PowerLyzer® UltraClean® Tissue & Cells RNA Isolation Kit

Catalog No.	Quantity
15055-50	50 Preps

Instruction Manual

PowerLyzer® Products

PowerLyzer® DNA and RNA Isolation kits combine either, glass, ceramic or metal bead tubes with MO BIO's trusted chemistry as an alternative to our traditional kits utilizing Garnet Bead Tubes for sample homogenization. These materials are much harder then garnet and when used with the PowerLyzer® 24 Bench Top Bead-Based Homogenizer or other bead beater, offer more robust mechanical shaking. Optimal lysis conditions will vary with each sample type. By providing more versatility for lysis, MO BIO's PowerLyzer® kits are a powerful tool in obtaining higher yields of DNA or RNA from some spores, yeast and fungi as well as some Gram positive strains of bacteria from a wide range of sample types. All PowerLyzer® DNA and RNA Isolation kits contain either glass or ceramic beads and are compatible with the PowerLyzer® 24 instrument.

Please Recycle

Version: 11132015

Table of Contents

Introduction

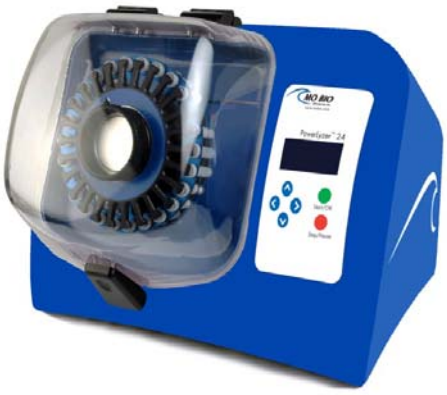
The PowerLyzer® UltraClean® Tissue & Cells RNA Isolation Kit differs from the original UltraClean® kit. This kit contains bead tubes with 2.8 mm ceramic beads that are optimized for quick and efficient lysis using robust bead based homogenizers like the PowerLyzer® 24 as well as Fast Prep® and Precellys® instruments. The PowerLyzer® UltraClean® Tissue & Cells RNA Isolation 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, minimizes the potential for cross-contamination between samples and enables up to 24 samples to be homogenized simultaneously. Typical yields from a variety of sources are listed in table 1.

Protocol Overview

UltraClean® silica spin column products utilize the novel MO BIO Laboratories flat bottom spin column design, which provides improved sample processing and yields. The bucket configuration allows for enhanced sample flow and membrane drying after wash steps since the entire membrane is accessible to air flow. Silica technology provides a robust and fast way to purify nucleic acids without the use of organic solvents or cesium chloride gradients.

With the PowerLyzer® UltraClean® Tissue & Cells RNA Isolation Kit, samples are first homogenized via bead based homogenizers such as the PowerLyzer® 24, in a specialized bead tube including 2.8 mm ceramic beads and lysis buffer that provides optimal RNA release without degradation. The lysate is next combined with ethanol to form the optimal binding conditions for spin filter membrane purification. The wash buffers remove protein, genomic DNA, and salts so that the final RNA is pure and ready to use. **Note: For complete on-column removal of genomic DNA, try our new On-Spin Column DNase I Kit (MO BIO Catalog# 15100-50).**

Optimized for Complete homogenization of any sample with the



PowerLyzer® 24
Bench Top Bead-Based Homogenizer
Cat#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 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 bead tubes prefilled with 2.8 mm ceramic beads to homogenize 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 PowerLyzer® UltraClean® Tissue & Cells 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, we recommend that for RNA from tissues you begin with the settings specified in this manual of 2 cycles at 45 seconds at setting 3500 RPM with a 30 second dwell between cycles.

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.

Expected Yields of RNA

Average yields of RNA for a variety of mouse tissues examined can be found in Table 1. 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. 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, genomic DNA removal using the On-Spin Column DNase I Kit (MO BIO Catalog# 15100-50) is strongly recommended.

