July 2018

RNeasy® Lipid Tissue Mini Handbook

For purification of total RNA from fatty tissues and all other types of tissue

Sample to Insight

QIAGEN

Contents

Handbook Revision History

Document	Changes	Date
HB-0144-001	Initial release.	February 2009
HB-0144-002	Changes to comply with GHS regulation, throughout the document.	January 2016
HB-0144-003	Removal of RNeasy Lipid Midi Kit (cat. no. 75842), including associated protocols, general kit information (Table 1 and Table 2) and ordering information. Update into Sample-to-Insight branding and edit into revised style.	January 2018
HB-0144-004	Corrected cross-reference in “Appendix C: Optional On-Column DNase Digestion with the RNase-Free DNase Set”	July 2018

Kit Contents

RNeasy Lipid Tissue Mini Kit	(50)
Catalog no.	**74804**
No. of preps	**50**
RNeasy Mini Spin Columns (each in a 2 ml collection tube)	50
Collection Tubes (1.5 ml)	50
Collection Tubes (2 ml)	50
QIAzol® Lysis Reagent*	50 ml
Buffer RW1*	45 ml
Buffer RPE† (concentrate)	11 ml
RNase-Free Water	10 ml
Quick-Start Protocol	1

* Contains a guanidine salt. Not compatible with disinfectants containing bleach. See page 6 for safety information.

† Before using for the first time, add 4 volumes of ethanol (96–100%) as indicated on the bottle to obtain a working solution.

Note: QIAzol Lysis Reagent is delivered separately.

Storage

The RNeasy Lipid Tissue Mini Kit should be stored dry at room temperature (15–25°C). All components are stable for at least 9 months under these conditions.

QIAzol Lysis Reagent can be stored at room temperature or at 2–8°C.

Intended Use

The RNeasy Lipid Tissue Mini Kit is intended for molecular biology applications. This product is 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.

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

QIAzol Lysis Reagent contains guanidine thiocyanate and Buffer RW1 contains a small amount of guanidine thiocyanate. Guanidine salts can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilt, clean with 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 Lipid Tissue Mini Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

The RNeasy Lipid Tissue Mini Kit is designed for optimal lysis of tissues rich in fat (such as brain and adipose tissue) and purification of high-quality total RNA >200 nt. The kit is also compatible with all other types of animal tissue.

Principle and procedure

The RNeasy Lipid Tissue Mini Kit integrates phenol/guanidine-based sample lysis and silica-membrane purification of total RNA. QIAzol Lysis Reagent, included in the kits, is a monophasic solution of phenol and guanidine thiocyanate, designed to facilitate lysis of fatty tissues and inhibit RNases. The high lysis efficiency of the reagent and the subsequent removal of contaminants by organic phase extraction enable the use of up to 100 mg of brain or adipose tissue per RNeasy Mini spin column (and up to 50 mg of other animal tissues).*

Tissue samples are homogenized in QIAzol Lysis Reagent. After addition of chloroform, the homogenate is separated into aqueous and organic phases by centrifugation. RNA partitions to the upper, aqueous phase while DNA partitions to the interphase and proteins to the lower, organic phase or the interphase.

The upper, aqueous phase is collected, and ethanol is added to provide appropriate binding conditions. The sample is then applied to an RNeasy Mini spin column, where the total RNA binds to the membrane, and phenol and other contaminants are efficiently washed away. Finally, high-quality RNA is eluted in RNase-free water (Figure 1, page 9).

* To ensure optimal RNA yields, the binding capacity of the RNeasy Mini spin column must not be exceeded. See the protocol for details.

With the RNeasy Lipid Tissue Mini Kit, all RNA molecules longer than 200 nucleotides are purified. The procedure provides enrichment for mRNA since most RNAs <200 nucleotides (such as 5.8S rRNA, 5S rRNA, and tRNAs, which together comprise 15–20% of total RNA) are selectively excluded. For purification of small RNA, including microRNA, from tissues and cells, we recommend using miRNeasy Kits.

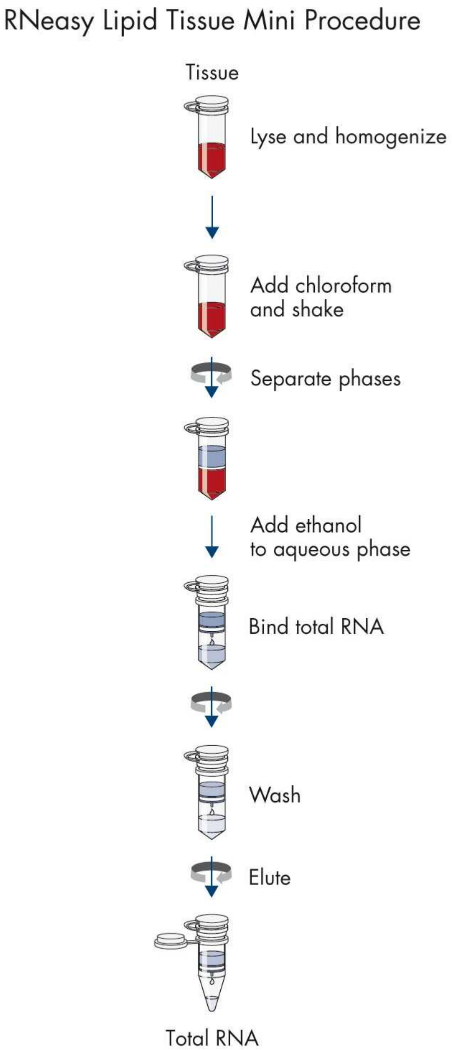

Figure 1. RNeasy Lipid Tissue Mini workflow.

