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RNeasy® PowerLyzer® Tissue & Cells Kit Handbook

For the isolation of total RNA from animal tissues or cells, optimized for use with bead-based homogenizers

Sample to Insight

QIAGEN

Contents

Kit Contents

RNeasy PowerLyzer Tissue & Cells Kit	**(50)**
Catalog no.	**15055-50**
Number of preps	**50**
PowerBead Tubes, Ceramic 2.8 mm	50
MB RNA Spin Columns	50
Solution TR1	33 ml
Solution TR2	30 ml
Solution WB	28 ml
Solution RW	2 x 28 ml
RNase-Free Water	10 ml
Collection Tubes (2 ml)	3 x 50
Quick Start Protocol	1

Storage

The RNeasy PowerLyzer Tissue & Cells Kit reagents and components 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 TR2 and Solution RW are flammable.**

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

Solution TR1 and Solution WB 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 PowerLyzer Tissue & Cells Kits is tested against predetermined specifications to ensure consistent product quality.

Introduction

The RNeasy PowerLyzer Tissue & Cells Kit provides a way to purify RNA from up to 25 mg of tissue or 1 x 10^7 cultured cells in a fraction of the time required by traditional polytron homogenization. This kit minimizes the potential for cross-contamination between samples and enables up to 24 samples to be homogenized simultaneously. The kit contains PowerBead Tubes with 2.8 mm ceramic beads that are optimized for quick and efficient lysis using robust bead-based homogenizers such as the PowerLyzer 24 Homogenizer as well as FastPrep® and Precellys® instruments.

Principle and procedure

Samples are first homogenized via bead-based homogenizers, such as the PowerLyzer 24 Homogenizer, in specialized bead tubes with 2.8 mm ceramic beads and lysis buffer that provides optimal RNA release without degradation. The lysate is then combined with ethanol to form optimal binding conditions for capture on a silica membrane using a spin-column format. Wash buffers remove protein, genomic DNA and salts. The RNA is then eluted using RNase-Free Water. The result is pure and ready-to-use RNA.

Silica spin-column technology provides a robust and fast way to purify nucleic acids without the use of organic solvents or cesium chloride gradients.

To remove genomic DNA contamination using a high-activity DNase I enzyme and a highly specific DNase removal resin, we recommend the DNase Max® Kit (cat. no. 15200-50). Refer to Appendix B: Additional Protocols for how to use the DNase Max Kit.

Optimized for homogenization with the PowerLyzer 24 Homogenizer

The RNeasy PowerLyzer Tissue & Cells Kit contains PowerBead Tubes with 2.8 mm ceramic beads, which allows for more options in choosing homogenization methods, including the use of the PowerLyzer 24 Homogenizer. The PowerLyzer's velocity and proprietary motion combine to provide the fastest homogenization possible, minimizing time spent processing samples.

Using the RNeasy PowerLyzer Tissue & Cells Kit with other homogenizers

To isolate RNA using the RNeasy PowerLyzer Tissue & Cells Kit with FastPrep or Precellys homogenizers, use the conversion chart (see Table 1 below) 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 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.

Table 1. Conversion chart for using other homogenizers with the RNeasy PowerLyzer Tissue & Cells Kit

PowerLyzer 24 (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.

