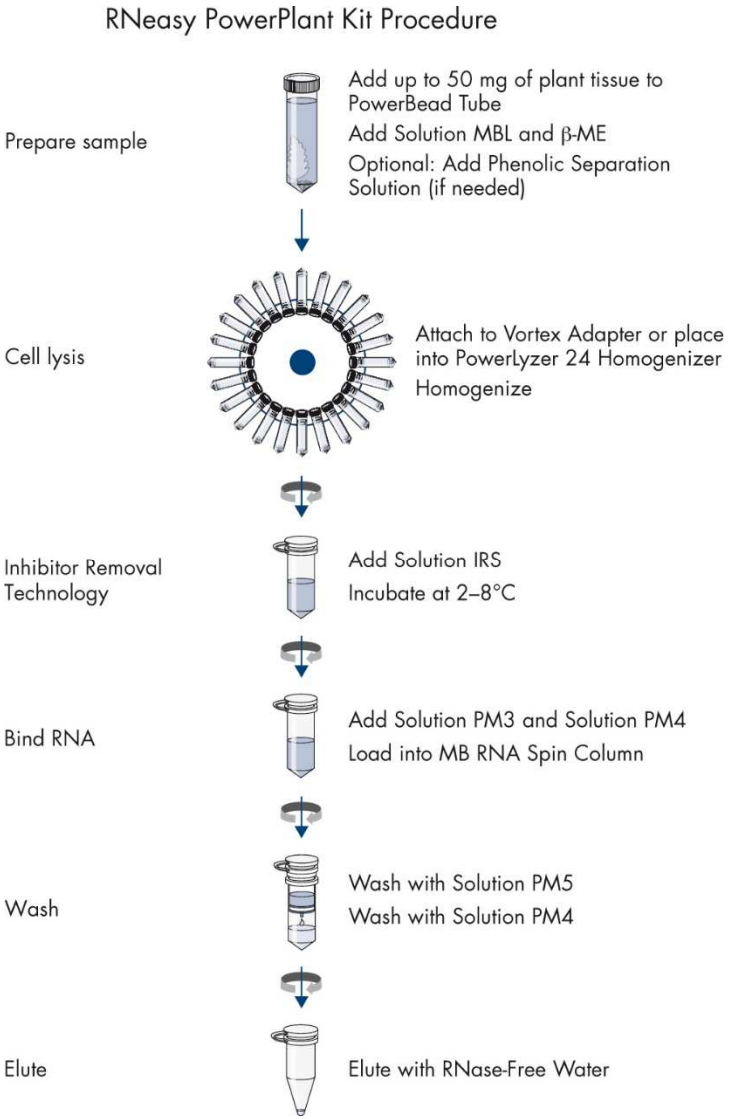

Figure 1. RNeasy PowerPlant Kit procedure.

Equipment and Reagents to Be Supplied by User

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

- Microcentrifuge (up to16,000 x *g*)
- PowerLyzer 24 Homogenizer or another bead homogenizer
- Pipette (50–600 µl)
- Vortex-Genie® 2
- Vortex Adapter for vortexing 1.7 or 2 ml tubes (cat. no. 13000-V1-24)
- β-mercaptoethanol (β-ME)

Protocol: Experienced User

Notes before starting

- Solution MBL must be warmed at 55°C for 5–10 minutes to dissolve precipitates prior to use. Use Solution MBL while still warm.
- Add the appropriate amount of β-mercaptoethanol (β-ME) to Solution MBL to produce a final concentration of 10 µl/ml of MBL/β-ME. For each prep, 600 µl of MBL/β-ME will be needed. Alternatively, add 594 µl of MBL/6 µl of β-ME directly to each bead tube.

Procedure

1. Place up to 50 mg of plant sample into a 2 ml PowerBead Tube (provided).
   Note: We recommended cutting samples into smaller pieces before weighing/loading.
2. Add 600 µl of Solution MBL/β-ME to the PowerBead Tube. You may prepare Solution MBL/β-ME in advance or add 594 µl of MBL and 6 µl of β-ME to each bead tube.
   Note: If sample is high in phenolics and you are using the Phenolic Separation Solution, reduce Solution MBL/β-ME 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 Homogenizer, we recommend a starting setting of 1 cycle at 4200 rpm for 45 s for leaf tissue and seeds.
   B. You may also homogenize using a Vortex Genie 2 and Vortex Adapter (cat. no. 13000-V1-24) for soft leaf tissue only. Set vortex on full speed for 10 min.
4. Centrifuge at 13,000 x *g* for 2 min 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 IRS and vortex briefly to mix. Incubate at 2–8°C for 5 min.
   Note: For plant samples that still contain PCR inhibitors after RNA purification, try adding up to 200 µl of Solution IRS.