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.

- Chloroform
- Ethanol (70% and 96–100%)*
- Sterile, RNase-free pipette tips
- Equipment for tissue disruption and homogenization (see page 12). We recommend either the TissueRuptor® II with TissueRuptor Disposable Probes or TissueLyser II (see Ordering Information, page 37)
- For stabilization of RNA in tissues (see "Handling and storing starting material", page 13): RNA*later*® RNA Stabilization Reagent (cat. no. 76104 or 76106) or Allprotect® Tissue Reagent (cat. no. 76405) or liquid nitrogen and dry ice
- 1.5 ml or 2 ml microcentrifuge tubes
- Microcentrifuges (with rotor for 2 ml tubes) for centrifugation at 4°C and at room temperature (15–25°C)
- Optional: RNase-Free DNase Set (cat. no. 79254) for on-column digestion of DNA during RNA purification

* Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.

Important Notes

Determining the amount of starting material

It is essential to use the correct amount of starting material in order to obtain optimal RNA yield and purity. The maximum amount that can be used is determined by:

- The type of tissue and its RNA content
- The volume of QIAzol Lysis Reagent required for efficient lysis
- The RNA binding capacity of the RNeasy Mini spin column

When processing samples containing high amounts of RNA, less than the maximum amount of starting material shown in Table 1 (next page) should be used, so that the RNA binding capacity of the RNeasy Mini spin column is not exceeded.

When processing samples containing low amounts of RNA, the maximum amount of starting material shown in Table 1 can be used. However, even though the RNA binding capacity of the RNeasy Mini spin column is not reached, the maximum amount of starting material must not be exceeded. Otherwise, lysis will be incomplete and cellular debris may interfere with the binding of RNA to the RNeasy Mini spin column membrane, resulting in lower RNA yield and purity.

More information on using the correct amount of starting material is given in the protocols. Table 2 (next page) shows expected RNA yields from various sources.

Table 1. RNeasy Mini spin column specifications

Specification	RNeasy Mini spin column
Maximum binding capacity	100 µg RNA
Maximum loading volume	700 µl
Minimum elution volume	30 µl
Maximum amount of starting tissue	≤100 mg

Note: If the binding capacity of the RNeasy Mini spin column is exceeded, RNA yields will not be consistent and may be reduced. If lysis of the starting material is incomplete, RNA yields will be lower than expected, even if the binding capacity of the RNeasy Mini spin column is not exceeded.

Table 2. Typical yields of total RNA

Mouse/rat tissue (10 mg)	Yield of total RNA (µg)*
Adipose tissue	0.5–2.5
Brain	5–20
Heart	5–25
Intestine	10–60
Kidney	5–40
Liver	15–80
Lung	5–15
Muscle	5–35
Skin	2–5
Spleen	15–100

* Amounts can vary due to factors such as species and developmental stage (especially with adipose tissues, large variations are possible due to developmental stage and location of the tissue). Since the RNeasy procedure enriches for mRNA and other RNA species >200 nucleotides, the total RNA yield does not include 5S rRNA, tRNA and other low-molecular-weight RNAs, which make up 15–20% of total cellular RNA.

Handling and storing starting material

RNA in harvested tissue is not protected until the sample is treated with RNA*later* RNA Stabilization Reagent, flash-frozen or disrupted and homogenized in the presence of RNase-inhibiting or denaturing reagents. If unprotected, unwanted changes in the gene expression profile will occur. It is therefore important that tissue samples are immediately frozen in liquid nitrogen and stored at –90 to –65°C or immediately immersed in RNA*later* RNA Stabilization Reagent at room temperature. An alternative to RNA*later* RNA Stabilization Reagent is Allprotect Tissue Reagent, which provides immediate stabilization of DNA, RNA and protein in tissue samples at room temperature.

Note: RNA*later* RNA Stabilization Reagent cannot be used to stabilize RNA in adipose tissue due to the high abundance of fat, but can be used to stabilize RNA in other fatty tissues, such as brain. Allprotect Tissue Reagent can stabilize adipose and brain tissue.

The procedures for tissue harvesting and RNA protection should be carried out as quickly as possible. Frozen tissue samples should not be allowed to thaw during handling or weighing.

Disrupting and homogenizing starting material

Efficient disruption and homogenization of the starting material is an absolute requirement for all total RNA purification procedures. Disruption and homogenization are two distinct steps:

- **Disruption**: Complete disruption of plasma membranes of cells and organelles is absolutely required to release all the RNA contained in the sample. Incomplete disruption results in significantly reduced RNA yields.
- **Homogenization**: Homogenization is necessary to reduce the viscosity of the lysates produced by disruption. Homogenization shears high-molecular-weight genomic DNA and other high-molecular-weight cellular components to create a homogeneous lysate. Incomplete homogenization results in inefficient binding of RNA to the RNeasy Mini spin column membrane and therefore significantly reduced RNA yields.