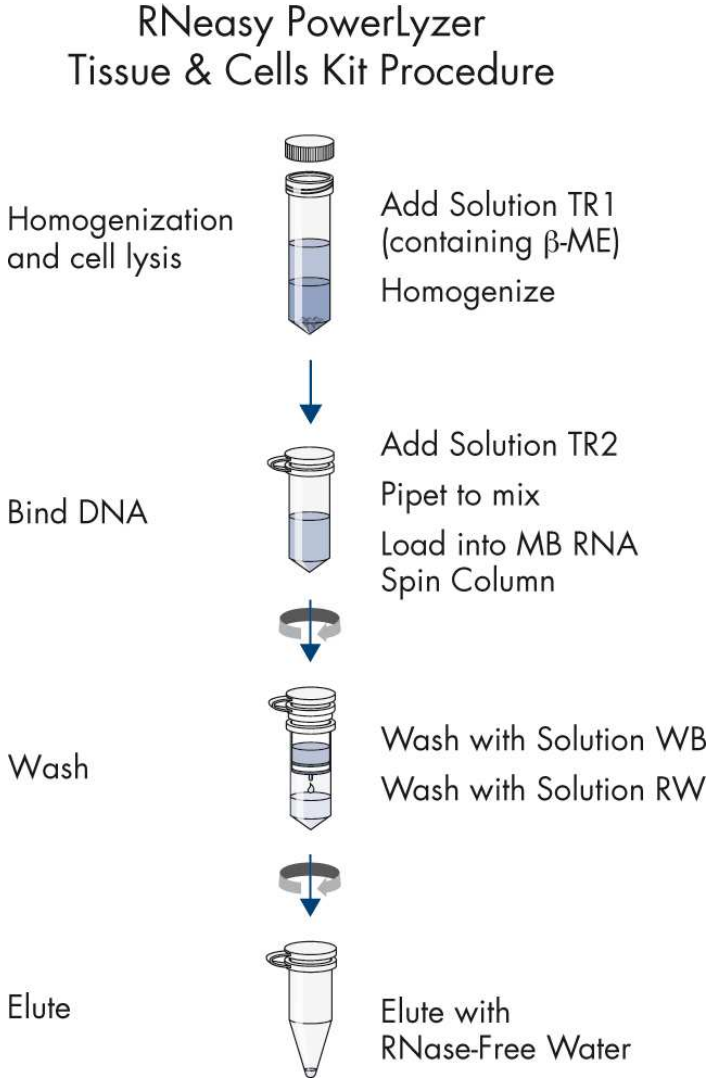

Figure 1. RNeasy PowerLyzer Tissue & Cells Kit procedure.

Equipment and Reagents to Be Supplied by User

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

- Microcentrifuge (13,000 x *g*)
- PowerLyzer 24 Homogenizer or another bead homogenizer
- Pipettor (5–600 µl)
- β-mercaptoethanol
- Rotor-stator or Polytron homogenizer (if using for homogenization step)
- Mortar and pestle (if using for homogenization step)
- Liquid nitrogen (if using for homogenization step)
- Phosphate-buffered saline (if using for homogenization step)

Important Notes Before Starting

PowerBead Tube identification

The high energies of the PowerLyzer 24 Homogenizer may affect labels on the caps of the PowerBead Tubes. Therefore, we recommend that you mark the sides of the PowerBead Tubes as well as the caps to ensure proper sample identification.

Homogenization methods

The RNeasy PowerLyzer Tissue & Cells Kit is optimized for use with the PowerLyzer 24 Homogenizer. The optimal setting is two cycles of 45 seconds at 3500 rpm with a 30-second dwell or pause in between cycles. Samples can be kept chilled in a bench-top cooler or on ice before homogenization to protect the RNA integrity while processing.

Amount of Solution TR1 to use

Table 2. Amount of Solution TR1 to use for the RNeasy PowerLyzer Tissue & Cells Kit

Amount of starting material	Volume of Solution TR1 (µl)
Tissue ≤ 12 mg	300
Tissue > 12 mg and up to 25 mg	600
Cells ≤ 5 x 10^6	300
Cells = 5 x 10^6 to 1 x 10^7	600

For spleen and thymus, do not use more than 10 mg of tissue per 300 µl of Solution TR1 to help decrease genomic DNA contamination. For liver samples, do not exceed 20 mg of tissue or you may exceed the binding capacity of the column. Up to 100 µg of RNA may be recovered from a single column.

For spleen, thymus and tissue culture cells, we recommend the DNase Max Kit (cat. no. 15200-50) to remove genomic DNA contamination.

Protocol: Experienced User

Important points before starting

- Warm Solution TR1 to 37°C for 5–10 minutes to dissolve any precipitate.
- Prepare Solution TR1 by adding 10 µl β-mercaptoethanol (β-ME) for every 1 ml of Solution TR1 for each sample to be processed. Solution TR1/β-ME should be prepared fresh each time according to the number of samples being processed.
- Perform all steps at room temperature (15–25°C). Use a standard microcentrifuge. If using a refrigerated centrifuge, do not allow the centrifuge to cool.
- Homogenized samples in Solution TR1 may be stored at –65 to –90°C for up to 2 months until ready to use.