Table 1.
Average yields of RNA from a variety of mouse tissues*

Tissue (10 mg)	Average yields (µg)
Liver	40-60
Spleen	30-60
Kidney	20-30
Lung	5-15
Heart	5-10
Muscle	10-30
Brain	5-20
Cells	varies

**Samples were stored in RNALater™ RNA Stabilization Reagent at -20°C*

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

Other Related Products	Catalog No.	Quantity
RNase-Free Gloves	1556-XS 1556-S 1556-M 1556-L	Bag of 150 Bag of 150 Bag of 150 Bag of 150
On-Spin Column DNase I Kit (RNase-Free)	15100-50	50 preps
UltraClean® Tissue & Cells DNA Isolation Kit	12334-50 12334-250	50 preps 250 preps
Ceramic Bead Tubes, 2.8 mm	13114-50	50 bead tubes
BiOstic® Paraffin Removal Reagent	12251-50	2 x 25 ml
PowerLyzer® Tube Holder	13156	1 unit
PowerLyzer® Tube Holder Stand	13157	1 unit

PowerLyzer® UltraClean® Tissue & Cells RNA Isolation Kit

Homogenization

- Add Solution TR1 (containing β-ME)
- Homogenize

Bind RNA

- Add Solution TR2
- Mix by pipetting
- Load into Spin Filter

Centrifuge

Wash

- Wash with Solution TR3
- Wash with Solution TR4

Centrifuge

Elute

- Elute with Solution TR5

Equipment and Reagents Required but Not Included in this Kit (for all Protocols)

PowerLyzer® 24 or other bead homogenizer
Microcentrifuge (13,000 x *g*)
Pipettors (5 – 600 µl)
β- mercaptoethanol

Sample Disruption and Homogenization for RNA Purification from Animal Tissues

Page 9 (step 2 of the protocol) describes methods and equipment that are suitable for tissue extraction. Depending on which method you choose, the following may be required.

- PowerLyzer® 24 (MO BIO Catalog# 13155)
- Rotor-stator or Polytron homogenizer
- Mortar and Pestle
- Liquid Nitrogen

For RNA from Animal Cells

Phosphate buffered saline and trypsin

Not Required but Recommended for Complete gDNA Removal

On-Spin Column DNase I Kit (MO BIO Catalog# 15100-50)

Kit Contents

	Kit Catalog #15055-50	
Components	**Catalog #**	**Amount**
PowerLyzer® Ceramic Bead Tubes, 2.8 mm	13114-50-CBT	50 tubes
Solution TR1	15000-50-1	33 ml
Solution TR2	15000-50-2	30 ml
Solution TR3	15000-50-3	28 ml
Solution TR4	15000-50-4	2 x 28 ml
Solution TR5	15000-50-5	8 ml
Spin Filters	15000-50-SF	50
2 ml Collection Tubes	15000-50-T	150

Kit Storage

Kit reagents and components should be stored 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: Solutions TR2 and TR4 are flammable.

Important Notes Before Starting

Information

- Solution TR1 may form a precipitate. Warm to 37°C for 5-10 minutes to dissolve.
- Prepare Solution TR1 by adding β- mercaptoethanol (βME)
  - Add 10 µl of β- mercaptoethanol (βME) for every 1 ml of the Solution TR1 for all samples to be processed.

    Note: Prepare Solution TR1 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.

    Note: βME will be stable in lysis buffer up to one month. You may add fresh βME to the lysis buffer if necessary.
- Perform all steps at room temperature (15-30°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 -80°C for up to 2 months until ready to use.

Table 2. Use the amount of Solution TR1 lysis buffer as specified in the table below:

Amount of starting material	Volume of Solution TR1
Tissue ≤ 12 mg	300 µl
Tissue > 12 mg - 25 mg	600 µl
Cells ≤ 5×10^6	300 µl
Cells 5×10^6- 1×10^7	600 µl

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 column binding capacity. Up to 100 µg of RNA may be recovered from a single column. For spleen, thymus and tissue culture cells, genomic DNA removal using the On-Spin Column DNase I Kit (MO BIO Catalog# 15100-50) is strongly recommended.

Bead Tube Identification

Due to the high energies of the PowerLyzer® 24, the potential of marring of the tops of the caps is possible, therefore it is recommended to mark the sides of the Ceramic Bead Tubes as well as the caps to ensure proper sample identification.

Homogenization Methods

The PowerLyzer® UltraClean® Tissue & Cells RNA Isolation Kit is optimized using the PowerLyzer® 24 bead beater as the homogenizer. The optimal setting is two cycles of 45 seconds at 3500 RPM with a 30 second dwell 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.

Experienced User Protocol

Please wear certified RNase-Free gloves (Catalog#1556) at all times.

Note: See Important Notes Before Starting section on the previous page for preparation of Solution TR1.

1. Properly identify each Ceramic Bead Tube on both the cap and on the side; See "**Important Notes Before Starting**" for more information.