Disruption and homogenization of tissue samples can be carried out rapidly and efficiently using either the TissueRuptor II (for processing samples individually) or the TissueLyser (for processing multiple samples simultaneously). Disruption and homogenization with the TissueRuptor II or TissueLyser generally results in higher RNA yields than with other methods.

Disruption and homogenization using the TissueRuptor II

The TissueRuptor II is a rotor–stator homogenizer that thoroughly disrupts and simultaneously homogenizes single tissue samples in the presence of lysis buffer in 15–90 seconds, depending on the toughness and size of the sample. The blade of the TissueRuptor disposable probe rotates at a very high speed, causing the sample to be disrupted and homogenized by a combination of turbulence and mechanical shearing.

For guidelines on using the TissueRuptor II, refer to the *TissueRuptor II Handbook*. For other rotor–stator homogenizers, refer to suppliers' guidelines.

Disruption and homogenization using the TissueLyser II

In bead-milling, tissues can be disrupted by rapid agitation in the presence of beads and lysis buffer. Disruption and simultaneous homogenization occur by the shearing and crushing action of the beads as they collide with the cells. The TissueLyser II disrupts and homogenizes up to 48 tissue samples simultaneously when used in combination with the TissueLyser Adapter Set 2 x 24, which holds 48 x 2 ml microcentrifuge tubes containing stainless steel beads of 5 mm mean diameter. For guidelines on using the TissueLyser II, refer to the *TissueLyser Handbook*. For other bead mills, refer to suppliers' guidelines.

Note: Tungsten carbide beads react with QIAzol Lysis Reagent and must not be used to disrupt and homogenize tissues.

The TissueLyser II can also disrupt and homogenize up to 192 tissue samples simultaneously when used in combination with the TissueLyser Adapter Set 2 x 96, which holds 192 x 1.2 ml microcentrifuge tubes containing stainless steel beads of 5 mm mean diameter. In this case, we recommend using the RNeasy 96 Universal Tissue Kit, which provides high-throughput RNA purification from all types of tissue – including fatty tissues – in 96-well format and is based on the same technology as the RNeasy Lipid Tissue Mini Kit.

Protocol: Purification of Total RNA Using the RNeasy Lipid Tissue Mini Kit

Determining the correct amount of starting material

It is essential to use the correct amount of tissue in order to obtain optimal RNA yield and purity. A maximum of 100 mg brain or adipose tissue can generally be processed. For these tissues, the RNA binding capacity of the RNeasy Mini spin column and the lysing capacity of QIAzol Lysis Reagent will not be exceeded by these amounts. For other tissues, a maximum of 50 mg tissue can generally be used. For tissues with high RNA content, such as liver, spleen and thymus, we recommend using no more than 30 mg tissue to ensure optimal RNA yields and to avoid exceeding the binding capacity of the RNeasy Mini spin column. Average RNA yields from various tissues are given in Table 2 (page 12).

If there is no information about the nature of your starting material, we recommend starting with no more than 30 mg tissue. Depending on RNA yield and purity, it may be possible to use up to 100 mg tissue in subsequent preparations.

IMPORTANT: Do not overload the RNeasy Mini spin column, as this will significantly reduce RNA yield and quality.

Weighing tissue is the most accurate way to quantify the amount of starting material. As a guide, a 4 mm cube (64 mm^3) of most animal tissues weighs 70–85 mg.

Important points before starting

- If using the RNeasy Lipid Tissue Mini Kit for the first time, read "Important Notes" (page 11).
- If working with RNA for the first time, read "Appendix A: General Remarks on Handling RNA", page 26).

- If using the TissueRuptor II, ensure that you are familiar with operating it by referring to the *TissueRuptor II User Manual* and *TissueRuptor II Handbook*.
- If using the TissueLyser II, ensure that you are familiar with operating it by referring to the operating instructions and *TissueLyser Handbook*.
- To freeze tissue for long-term storage (several months), flash-freeze in liquid nitrogen and immediately transfer to –90 to –65°C. Do not allow tissues to thaw during weighing or handling prior to disruption in QIAzol Lysis Reagent. Homogenized tissue lysates from step 3 can also be stored at –90 to –65°C for several months. Incubate frozen lysates at 37°C in a water bath until completely thawed and salts are dissolved before continuing with the protocol. Avoid prolonged incubation, which may compromise RNA integrity.
- Generally, DNase digestion is not required since integrated QIAzol and RNeasy technologies efficiently remove most of the DNA without DNase treatment. However, further DNA removal may be necessary for certain RNA applications that are sensitive to very small amounts of DNA (e.g., TaqMan® real-time RT-PCR analysis with a low-abundance target). In these cases, residual DNA can be removed by optional on-column DNase-digestion using the RNase-Free DNase Set (see "Appendix C: Optional On-Column DNase Digestion with the RNase-Free DNase Set", page 34). Alternatively, for real-time two-step RT-PCR applications, the QuantiNova® Reverse Transcription Kit (cat. no. 205411) provides cDNA synthesis with integrated removal of genomic DNA contamination.
- QIAzol Lysis Reagent and Buffer RW1 contain a guanidine salt and are therefore not compatible with disinfecting reagents containing bleach. See page 6 for safety information.
- Except for phase separation (step 15), all protocol and centrifugation steps should be performed at room temperature (15–25°C). During the procedure, work quickly.