Procedure

1. Properly label each PowerBead Tube on both the cap and on the side.
2. Homogenize tissue samples following ONLY one of the methods (a–d) described below. For alternative homogenization methods, please contact QIAGEN Technical Services.

a) PowerLyzer 24 or other bead beater

1) In a PowerBead Tube, Ceramic 2.8 mm (provided), add 300 or 600 µl of Solution TR1/β-ME, according to Table 2 (page 10) in the Handbook. Chill the tubes on ice or in a cooling block.
2) Weigh and add tissues to the PowerBead Tube. Keep chilled until ready to homogenize.
3) Place PowerBead Tubes into the tube holder of the PowerLyzer 24 Homogenizer. The tubes must be balanced in the tube holder. Homogenize the tissue for 2 cycles at 3500 rpm for 45 s each with 30 s dwells between cycles.
4) Centrifuge the PowerBead Tubes containing the tissue lysate at 13,000 x *g* for 1 min. Transfer the lysate to a new 2 ml Collection Tube (provided)

b) Rotor-stator or Polytron homogenizer

1) Weigh the tissues and place into a vessel aptly sized for your homogenizer.

2) Add 300 or 600 µl of Solution TR1/β-ME, according to Table 2 (page 10) in the handbook.
3) Homogenize for 30–40 s until the tissue is completely liquefied and no visible particulates remain.
4) Transfer the lysate to a new 2 ml Collection Tube (provided).

c) Liquid nitrogen and mortar and pestle

1) Weigh the tissues and place into the pre-chilled mortar.
2) Add liquid nitrogen and homogenize the tissue to a fine powder.
3) Resuspend powdered tissue with 300 or 600 µl of Solution TR1/β-ME, according to Table 2 (page 10) in the Handbook. Transfer to a 2 ml Collection Tube (provided).
4) Shear genomic DNA using a 20-gauge needle on a 1 cc syringe by moving the lysate in and out of the syringe at least 10 times or until the sample loses viscosity.

d) Homogenization of cells

1) Collect cells from culture medium and perform a cell count to determine the correct volume of Solution TR1 to use.
2) Pellet cells at 2000 x *g* for 5 min. Wash cells once with phosphate buffered saline to remove the culture medium. Pellet the cells again at 2000 x *g* for 5 min.
3) Add 300 or 600 µl of Solution TR1/β-ME, according to Table 2 (page 10) in the handbook, and transfer sample to a 2 ml Collection Tube (provided).
4) Vortex for 2 min to resuspend cells. No visible cell debris should remain.

3. Add 1 equal volume (300 or 600 µl) of Solution TR2 to the lysate. Mix by pipetting.
4. Transfer 600 µl of lysate to an MB RNA Spin Column. Centrifuge at ≥10,000 x *g* for 1 min. Discard flow through and place the MB RNA Spin Column back into the 2 ml Collection Tube. If you used 600 µl each of Solutions TR1 and TR2, repeat this step.
5. Wash the MB RNA Spin Column with 500 µl of Solution WB. Centrifuge for 1 min at ≥ 10,000 x *g*. Transfer the Spin Filter to a new 2 ml Collection Tube (provided).
6. Wash the MB RNA Spin Column with 500 µl of Solution RW. Centrifuge for 1 min at ≥ 10,000 x *g*. Discard flow through. Place Spin Filter back into the 2 ml Collection Tube.
7. Repeat step 6.

8. Centrifuge the MB RNA Spin Column for 2 min at 13,000 x *g* to dry the membrane. Transfer the MB RNA Spin Column to a new 2 ml Collection Tube (provided).
9. Add 50–100 µl of RNase-Free Water directly onto the MB RNA Spin Column membrane. Incubate for 1 min at room temperature. Centrifuge for 1 min at ≥ 10,000 x *g*. The RNA is now ready for downstream applications and can be stored at –65 to –90°C.