2. Homogenize tissue samples following ONLY one of the methods a-d described below. For alternative homogenization methods contact MO BIO Laboratories Technical Services (technical@mobio.com).

   a) PowerLyzer® 24 or other bead beater

   In a PowerLyzer® Ceramic Bead Tube, 2.8 mm (provided), add either **300 µl or 600 µl of Solution TR1** containing βME, according to Table 2. Chill the tubes on ice or in a cooling block.

   1) Weigh the tissues and add them to the Bead Tube. Keep chilled until ready to homogenize.
   2) Place Bead Tubes into the Tube Holder of the PowerLyzer® 24. The tubes must be balanced (evenly spaced) on the Tube Holder. Homogenize the tissue for 2 cycles at speed 3500 rpm for 45 seconds each with a 30 second dwell between cycles.
   3) Centrifuge the Bead Tubes containing the tissue lysate at 13,000 x *g* for 1 minute to collect the lysate. Transfer the lysate to a new 2 ml Collection Tube (provided).

   b) Rotor-stator or Polytron homogenizer

   1) Weigh the tissues and place into an appropriate sized vessel for your homogenizer.
   2) Add **300 µl or 600 µl of Solution TR1** containing βME, to the vessel, according to Table 2.
   3) Homogenize for 30-40 seconds 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 the powdered tissue with **300 µl or 600 µl of Solution TR1** containing βME, according to Table 2, and 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 more 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 minutes and wash the cells once with phosphate buffered saline to remove the culture medium. Pellet the cells again at 2000 x *g* for 5 minutes.
   3) Add **300 µl or 600 µl of Solution TR1** containing βME, to the cell pellet according to Table 2 and transfer the sample to a 2 ml Collection Tube (provided).
   4) Vortex for 2 minutes to resuspend and homogenize the cells. No visible cell debris should remain.

3. Add **1 equal volume (300 µl or 600 µl) of Solution TR2** to the lysate and mix by pipetting.

4. Transfer 600 µl of lysate to the Spin Filter. Centrifuge for 1 minute at ≥10,000 x *g*. Discard the flow-through and place the Spin Filter back into the 2 ml Collection Tube. If you used 600 µl of Solution TR1 and Solution TR2, repeat with a second loading of lysate. Centrifuge for 1 minute at ≥ 10,000 x *g* and discard the flow-through. Place the Spin Filter back into the same 2 ml Collection Tube.
5. Wash the Spin Filter with **500 µl of Solution TR3**. Centrifuge for 1 minute at ≥ 10,000 x *g*. Transfer the Spin Filter to a new 2 ml Collection Tube (provided).

   Note: An optional on-column DNA removal procedure may be inserted after this step of the protocol. See page 13 for instructions.

6. Wash the Spin Filter with **500 µl of Solution TR4**. Centrifuge for 1 minute at ≥ 10,000 x *g*. Discard the flow-through and place the Spin Filter back into the same 2 ml Collection Tube.
7. Repeat step 6.
8. Centrifuge the Spin Filter in the empty 2 ml Collection Tube for 2 minutes at 13,000 x *g* to dry the membrane. Transfer the Spin Filter to a new 2 ml Collection Tube (provided).
9. To elute the RNA, add **50-100 µl of Solution TR5** directly onto the Spin Filter membrane and allow it to incubate for 1 minute at room temperature. Centrifuge for 1 minute at ≥ 10,000 x *g*.

   We recommend storing RNA frozen (-20°C or -80°C). RNase-Free Water contains no EDTA.

Thank you for choosing the PowerLyzer® UltraClean® Tissue & Cells RNA Isolation Kit.

Detailed Protocol (Describes what is happening at each step)

Please wear certified RNase-Free gloves (Catalog#1556) at all times

Note: See Important Notes Before Starting section for preparation of Solution TR1.

1. Properly identify each Ceramic Bead Tube on both the cap and on the side; See "**Important Notes Before Starting**" for more information.

2. Homogenization steps in **Solution TR1 (see steps 2 a – d on page 9)**

   What's happening: The matrix of the tissue is dissolved into single cells that are lysed in the Solution TR1 releasing the RNA. Genomic DNA is sheared into small sizes to enhance removal from the Spin Filter.

3. Add **1 equal volume (300 µl or 600 µl) of Solution TR2** to the lysate and mix by pipetting.

   What's happening: Add the same volume of TR2 that you added of TR1 in step 2. Solution TR2 is 70% ethanol and prepares optimal binding conditions for RNA capture on the Spin Filter membrane.