Things to do before starting

- Buffer RPE is supplied as a concentrate. Before using for the first time, add 4 volumes of ethanol (96–100%) as indicated on the bottle to obtain a working solution.

- If performing optional on-column DNase digestion, prepare DNase I stock solution as described in "Appendix C: Optional On-Column DNase Digestion with the RNase-Free DNase Set", page 34.

Procedure

1. If using the TissueLyser II, add one stainless steel bead (5 mm mean diameter) per 2 ml microcentrifuge tube (not supplied). If working with tissues that are not stabilized in RNA*later* RNA Stabilization Reagent or Allprotect Tissue Reagent, place the tubes on dry ice.
2. Excise the tissue sample from the animal or remove it from storage. Determine the amount of tissue. Do not use more than 100 mg. Proceed immediately to step 3.

   Note: Weighing tissue is the most accurate way to determine the amount.

   Note: If the tissue sample was stored in RNA*later* RNA Stabilization Reagent or Allprotect Tissue Reagent, remove it from the reagent using forceps and be sure to remove any excess reagent or crystals that may have formed.

   Note: RNA in harvested tissues is not protected until the tissues are treated with RNA*later* RNA Stabilization Reagent or Allprotect Tissue Reagent, flash-frozen or disrupted and homogenized in step 3. Frozen tissues should not be allowed to thaw during handling. The relevant procedures should be carried out as quickly as possible.
3. Disrupt the tissue and homogenize the lysate using **EITHER** the TissueRuptor II (follow steps 4 and 5) **OR** the TissueLyser II (follow steps 6–11).

   See "Disrupting and homogenizing starting material", page 14, for more details on disruption and homogenization.

   Note: Incomplete homogenization leads to significantly reduced RNA yields and can cause clogging of the RNeasy Mini spin column. Homogenization with the TissueRuptor II or TissueLyser II generally results in higher RNA yields than with other methods.

Disruption and homogenization using the TissueRuptor II

4. Place the tissue in a suitably sized vessel containing 1 ml QIAzol Lysis Reagent.

   Note: Use a suitably sized vessel with sufficient extra headspace to accommodate foaming, which may occur during homogenization.

   Generally, round-bottomed tubes allow more efficient disruption and homogenization than conical-bottomed tubes.

5. Place the tip of the disposable probe into the vessel and operate the TissueRuptor II at full speed until the lysate is uniformly homogeneous (usually 20–40 s). Proceed to step 12.

   Note: To avoid damage to the TissueRuptor II and disposable probe during operation, make sure the tip of the probe remains submerged in the buffer.

   Note: Foaming may occur during homogenization, especially of brain tissue. If this occurs, let the homogenate stand at room temperature for 2–3 min until the foam subsides before continuing with the procedure.

Disruption and homogenization using the TissueLyser II

6. Place the tissues in the tubes prepared in step 1.
7. If the tubes were stored on dry ice, place them at room temperature. Then immediately add 1 ml QIAzol Lysis Reagent per tube.
8. Place the tubes in the TissueLyser Adapter Set 2 x 24.
9. Operate the TissueLyser II for 2 min at 20 Hz.

   Note: The time depends on the tissue being processed and can be extended until the tissue is completely homogenized.

10. Disassemble the adapter set, rotate the rack of tubes so that the tubes nearest to the TissueLyser II are now outermost, and reassemble the adapter set. Operate the TissueLyser II for another 2 min at 20 Hz.

    Note: Rearranging the tubes allows even homogenization.

11. Carefully pipet the lysates into new microcentrifuge tubes (not supplied). Proceed to step 12.

    Important: Do not reuse the stainless steel beads.

Preparation of total RNA

12. Place the tube containing the homogenate on the benchtop at room temperature (15–25°C) for 5 min.

    Note: This step promotes dissociation of nucleoprotein complexes.

13. Add 200 µl chloroform. Securely cap the tube containing the homogenate and shake it vigorously for 15 s.

    Note: Thorough mixing is important for subsequent phase separation.

14. Place the tube containing the homogenate on the benchtop at room temperature for 2–3 min.

15. Centrifuge at 12,000 x *g* for 15 min at 4°C. After centrifugation, heat the centrifuge to room temperature (15–25°C) if the same centrifuge will be used in the later steps of this procedure.

    Note: After centrifugation, the sample separates into three phases: an upper, colorless, aqueous phase containing RNA; a white interphase; and a lower, red, organic phase. For tissues with an especially high fat content, an additional, clear phase may be visible below the red, organic phase. The volume of the aqueous phase should be approximately 600 µl.

16. Transfer the upper, aqueous phase to a new tube (not supplied). Add 1 volume (usually 600 µl) of 70% ethanol, and mix thoroughly by vortexing. Do not centrifuge. Proceed immediately to step 17.

    Note: The volume of lysate may be less than 600 µl due to loss during homogenization and centrifugation.