Protocol: Detailed

Important point before starting

- Warm Solution TR1 to 37°C for 5–10 minutes to dissolve any precipitate.
- Prepare Solution TR1 by adding 10 µl β-mercaptoethanol (β-ME) for every 1 ml of Solution TR1 for each sample to be processed. Solution TR1/β-ME should be prepared fresh each time according to the number of samples being processed.
- Perform all steps at room temperature (15–25°C). Use a standard microcentrifuge. If using a refrigerated centrifuge, do not allow the centrifuge to cool.
- Homogenized samples in Solution TR1 may be stored at –65 to –90°C for up to 2 months until ready to use.

Procedure

1. Properly label each PowerBead Tube on both the cap and on the side.
2. Homogenize tissue samples following ONLY one of the methods (a–d) described below. For alternative homogenization methods, please contact QIAGEN Technical Services.
   Note: The matrix of the tissue samples is dissolved into single cells that are then lysed by Solution TR1, which releases the cellular RNA. Genomic DNA is sheared into small sizes to enhance removal from the MB RNA Spin Column filter membrane.

   a) PowerLyzer 24 or other bead beater

   1) In a PowerBead Tube, Ceramic 2.8 mm (provided), add 300 or 600 µl of Solution TR1/β-ME, according to Table 2 (page 10) in the handbook. Chill the tubes on ice or in a cooling block.
   2) Weigh and add tissues to the PowerBead Tube. Keep chilled until ready to homogenize.
   3) Place PowerBead Tubes into the tube holder of the PowerLyzer 24 Homogenizer. The tubes must be balanced in the tube holder. Homogenize the tissue for 2 cycles at 3500 rpm for 45 s each with 30 s dwells between cycles.
   4) Centrifuge the PowerBead Tubes containing the tissue lysate at 13,000 x *g* for 1 min. Transfer the lysate to a new 2 ml Collection Tube (provided)

b) Rotor-stator or Polytron homogenizer

1) Weigh the tissues and place into a vessel aptly sized for your homogenizer.
2) Add 300 or 600 µl of Solution TR1/β-ME, according to Table 2 (page 10) in the handbook.
3) Homogenize for 30–40 s until the tissue is completely liquefied and no visible particulates remain.
4) Transfer the lysate to a new 2 ml Collection Tube (provided).

c) Liquid nitrogen and mortar and pestle

1) Weigh the tissues and place into the pre-chilled mortar.
2) Add liquid nitrogen and homogenize the tissue to a fine powder.
3) Resuspend powdered tissue with 300 or 600 µl of Solution TR1/β-ME, according to Table 2 (page 10) in the handbook. Transfer to a 2 ml Collection Tube (provided).
4) Shear genomic DNA using a 20-gauge needle on a 1 cc syringe by moving the lysate in and out of the syringe at least 10 times or until the sample loses viscosity.

d) Homogenization of cells

1) Collect cells from culture medium and perform a cell count to determine the correct volume of Solution TR1 to use.
2) Pellet cells at 2000 x *g* for 5 min. Wash cells once with phosphate buffered saline to remove the culture medium. Pellet the cells again at 2000 x *g* for 5 min.
3) Add 300 or 600 µl of Solution TR1/β-ME, according to Table 2 (page 10) in the handbook, and transfer sample to a 2 ml Collection Tube (provided).
4) Vortex for 2 min to resuspend cells. No visible cell debris should remain.

3. Add 1 equal volume (300 or 600 µl) of Solution TR2 to the lysate. Mix by pipetting.
   Note: Solution TR2 is 70% ethanol and prepares optimal binding conditions for RNA capture on the MB RNA Spin Column filter membrane.
4. Transfer 600 µl of lysate to an MB RNA Spin Column. Centrifuge at ≥10,000 x *g* for 1 min. Discard flow-through and place the MB RNA Spin Column back into the 2 ml Collection Tube. If you used 600 µl each of Solutions TR1 and TR2, repeat this step.
   Note: The RNA binds to the MB RNA Spin Column filter membrane.