4. Transfer 600 µl of lysate onto the Spin Filter. Centrifuge for 1 minute at ≥ 10,000 x *g*. Discard the flow-through and place the Spin Filter back into the 2 ml Collection Tube. If you used 600 µl of Solution TR1 and Solution TR2, repeat with a second loading of lysate. Centrifuge for 1 minute at ≥ 10,000 x *g* and discard the flow-through. Place the Spin Filter back into the same 2 ml Collection Tube.

   What's happening: The RNA is bound to the Spin Filter by passing it through the membrane.

5. Wash the Spin Filter with **500 µl of Solution TR3**. Centrifuge for 1 minute at ≥ 10,000 x *g*. Transfer the Spin Filter to a new 2 ml Collection Tube (provided).

   What's happening: Solution TR3 is a wash buffer that removes protein from the column.

   Note: An optional on-column DNA removal procedure may be inserted after this step of the protocol. See page 13 for instructions

   What's happening: The On-Spin Column DNase I Kit will remove any remaining genomic DNA from the Spin Filter membrane that did not wash off in Solution TR3. Some tissues (ex. spleen and thymus) may have higher than normal amounts of genomic DNA and require DNase I digestion.

6. Wash the Spin Filter with **500 µl of Solution TR4**. Centrifuge for 1 minute at ≥ 10,000 x *g*. Discard the flow-through and place the Spin Filter back into the same 2 ml Collection Tube.

7. Repeat step 6.

   What's happening: Solution TR4 contains ethanol and removes the salts from the Solution TR3.

8. 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 (provided).

   What's happening: This dries the membrane completely so that the RNA may be released during the elution step with RNase-Free Water (Solution TR5).

9. To elute the RNA, add **50-100 µl of Solution TR5** directly onto the Spin Filter membrane and allow it to incubate for 1 minute at room temperature. Centrifuge for 1 minute at ≥ 10,000 x *g*. RNA is now ready to use or may be stored at -80°C or -20°C.

   What's happening: Either 50 or 100 µl will work for this RNA recovery step. Using 50 µl gives more concentrated RNA or using 100 µl gives slightly higher overall yields. RNA is eluted in RNase-Free Water (Solution TR5) and is ready to be used for enzymatic applications or can be stored until needed.

   We recommend storing RNA frozen (-20°C or -80°C). RNase-Free Water contains no EDTA.

Thank you for choosing the PowerLyzer® UltraClean® Tissue & Cells 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 and mix gently. 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 TR3 wash step (step 5), centrifuge for 2 minutes at 10,000 x *g* to thoroughly remove all traces of wash buffer from the Spin Filter membrane.
Note: This is step 11 on the protocol page 14 for using Tri Reagent® or TRIzol® reagent.

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 **45 µl of Solution D1 and 5 µl of DNase I).** Incubate at room temperature for 15 minutes.

C. To the Spin Filter column add **400 µl Solution D2** and centrifuge at 10,000 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 10,000 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 10,000 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 10,000 x *g* to completely dry the membrane. Continue with the elution (step 9) on page 10 of the **Experienced Users** protocol and page 12 of the **Detailed Protocol**.

B. Protocol for RNA Clean up after using TRIzol® or Tri Reagent®:

1. In a Ceramic Bead Tube, 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 the PowerLyzer® 24 for 2 cycles at 45 seconds at setting 3500 RPM with a 30 second dwell between cycles.
3. Allow the sample to sit at room temperature for 5 minutes 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 seconds.
5. Incubate at room temperature for 2-3 minutes.
6. Centrifuge the tubes at 12,000 x *g* for 15 minutes at 4°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 Spin Filter column. Centrifuge for 1 minute at ≥ 10,000 x *g*. Discard the flow-through and place the Spin Filter back into the 2 ml Collection Tube. Repeat with the rest of the lysate and centrifuge for 1 minute at ≥ 10,000 x *g*.
10. Discard the flow-through. Place the Spin Filter back into the same 2 ml Collection Tube.
11. Wash the Spin Filter with **500 µl of Solution TR3**. Centrifuge for 1 minute at ≥ 10,000 x *g*. Transfer the Spin Filter to a new 2 ml Collection Tube.

    Note: An optional on-column DNA removal procedure may be inserted after this step of the protocol. See page 13 for instructions

12. Wash the Spin Filter with **500 µl of Solution TR4**. Centrifuge for 1 minute at ≥ 10,000 x *g*. Discard the flow-through and place the Spin Filter 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 Solution TR5** directly onto the Spin Filter membrane and allow it to incubate for 1 minute at room temperature. Centrifuge for 1 minute at ≥ 10,000 x *g*. RNA is now ready to use or may be stored at -80°C or -20°C.