    Note: Precipitates may be visible after addition of ethanol. Resuspend precipitates completely by vigorous shaking and proceed immediately to step 17.

17. Transfer up to 700 µl of the sample to an RNeasy Mini spin column placed in a 2 ml collection tube (supplied). Close the lid gently, and centrifuge for 15 s at 8000 x *g* (10,000 rpm) at room temperature (15–25°C). Discard the flow-through.*

    Reuse the collection tube in step 18.

* Flow-through contains QIAzol Lysis Reagent and is therefore not compatible with bleach. See page 6 for safety information.

18. Repeat step 17 using the remainder of the sample. Discard the flow-through.*

Reuse the collection tube in step 19.

Optional: If performing optional on-column DNase digestion (see "Important points before starting", page 16), follow steps 1–4 (page 34) after performing this step.

19. Add 700 µl Buffer RW1 to the RNeasy Mini spin column. Close the lid gently and centrifuge for 15 s at 8000 x *g* (10,000 rpm) to wash the membrane. Discard the flow-through.*

Reuse the collection tube in step 20.

After centrifugation, carefully remove the RNeasy Mini spin column from the collection tube so that the column does not contact the flow-through. Be sure to empty the collection tube completely.*

Skip this step if performing optional on-column DNase digestion (page 33).

20. Add 500 µl Buffer RPE to the RNeasy Mini spin column. Close the lid gently and centrifuge for 15 s at 8000 x *g* (10,000 rpm) to wash the membrane. Discard the flow-through.

Reuse the collection tube in step 21.

Note: Buffer RPE is supplied as a concentrate. Ensure that ethanol is added to Buffer RPE before use (see "Things to do before starting", page 17).

21. Add 500 µl Buffer RPE to the RNeasy Mini spin column. Close the lid gently and centrifuge for 2 min at 8000 x *g* (10,000 rpm) to wash the membrane.

Note: The long centrifugation dries the spin column membrane, ensuring that no ethanol is carried over during RNA elution. Residual ethanol may interfere with downstream reactions.

Note: After centrifugation, carefully remove the RNeasy Mini spin column from the collection tube so that the column does not contact the flow-through. Otherwise, carryover of ethanol will occur.

* Flow-through contains Buffer RW1 and is therefore not compatible with bleach. See page 6 for safety information.

22. **Optional**: Place the RNeasy Mini spin column in a new 2 ml collection tube (supplied) and discard the old collection tube with the flow-through. Close the lid gently and centrifuge at full speed for 1 min.

    Perform this step to eliminate any possible carryover of Buffer RPE or if residual flow-through remains on the outside of the RNeasy Mini spin column after step 21.

23. Place the RNeasy Mini spin column in a new 1.5 ml collection tube (supplied). Add 30–50 µl RNase-free water directly to the spin column membrane. Close the lid gently. To elute the RNA, centrifuge for 1 min at 8000 x *g* (10,000 rpm).

24. Repeat step 23 using another volume of RNase-free water, or using the eluate from step 23 (if high RNA concentration is required). Reuse the collection tube from step 23.

    If using the eluate from step 23, the RNA yield will be 15–30% less than that obtained using a second volume of RNase-free water, but the final RNA concentration will be higher.

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
Phases do not separate completely	
a) No chloroform added or chloroform not pure	Make sure to add chloroform that does not contain isoamyl alcohol or other additives.
b) Homogenate not sufficiently mixed before centrifugation	After addition of chloroform (step 13), the homogenate must be vigorously shaken. If the phases are not well separated, shake the tube vigorously for at least 15 s, and repeat the incubation and centrifugation in steps 14 and 15.
c) Organic solvents in samples used for RNA purification	Make sure that the starting sample does not contain organic solvents (e.g., ethanol, DMSO), strong buffers, or alkaline reagents. These can interfere with the phase separation.
Clogged RNeasy Mini spin column	
a) Inefficient disruption and/or homogenization	See "Disrupting and homogenizing starting material" (page 14) for details on disruption and homogenization methods. Increase *g*-force and centrifugation time, if necessary. In subsequent preparations, reduce the amount of starting material (see page 11 and protocol) and/or increase the homogenization time.
b) Too much starting material	In subsequent preparations, reduce the amount of starting material. It is essential to use the correct amount of starting material (see page 11 and protocol).
c) Centrifugation temperature too low	Except for phase separation (step15), all centrifugation steps should be performed at 15–25°C. Some centrifuges may cool to below 20°C even when set at 20°C. This can cause formation of precipitates that can clog the RNeasy Mini spin column. If this happens, set the centrifugation temperature to 25°C. Warm the ethanol-containing lysate to 37°C before transferring to the RNeasy Mini spin column.