7. Centrifuge the tubes at 13,000 x *g* for 2 min. Avoiding the pellet, transfer the supernatant to a clean 2 ml Collection Tube. Transfer no more than 650 µl at this step.
8. Add 650 µl of Solution PM3 and 650 µl of Solution PM4. Vortex briefly to mix.
   Note: To purify small RNAs, such as microRNAs and siRNAs, 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 the 100% ethanol.
9. Load 650 µl of supernatant onto an MB RNA Spin Column and centrifuge at 13,000 x *g* for 1 min. Discard the flow-through and place the Spin Column back into the 2 ml Collection Tube. Repeat until all the supernatant has been loaded onto the Spin Column.
   Note: A total of three loads for each sample processed are required (four loads if an additional volume of 100% ethanol is added for the miRNA and siRNA protocol).
10. Shake to mix Solution PM5. Add 600 µl of Solution PM5 to the MB RNA Spin Column and centrifuge at 13,000 x *g* for 1 min.
    Optional: If the RNase-Free DNase Set (cat. no. 79254) was purchased separately, it should be incorporated after step 10.
11. Discard the flow-through, place the MB RNA Spin Column back into the 2 ml Collection Tube and add 600 µl of Solution PM4. Centrifuge at 13,000 x *g* for 1 min.
12. Discard the flow-through and centrifuge again at 16,000 x *g* for 2 min.
13. Place the MB RNA Spin Column filter into a clean 2 ml Collection Tube.
14. Add 50–100 µl of RNase-Free Water (provided) to the center of the white Spin Column membrane. Incubate at room temperature for 1 min.
    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.
15. Centrifuge at 13,000 x *g* for 1 min. Discard the MB RNA Spin Column. The RNA is now ready for downstream applications and may be stored at –65° to –90°C.

Protocol: Detailed

Notes before starting

- Solution MBL must be warmed at 55°C for 5–10 minutes to dissolve precipitates prior to use. Use Solution MBL while still warm.
- Add the appropriate amount of β-mercaptoethanol (β-ME) to Solution MBL to produce a final concentration of 10 µl/ml of MBL/β-ME. For each prep, 600 µl of MBL/β-ME will be needed. Alternatively, add 594 µl of MBL/6 µl of β-ME directly to each bead tube.

Procedure

1. Place up to 50 mg of plant sample into a 2 ml PowerBead Tube (provided).
   Note: We recommended cutting samples into smaller pieces before weighing/loading.
2. Add 600 µl of Solution MBL/β-ME to the PowerBead Tube. You may prepare Solution MBL/β-ME in advance or add 594 µl of MBL and 6 µl of β-ME to each bead tube.
   Note: If sample is high in phenolics and you are using the Phenolic Separation Solution, reduce Solution MBL/β-ME to 550 µl and add 50 µl of the Phenolic Separation Solution.
   Note: Solution MBL is an RNA lysis buffer that protects RNA during homogenization and provides the buffer conditions required for IRT. β-ME is used as an additional RNase inhibitor to fully protect the RNA during the initial steps.
   Note: Phenolic Separation Solution (PSS) prevents cross-linking of nucleic acids to oxidized phenolic compounds which are removed by IRT. Not all samples require PSS. Table 2 on page 9 lists some samples for which PSS might increase RNA yields.
3. For the highest yields of RNA, a high-powered bead beater is recommended.
   A. On the PowerLyzer 24 Homogenizer, we recommend a starting setting of 1 cycle at 4200 rpm for 45 s for leaf tissue and seeds.
   B. You may also homogenize using a Vortex Genie 2 and Vortex Adapter (cat. no. 13000-V1-24) for soft leaf tissue only. Set vortex on full speed for 10 min.
   Note: Rapid and efficient homogenization is critical to purify high-quality RNA. For the highest yields, we recommend using the PowerLyzer 24 Homogenizer, which quickly

pulverizes the sample and breaks genomic DNA. Using the Vortex Genie 2 and a Vortex Adapter is suitable for soft leaf tissue.

4. Centrifuge at 13,000 x *g* for 2 min at room temperature.
   Note: Centrifugation clears the lysate of any remaining particulates 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 IRS and vortex briefly to mix. Incubate at 2–8°C for 5 min.
   Note: For plant samples that still contain PCR inhibitors after RNA purification, try adding up to 200 µl of Solution IRS.
   Note: Solution IRS has IRT, which removes inhibiting compounds, such as polyphenolics and carbohydrates from the lysate.
7. Centrifuge the tubes at 13,000 x *g* for 2 min. Avoiding the pellet, transfer the supernatant to a clean 2 ml Collection Tube. Transfer no more than 650 µl at this step.
8. Add 650 µl of Solution PM3 and 650 µl of Solution PM4. Vortex briefly to mix.
   Note: To purify small RNAs, such as microRNAs and siRNAs, 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 the 100% ethanol.
   Note: Solution PM3 contains binding salts necessary to capture RNA on the MB RNA Spin Column membrane. Solution PM4 is 100% ethanol. Equal volumes of Solutions PM3 and PM4 are added for optimal binding of both rRNA and mRNA.
9. Load 650 µl of supernatant onto an MB RNA Spin Column and centrifuge at 13,000 x *g* for 1 min. Discard the flow-through and place the Spin Column back into the 2 ml Collection Tube. Repeat until all the supernatant has been loaded onto the Spin Column.
   Note: A total of three loads for each sample processed are required (four loads if an additional volume of 100% ethanol is added for the miRNA and siRNA protocol).
   Note: RNA is bound to the MB RNA Spin Column membrane. Salts and any remaining impurities from the plant material flow through.
10. Shake to mix Solution PM5. Add 600 µl of Solution PM5 to the MB RNA Spin Column and centrifuge at 13,000 x *g* for 1 min.