5. Wash the MB RNA Spin Column with 500 µl of Solution WB. Centrifuge for 1 min at ≥ 10,000 x *g*. Transfer the Spin Filter to a new 2 ml Collection Tube (provided).
   Note: Solution WB removes proteins from the MB RNA Spin Column filter membrane.
   Note: The DNase Max Kit may be used after this step to remove genomic DNA that may be contaminating the RNA in the MB RNA Spin Column filter membrane.
6. Wash the MB RNA Spin Column with 500 µl of Solution RW. Centrifuge for 1 min at ≥ 10,000 x *g*. Discard flow through. Place Spin Filter back into the 2 ml Collection Tube (provided).
   Note: Solution RW contains ethanol and removes salts that are present in Solution WB.
7. Repeat step 6.
8. Centrifuge the MB RNA Spin Column for 2 min at 13,000 x *g* to dry the membrane. Transfer the MB RNA Spin Column to a new 2 ml Collection Tube (provided).
   Note: This step dries the MB RNA Spin Column filter membrane completely so that the RNA may be released efficiently during the elution step with RNase-Free Water.
9. Add 50–100 µl of RNase-Free Water directly onto the MB RNA Spin Column membrane. Incubate for 1 min at room temperature. Centrifuge for 1 min at ≥ 10,000 x *g*. The RNA is now ready for downstream applications and can be stored at –65 to –90°C.
   Note: Either 50 µl or 100 µl of RNase-Free Water will work for the elution step. Using 50 µl yields more concentrated RNA while using 100 µl yields slightly higher amounts. The eluted RNA is ready to be used in enzymatic applications, or it can be stored until needed.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving 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 a well when loading a gel	This usually occurs because Solution RW, which contains ethanol, remains in the final sample. Avoid transferring any Solution RW to the elution step. To ensure complete drying of the MB RNA Spin Column membrane after washing with Solution RW, centrifuge the column in a clean 2 ml Collection Tube for an additional minute after the final wash step. 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 with drying of the membrane. Increase the centrifugation times by another minute.
b)	RNA has low $A_{260/280}$ ratio	The $A_{260/280}$ ratio for pure RNA should be 1.9–2.1. An $A_{260/280}$ ratio below 1.6 may have significant protein contamination. A low $A_{260/280}$ ratio can also occur when the sample is measured in water. The low pH of water can influence the A_{280} reading and cause reduced sensitivity to protein contamination. Re-measure the $A_{260/280}$ after diluting the RNA in 10 mM Tris, pH 7.5. Low $A_{260/280}$ ratios also may occur if excess tissue was used or not enough Solution TR1 was used for homogenization. Do not exceed 25 mg of tissue and use enough Solution TR1 to ensure complete lysis of the sample. Make sure to perform the wash step using Solution WB.
c)	DNA contamination of RNA	To remove genomic DNA from RNA preps, we recommend the DNase Max Kit (cat. no. 15200-50). The DNase Max Kit uses a high-velocity DNase enzyme, which is efficiently removed post digestion using a resin that inactivates and binds to the enzyme. RNA is purified from DNA without the use of inhibitors (EDTA) or heat and is ready to use.

		Comments and suggestions
d)	Low yields or RNA degradation	Expected RNA yields for a variety of mouse tissues are shown in Table 3 (page 19). If the yields or integrity of RNA obtained are lower than expected, the following reasons may apply: • Homogenization of the sample to release RNA is critical. For best results, use the PowerLyzer 24 Homogenizer. Ensure that the sample is completely homogeneous. • Do not use more than 25 mg of tissue per prep to avoid overloading the capacity of the MB RNA Spin Column. That can cause clogging and lead to lower RNA yields and purity. • Tissue samples must be stored at –65 to –90°C or in liquid nitrogen immediately upon collection to preserve the RNA. The use of RNA stabilizing reagents, such as RNA*later*™, is suitable for collection of samples at room temperature and subsequent storage at –15 to –30°C. • Homogenization should be performed quickly upon removal of tissue samples from storage or from RNA stabilizing reagents. • For samples that are difficult to homogenize with standard methods (e.g., muscle, skin or cartilage), call QIAGEN Technical Services for alternative protocols. • RNA will not always run correctly on non-denaturing gels and may show smearing due to secondary structure. Run RNA on a denaturing gel according to instructions in Appendix C: Protocol for Formaldehyde Gel Electrophoresis. Alternatively, to visualize RNA on a non-denaturing agarose gel, heat denature the RNA prior to loading by performing the steps for "RNA Sample Preparation" in Appendix C: Protocol for Formaldehyde Gel Electrophoresis. • RNA should only be heated in water or in a loading buffer that contains EDTA to chelate divalent cations that can cause hydrolysis of RNA. • The $A_{260/280}$ ratio is a good indicator of RNA quality. The A_{260} value will increase as RNA is digested into smaller fragments and single nucleotides. An $A_{260/280}$ ratio above 2.3 may indicate RNA degradation. • If you are using the Agilent® BioAnalyzer® to visualize RNA, make sure to perform the heat denaturation step prior to loading samples into the chip to obtain accurate RNA profiles.
e)	Concentrating eluted RNA	The final volume of eluted RNA will be 50–100 µl. The RNA may be concentrated by adding 5–10 µl of 3 M NaCl and inverting 3–5 times to mix. Next, add 250 µl of 100% cold ethanol and invert 3–5 times to mix. Incubate at –15 to –30°C for 20 minutes and centrifuge at 16,000 x *g* for 20 minutes at room temperature. Decant all liquid. Remove all residual ethanol in a speed vac, dessicator or ambient air. Resuspend precipitated RNA in desired volume of RNase-Free water.
f)	Storing RNA	RNA is eluted in RNase-Free Water (provided) and must be stored at –65 to –90°C to prevent degradation. RNA can be precipitated in ethanol and stored at –15 to –30°C to ensure minimal degradation during long term storage.