		Comments and suggestions
Low RNA yield		
a)	Inefficient disruption and/or homogenization	See "Disrupting and homogenizing starting material" (page 14) for details on disruption and homogenization methods.
b)	Too much starting material	In subsequent preparations, reduce the amount of starting material. It is essential to use the correct amount of starting material (see page 11 and protocol).
c)	RNA still bound to RNeasy Mini spin column membrane	Repeat RNA elution, but incubate the RNeasy Mini spin column on the benchtop for 10 min with RNase-free water before centrifuging.
d)	Centrifugation temperature too low	Except for phase separation (step 15), all centrifugation steps should be performed at 15–25°C. Some centrifuges may cool to below 20°C even when set at 20°C. This can cause formation of precipitates that can clog the RNeasy Mini spin column. If this happens, set the centrifugation temperature to 25°C. Warm the ethanol-containing lysate to 37°C before transferring to the RNeasy Mini spin column.
Low or no recovery of RNA		
	RNase-free water incorrectly dispensed	Add RNase-free water to the center of the RNeasy Mini spin column membrane to ensure that the membrane is completely covered.
Low A_{260}/A_{280} value		
a)	Insufficient QIAzol Lysis Reagent used for homogenization	In subsequent preparations, reduce the amount of starting material and/or increase the volume of QIAzol Lysis Reagent and the homogenization time.
b)	Sample not incubated for 5 min after homogenization	Place the sample at room temperature (15–25°C) for 5 min after homogenization as indicated in the protocol (step 12). This step is important to promote dissociation of nucleoprotein complexes.
c)	Water used to dilute RNA for A_{260}/A_{280} measurement	Use 10 mM Tris·Cl, pH 7.5, not RNase-free water, to dilute the sample before measuring purity (see "Appendix B: Storage, Quantification, and Determination of Quality of RNA", page 29).
RNA degraded		
a)	Inappropriate handling of starting material	For frozen tissue samples, ensure that they were flash-frozen immediately in liquid nitrogen and properly stored at –70°C.Perform the RNeasy procedure quickly, especially the first few steps. See "Appendix A: General Remarks on Handling RNA", page 26 and "Handling and storing starting material", page 13.

		Comments and suggestions
b)	RNase contamination	Although all RNeasy buffers have been tested and are guaranteed RNase-free, RNases can be introduced during use. Be certain not to introduce any RNases during the RNeasy procedure or later handling. See ""Appendix A: General Remarks on Handling RNA", page 26. Do not put RNA samples into a vacuum dryer that has been used in DNA preparation where RNases may have been used.

DNA contamination in downstream experiments

a)	Phase separation performed at too high a temperature	The phase separation (step 15) should be performed at 4°C to allow optimal phase separation and removal of genomic DNA from the aqueous phase. Make sure that the centrifuge does not heat above 10°C during the centrifugation.
b)	Interphase contamination of the aqueous phase	Contamination of the aqueous phase with the interphase results in an increased DNA content in the RNA eluate. Make sure to transfer the aqueous phase without interphase contamination.
c)	Insufficient QIAzol Lysis Reagent used for homogenization	In subsequent preparations, reduce the amount of starting material and/or increase the volume of QIAzol Lysis Reagent and the homogenization time.
d)	No DNase treatment	Perform optional on-column DNase digestion using the RNase-Free DNase Set (see "Appendix C: Optional On-Column DNase Digestion with the RNase-Free DNase Set", page 34) at step 18 of the protocol. Alternatively, perform cDNA synthesis with integrated DNA removal.

RNA does not perform well in downstream experiments

a)	Salt carryover during elution	Ensure that Buffer RPE is at 15–25°C.
b)	Ethanol carryover	During the second wash with Buffer RPE (step 21), be sure to dry the RNeasy Mini spin column membrane by centrifuging at 8000 x *g* (10,000 rpm) for 2 min at 15–25°C. After centrifugation, carefully remove the column from the collection tube so that the column does not contact the flow-through. Otherwise, carryover of ethanol will occur. To eliminate any chance of possible ethanol carryover, place the RNeasy Mini spin column in a new 2 ml collection tube and perform the optional 1-minute centrifugation step as described in step 22 of the protocol.

Appendix A: General Remarks on Handling RNA

Handling RNA

Ribonucleases (RNases) are very stable and active enzymes that generally do not require cofactors to function. Since RNases are difficult to inactivate and even minute amounts are sufficient to destroy RNA, do not use any plasticware or glassware without first eliminating possible RNase contamination. Great care should be taken to avoid inadvertently introducing RNases into the RNA sample during or after the purification procedure. In order to create and maintain an RNase-free environment, the following precautions must be taken during pretreatment and use of disposable and nondisposable vessels and solutions while working with RNA.

General handling

Proper microbiological, aseptic technique should always be used when working with RNA. Hands and dust particles may carry bacteria and molds and are the most common sources of RNase contamination. Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contamination from the surface of the skin or from dusty laboratory equipment. Change gloves frequently and keep tubes closed whenever possible. Keep purified RNA on ice when aliquots are pipetted for downstream applications.

Disposable plasticware

The use of sterile, disposable polypropylene tubes is recommended throughout the procedure. These tubes are generally RNase-free and do not require pretreatment to inactivate RNases.

Nondisposable plasticware

Nondisposable plasticware should be treated before use to ensure that it is RNase-free. Plasticware should be thoroughly rinsed with 0.1 M NaOH, 1 mM EDTA* followed by RNase-free water (see "Solutions", page 28). Alternatively, chloroform-resistant plasticware can be rinsed with chloroform* to inactivate RNases.