C. Protocol for RNA clean up and concentration using already purified RNA

1. Resuspend the RNA in a volume of 100 µl with 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 the 700 µl of sample onto a Spin Filter and centrifuge at 10,000 x *g* for 1 minute. Discard the flow-through and place the Spin Filter back into the same 2 ml Collection Tube.
5. Add 500 µl of Solution TR4 and centrifuge at 10,000 x *g* for 1 minute to wash the membrane. Discard the flow-through and place the Spin Filter back into the 2 ml Collection Tube.
   Note: The Solution TR3 step is not required for RNA Clean up
6. Repeat step 5.
7. Centrifuge to dry the membrane for 2 minutes at 13,000 x *g*.
8. Transfer the Spin Filter to a new 2 ml Collection Tube and elute with 50-100 µl of Solution TR5 (RNase-Free Water).

Hints and Troubleshooting Guide

Low Yields or RNA Degradation

Expected yields for a variety of mouse tissues are shown in Table 1. If the yields or integrity of RNA obtained are lower than expected, the following reasons may apply:

- Homogenization of the sample to release the RNA is critical. For best results, homogenize using the PowerLyzer® 24 homogenizer. The sample should be completely homogeneous after homogenization.
- Do not use more than 25 mg of tissue per prep to avoid overloading the capacity of the column and causing clogging that can lead to lower yields and purity.
- Tissue samples must be stored at -80°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 storage of samples at -20 °C.
- Homogenization should be performed quickly upon removal of the tissue from storage or from RNA*Later*™.
- For samples that are difficult to homogenize with standard methods (for example, muscle, skin, or cartilage), call technical service for an alternative protocol.
- RNA will not always run correctly on non-denaturing gels and may show smearing due to RNA secondary structure. Run RNA on a denaturing gel according to the "**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**" under the section below called "**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 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.
- If you are using the Agilent BioAnalyzer to visualize RNA, make sure to perform the heat denaturation step prior to loading the sample into the chip to obtain accurate RNA profiles.

RNA Floats Out of Well When Loaded on a Gel

Residual Solution TR4 may be in the final sample.

- To ensure complete drying of the membrane after Solution TR4, centrifuge the Spin Filter in a clean 2 ml Collection Tube for an additional minute after the final wash step. Ethanol precipitation is the best way to remove residual Solution TR4. (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 by another minute.

RNA Has Low 260/280 Ratio

The ratio for pure RNA should be 1.9-2.1. $A_{260/280}$ ratio below 1.6 may have significant protein contamination.

- A low ratio can occur when the sample is measured 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.
- Low 260/280 ratios may occur if excess tissue was used or not enough Solution TR1 was used for the homogenization. Do not exceed 25 mg of tissue and increase the amount of Solution TR1 to ensure complete lysis of the sample.
- Make sure to perform the Solution TR3 wash step.

Hints and Troubleshooting Guide cont.

*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 PowerLyzer® UltraClean® Tissue & Cells RNA Isolation Kit may be used with the On-Spin Column DNase I Kit for on-column genomic DNA removal. When used with the DNase I Buffer 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.
- The digest may be extended to 30 minutes if genomic DNA contamination is high. This will not hurt the RNA.
- Homogenization of genomic DNA reduces the contamination on the column. If you are using a bead beater other than the PowerLyzer® 24, you may need to optimize the number of cycles of homogenization that sufficiently homogenizes your tissue. Increase the time of the beating and try using 2-3 cycles of homogenization.
- If working with spleen or thymus tissue, decrease the amount of sample and increase the volume of Solution TR1. Always perform the DNase I digest step.

Concentrating the RNA

Your final volume will be 50 µl. If this is too dilute for your purposes, add 5 µl of 3 M NaAcetate and mix. Then add 150 µl 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.

Storing RNA

RNA is eluted in RNase-Free Water (Solution TR5) and should be used immediately or stored at -20°C or -80°C to avoid degradation. RNA can be precipitated in ethanol 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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Description	Catalog No.	Quantity
PowerLyzer® 24 homogenizer	13155	1 unit
UltraClean® Tissue & Cells RNA Isolation Kit	15000-50	50 preps
UltraClean® Tissue & Cells DNA Isolation Kit	12334-50 12334-250	50 preps 250 preps
RTS DNase Kit	15200-50	50 preps
On-Spin Column DNase I Kit (RNase-Free)	15100-50	50 preps
Dye Dots®	15020-10 15020-20	10 plates 20 plates