Appendix A: Expected RNA Yields

Average yields of RNA for a variety of mouse tissues is provided in Table 3. Yields may vary based on the age of the animal, method of tissue storage, and the growth stage of cells in culture.

Liver tissues contain the highest yields of RNA and spleen and thymus contain highest levels of genomic DNA. If you are working with a tissue for the first time and are unsure of the yields, start with 10 mg of sample. For liver samples, do not exceed 20 mg of tissue or you may exceed the column binding capacity. Up to 100 µg of RNA may be recovered from a single column. For spleen, thymus and tissue culture cells, we strongly recommend using the DNase Max Kit (cat. no. 15200-50) to remove genomic DNA.

Table 3. Average RNA yields for a variety of mouse tissues

Tissue (10 mg)	Average RNA yield (µg)
Liver	40–60
Spleen	30–60
Kidney	20–30
Lung	5–15
Heart	5–10
Muscle	10–30
Brain	5–20
Cells	Varies

Note: Samples were stored in RNA*later* stabilization reagent at –15 to –30°C.

Appendix B: Additional Protocols

Protocol for RNA cleanup after using TRIzol™ or TRI Reagent®

1. In a PowerBead Tube, Ceramic 2.8 mm weigh up to 100 mg of tissue and add 1 ml of TRIzol or TRI Reagent.
2. Homogenize the tissue sample using a PowerLyzer 24 Homogenizer for 2 cycles at 45 s at setting 3500 rpm with a 30 second dwell between cycles.
3. Allow the sample to sit at room temperature for 5 min to allow for dissociation of nucleoprotein complexes.
4. Add 200 µl of chloroform per 1 ml TRIzol Reagent. Cap the tubes and shake vigorously for 20 s.
5. Incubate at room temperature for 2–3 min.
6. Centrifuge the tubes at 12,000 x *g* for 15 min at 2–8°C to separate the phases.
   Note: The mixture will separate into a lower red phase containing the phenol-chloroform, an interphase, and an upper aqueous phase containing the RNA.
7. Collect the upper aqueous phase (approximately 600 µl) and transfer to a new tube.
8. Add one volume of Solution TR2. Mix by vortexing.
9. Transfer 600 µl of lysate onto the MB RNA Spin Column. Centrifuge at ≥ 10,000 x *g* for 1 min. Discard the flow-through and place the MB RNA Spin Column back into the 2 ml Collection Tube. Repeat with the rest of the lysate and centrifuge at ≥ 10,000 x *g* for 1 min.
10. Discard the flow-through. Place the MB RNA Spin Column back into the same 2 ml Collection Tube.
11. Wash the MB RNA Spin Column with 500 µl of Solution WB. Centrifuge for 1 min at ≥ 10,000 x g. Transfer the MB RNA Spin Column to a new 2 ml Collection Tube.
    Note: The DNase Max Kit may be used after this step to remove genomic DNA that may be contaminating the RNA in the MB RNA Spin Column filter membrane.