Glassware

Glassware should be treated before use to ensure that it is RNase-free. Glassware used for RNA work should be cleaned with a detergent,* thoroughly rinsed, and oven baked at 240°C for at least 4 hours (overnight, if more convenient) before use. Autoclaving alone will not fully inactivate many RNases. Alternatively, glassware can be treated with DEPC* (diethyl pyrocarbonate). Fill glassware with 0.1% DEPC (0.1% in water), allow to stand overnight (12 hours) at 37°C and then autoclave or heat to 100°C for 15 minutes to eliminate residual DEPC.

Electrophoresis tanks

Electrophoresis tanks should be cleaned with detergent solution (e.g., 0.5% SDS),* thoroughly rinsed with RNase-free water, and then rinsed with ethanol† and allowed to dry.

* 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.

† Plastics used for some electrophoresis tanks are not resistant to ethanol. Take proper care and check the supplier's instructions.

Solutions

Solutions (water and other solutions) should be treated with 0.1% DEPC. DEPC is a strong, but not absolute, inhibitor of RNases. It is commonly used at a concentration of 0.1% to inactivate RNases on glass or plasticware or to create RNase-free solutions and water. DEPC inactivates RNases by covalent modification. Add 0.1 ml DEPC to 100 ml of the solution to be treated and shake vigorously to bring the DEPC into solution. Let the solution incubate for 12 hours at 37°C. Autoclave for 15 minutes to remove any trace of DEPC. DEPC will react with primary amines and cannot be used directly to treat Tris* buffers. DEPC is highly unstable in the presence of Tris buffers and decomposes rapidly into ethanol and CO_2. When preparing Tris buffers, treat water with DEPC first, and then dissolve Tris to make the appropriate buffer. Trace amounts of DEPC will modify purine residues in RNA by carbethoxylation. Carbethoxylated RNA is translated with very low efficiency in cell-free systems. However, its ability to form DNA:RNA or RNA:RNA hybrids is not seriously affected unless a large fraction of the purine residues have been modified. Residual DEPC must always be eliminated from solutions or vessels by autoclaving or heating to 100°C for 15 minutes.

Note: RNeasy buffers are guaranteed RNase-free without using DEPC treatment and are therefore free of any DEPC contamination.

* 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.

Appendix B: Storage, Quantification, and Determination of Quality of RNA

Storage of RNA

Purified RNA may be stored at –30 to –15°C or –90 to –65°C in RNase-free water. Under these conditions, no degradation of RNA is detectable after 1 year.

Quantification of RNA

The concentration of RNA should be determined by measuring the absorbance at 260 nm (A_{260}) in a spectrophotometer (see "Spectrophotometric quantification of RNA" below). For small amounts of RNA, however, it may be difficult to determine amounts photometrically. Small amounts of RNA can be quantified using the QIAxpert®, QIAxcel® Advanced System or Agilent® 2100 bioanalyzer, quantitative RT-PCR or fluorometric quantification.

Spectrophotometric quantification of RNA

To ensure significance, A_{260} readings should be greater than 0.15. An absorbance of 1 unit at 260 nm corresponds to 44 µg of RNA per ml ($A_{260} = 1 \rightarrow 44$ µg/ml). This relation is valid only for measurements at a neutral pH. Therefore, if it is necessary to dilute the RNA sample, this should be done in a buffer with neutral pH.* As discussed below (see "Purity of RNA", page 31), the ratio between the absorbance values at 260 and 280 nm gives an estimate of RNA purity.

* 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.

When measuring RNA samples, be certain that cuvettes are RNase-free, especially if the RNA is to be recovered after spectrophotometry. This can be accomplished by washing cuvettes with 0.1 M NaOH, 1 mM EDTA,* followed by washing with RNase-free water (see "Solutions", page 28). Use the buffer in which the RNA is diluted to zero the spectrophotometer. An example of the calculation involved in RNA quantification is shown below:

Volume of RNA sample = 100 µl

Dilution = 10 µl RNA sample + 490 µl 10 mM Tris·Cl,* pH 7.0 (1/50 dilution)

Measure absorbance of diluted sample in a 1 ml cuvette (RNase-free)

A_{260} = 0.2

Concentration of RNA sample	= 44 µg/ml x A_{260} x dilution factor
	= 44 µg/ml x 0.2 x 50
	= 440 µg/ml
Total amount	= concentration x volume in milliliters
	= 440 µg/ml x 0.1 ml
	= 44 µg of RNA

* 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.

Purity of RNA

The ratio of the readings at 260 nm and 280 nm (A_{260}/A_{280}) provides an estimate of the purity of RNA with respect to contaminants that absorb in the UV spectrum, such as protein. However, the A_{260}/A_{280} ratio is influenced considerably by pH. Since water is not buffered, the pH and the resulting A_{260}/A_{280} ratio can vary greatly. Lower pH results in a lower A_{260}/A_{280} ratio and reduced sensitivity to protein contamination.* For accurate values, we recommend measuring absorbance in 10 mM Tris·Cl, pH 7.5. Pure RNA has an A_{260}/A_{280} ratio of 1.9–2.1† in 10 mM Tris·Cl, pH 7.5. Always be sure to calibrate the spectrophotometer with the same solution used for dilution.