Optional: If the RNase-Free DNase Set (cat. no. 79254) was purchased separately, it should be incorporated after step 10.

Note: Solution PM5 is a wash buffer and removes the salts in Solution PM3 from the MB RNA Spin Column filter membrane in preparation for the DNase digest in the next step

11. Discard the flow-through, place the MB RNA Spin Column back into the 2 ml Collection Tube and add 600 µl of Solution PM4. Centrifuge at 13,000 x *g* for 1 min.
    Note: Solution PM4 is 100% ethanol and is used to completely flush the MB RNA Spin Column filter membrane of any remaining salts. Solution PM4 evaporates quickly and prepares the membrane for elution.
12. Discard the flow-through and centrifuge again at 16,000 x *g* for 2 min.
    Note: This final spin is to completely dry the MB RNA Spin Column filter membrane and remove all traces of ethanol before the elution step.
13. Place the MB RNA Spin Column filter into a clean 2 ml Collection Tube.
14. Add 50–100 µl of RNase-Free Water (provided) to the center of the white MB RNA Spin Column membrane. Incubate at room temperature for 1 min.
    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.
15. Centrifuge at 13,000 x *g* for 1 min. Discard the MB RNA Spin Column. The RNA is now ready for downstream applications and may be stored at –65° to –90°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**. 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
RNA		
a)	RNA floats out of 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.
b)	Concentrating eluted RNA	The final volume of eluted RNA will be 50–100 µl. The RNA may be concentrated by adding 5 µl 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°C for 15 minutes or –20°C for 2 hours to overnight. Centrifuge at 10,000 x *g* for 10–15 min at 2–8°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.
c)	Storing RNA	RNA is eluted in RNase-Free water and must be stored at –20°C to –80°C to prevent degradation.
d)	Removing genomic DNA	This protocol will result in the co-isolation of RNA and DNA. To remove the genomic DNA, we recommend the DNase Max Kit (cat. no. 15200-50). The DNase Max Kit uses a high-velocity, room temperature-stable DNase followed by a gentle and efficient resin-based DNase removal system. RNA is purified without the use of heat or EDTA.

		Comments and suggestions
Sample processing		
a)	Preparing plant tissue	Before placing samples into PowerBead Tubes we recommend cutting the plant material into smaller pieces, which will increase the homogenization efficiency. Young and fresh tissues are ideal for isolating the maximum amount of high-quality RNA. The polyphenolic content of plant material increases with age, and 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, which leads to the oxidation of phenolic compounds. These compounds can interact irreversibly with proteins and nucleic acids, and inhibit the translational activity of RNA and interfere with the quantitation of total DNA and RNA by UV absorption. Therefore, it is essential to consider the age and type of plant tissues processed before starting RNA isolation.
b)	Amount of plant tissue to process	We recommend using up to 50 mg of fresh or frozen tissue samples as starting material. 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 MB RNA Spin Column filters. If working with dehydrated or freeze-dried samples, begin with 25 mg of tissue.
c)	Processing frozen plant 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 transferring to a PowerBead Tube. Resuspend the powdered material in Solution MBL/β-ME to ensure that cells are fully lysed, proteins denatured and nucleic acids are available for isolation and purification.

Appendix A: 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 µl saturated aqueous Bromophenol Blue solution
  - 80 µl 0.5 M EDTA (pH 8.0)
  - 720 µl 37% formaldehyde
  - 2 ml 100% glycerol
  - 3084 µl 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 µl of 5 mg/ml ethidium bromide. Swirl to mix and pour

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 µl of 5x RNA loading dye for each 8 µl 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

Product	Contents	Cat. no.
RNeasy PowerPlant Kit (50)	For 50 preps: Isolation of total RNA from plant and seed samples, including those high in polyphenols and polysaccharides	13500-50
Related products		
DNase Max Kit (384)	For 384 preps: Removal of genomic DNA contamination in RNA preparations using a high-activity DNase I enzyme and a highly specific DNase removal resin	15200-50
DNeasy® PowerPlant Pro Kit (50)	For 50 preps: Isolation of genomic DNA from plant and seed samples, removes polyphenolics and polysaccharides	13400-50
DNeasy PowerPlant Pro HTP 96 Kit (384)	384 preps: High-throughput isolation of genomic DNA from plant and seed samples	13496-4
Vortex Adapter	For vortexing 1.7 ml or 2 ml tubes using the Vortex-Genie 2 Vortex	13000-V1-24
PowerLyzer 24 Homogenizer	For complete lysis and homogenization of any biological sample	13155

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Notes

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