12. Wash the Spin Filter with 500 µl of Solution RW. Centrifuge at ≥ 10,000 x *g* for 1 min. Discard the flow-through and place the MB RNA Spin Column back into the same 2 ml Collection Tube.
13. Repeat step 12.
14. Centrifuge the Spin Filter in the empty 2 ml Collection Tube for 2 minutes at 13,000 x *g*. Transfer the Spin Filter to a new 2 ml Collection Tube.
15. To elute the RNA, add 50–100 µl of RNase-Free Water directly onto the MB RNA Spin Column filter membrane and allow it to incubate for 1 min at room temperature. Then centrifuge for 1 min at ≥ 10,000 x *g*.
    Note: The RNA may be stored at –65 to –90°C.

Protocol for RNA cleanup and concentration using already-purified RNA

1. Resuspend the RNA in 100 µl of RNase-Free Water.
2. Add 350 µl of Solution TR1 and mix with pipetting.
3. Add 250 µl of Solution TR2 and mix by pipetting.
4. Load all 700 µl of the sample onto a MB RNA Spin Column and centrifuge for 1 min at 10,000 x *g*. Discard the flow-through and place the MB RNA Spin Column back into the same 2 ml Collection Tube.
5. Add 500 µl of Solution RW and centrifuge at 10,000 x *g* for 1 minute to wash the membrane. Discard the flow-through and place the MB RNA Spin Column back into the 2 ml Collection Tube.
   Note: Solution WB is not required for RNA cleanup.
6. Repeat step 5.
7. Centrifuge to dry the membrane for 2 min at 13,000 x *g*.
8. Transfer the MB RNA Spin Column to a new 2 ml Collection Tube and elute with 50–100 µl of RNase-Free Water.

Protocol to remove genomic DNA contamination using the DNase Max Kit

Notes before starting

- DO NOT VORTEX the DNase I. It will denature the enzyme and decrease its activity.
- Just before use, resuspend the DNase Removal Resin by inverting or vortexing until the slurry is homogeneous.

DNase Reaction

1. Mix 1 µl of DNase I enzyme (10 units) and enough 10x DNase Buffer to achieve a final concentration of 1x DNase Buffer in the digestion reaction.
   Examples: For 50 µl digestion reactions, use 1 µl of DNase I enzyme and 5 µl of 10x DNase Buffer. For 100 µl reactions, use1 µl DNase I enzyme and 10 µl of 10x DNase Buffer.
2. Bring the reaction to final volume using RNase-Free water (provided). Mix by pipetting up and down.
3. Incubate at 37°C for 20 min.

DNase Removal

4. Add 5 µl of homogeneous DNase Removal Resin per 10 units of DNase I for a 50 µl reaction, or 10 µl of DNase Removal Resin for every 100 µl reaction, whichever is greater.
5. Incubate for 10 min at room temperature. Invert or flick to resuspend every 1–2 min or place the tubes on a Vortex Adapter (cat. no. 13000-V1) attached to a Vortex Genie® 2 and set the vortex between speed 5–6 to agitate the resin and promote binding of the DNase.
   Note: The solution should agitate without splashing.
6. Centrifuge at 13,000 x *g* for 1 min to pellet the resin.

Transfer the supernatant to a new tube, taking care not to transfer any of the resin. The RNA is now ready to use for RT-PCR and further analysis.

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 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 PowerLyzer Tissue & Cells Kit (50)	For 50 preps: Isolation of total RNA from animal tissues or cells, optimized for use with bead-based homogenizers	15055-50
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
RNeasy PowerClean Pro Cleanup Kit (50)	For 50 preps: Remove PCR inhibitors from purified RNA in just 7 minutes	13997-50
RNeasy PowerSoil® Total RNA Kit (50)	For 25 preps: Isolate high-quality total RNA from all soil types	12866-25
RNeasy PowerPlant® Kit (50)	For 50 preps: Isolate total RNA from plant and seed samples, including those high in polyphenols and polysaccharides	13500-50
RNeasy PowerWater® Kit (50)	For 50 preps: Isolate total RNA from filtered water samples, including turbid water	14700-50-NF
PowerLyzer 24 Homogenizer	For complete lysis and homogenization of any biological sample	13155

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Notes

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