For determination of RNA concentration, however, we recommend dilution of the sample in a buffer with neutral pH since the relationship between absorbance and concentration (A_{260} reading of 1 = 44 µg/ml RNA) is based on an extinction coefficient calculated for RNA at neutral pH (see "Spectrophotometric quantification of RNA", page 29).

To assess the purity of RNA (A_{260}/A_{280}) we recommend using the QIAxpert. The QIAxpert is an innovative µ-volume UV/Vis spectrophotometer that overcomes the limitations of classic spectrophotometry and purity assessment using absorbance ratios. Using reference spectra of known contaminants, a state-of-the-art software algorithm on the QIAxpert instrument performs a deconvolution of measured UV/Vis spectra according to the Beer Lambert law for mixtures, stating that the absorption spectrum of a mixture is a linear combination of the spectra of its individual constituents. This feature, known as Spectral Content Profiling (SCP), allows dye-free and easy differentiation between DNA, RNA and other UV/Vis absorbing contaminants in complex biological samples).

* 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.

† Values up to 2.3 are routinely obtained for pure RNA (in 10 mM Tris·Cl, pH 7.5) with some spectrophotometers.

DNA contamination

No currently available purification method can guarantee that RNA is completely free of DNA, even when it is not visible on an agarose gel. While RNeasy Kits will remove the vast majority of cellular DNA, trace amounts may still remain, depending on the amount and nature of the sample.

For analysis of very low abundance targets, any interference by residual DNA contamination can be detected by performing real-time RT-PCR control experiments in which no reverse transcriptase is added prior to the PCR step.

To prevent any interference by DNA in real-time RT-PCR applications, such as with Rotor-Gene® and Applied Biosystems® instruments, we recommend designing primers that anneal at intron splice junctions so that genomic DNA will not be amplified. QuantiTect® Primer Assays from QIAGEN are designed for SYBR® Green-based real-time RT-PCR analysis of RNA sequences (without detection of genomic DNA) where possible (see **www.qiagen.com/GeneGlobe**). For real-time RT-PCR assays where amplification of genomic DNA cannot be avoided, we recommend using the QuantiTect Reverse Transcription Kit (cat. no. 205311) for reverse transcription. The kit integrates fast cDNA synthesis with rapid removal of genomic DNA contamination.

For other sensitive applications, DNase digestion of the purified RNA with RNase-free DNase is recommended. A protocol for optional on-column DNase digestion using the RNase-Free DNase Set is provided in "Appendix C: Optional On-Column DNase Digestion with the RNase-Free DNase Set", page 34. The DNase is efficiently washed away in subsequent wash steps.

Integrity of RNA

The integrity and size distribution of total RNA purified with RNeasy Lipid Tissue Kits can be checked by denaturing agarose gel electrophoresis and ethidium bromide staining* or by using the QIAxcel Advanced System, Agilent Tapestation or Agilent 2100 bioanalyzer. The respective ribosomal RNAs should appear as sharp bands or peaks. The apparent ratio of 28S rRNA to 18S rRNA should be approximately 2:1. If the ribosomal bands or peaks of a specific sample are not sharp, but appear as a smear towards smaller sized RNAs, it is likely that the sample suffered major degradation either before or during RNA purification.

The QIAxcel Advanced System also provides an assessment of RNA integrity using an RNA integrity score (RIS). The RIS gives an objective quality measurement for eukaryotic RNA samples and allows easy interpretation of sample integrity. The RIS is a value from 1 to 10 where a value of 10 indicates completely intact RNA. Similarly, the Agilent 2100 bioanalyzer offers an RNA Integrity Number (RIN) as a measure of RNA integrity. Ideally, the RIN should be close to 10, but in many cases (particularly with tissue samples), how well the original sample is preserved greatly influences RNA quality.

* 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.

Appendix C: Optional On-Column DNase Digestion with the RNase-Free DNase Set

The RNase-Free DNase Set (cat. no. 79254) provides efficient on-column digestion of DNA during RNA purification. The DNase is efficiently removed in subsequent wash steps.

Note: Standard DNase buffers are not compatible with on-column DNase digestion. Use of other buffers may affect the binding of RNA to the RNeasy membrane, reducing RNA yield and integrity.

Lysis and homogenization of the sample and binding of RNA to the RNeasy membrane are performed according to the standard protocol. After washing with a reduced volume of Buffer RW1, the RNA is treated with DNase I while bound to the RNeasy membrane. The DNase I is removed by a second wash with Buffer RW1. Washing with Buffer RPE and elution of RNA are then performed according to the standard protocol.

Important points before starting

- Generally, DNase digestion is not required since integrated QIAzol and RNeasy technologies efficiently remove most of the DNA without DNase treatment. However, further DNA removal may be necessary for certain RNA applications that are sensitive to very small amounts of DNA (e.g., TaqMan RT-PCR analysis with a low-abundant target). DNA can also be removed by a DNase digestion following RNA purification.
- **Do not vortex the reconstituted DNase I**. DNase I is especially sensitive to physical denaturation. Mixing should only be carried out by gently inverting the tube.

Things to do before starting

- Prepare DNase I stock solution before using the RNase-Free DNase Set for the first time. Dissolve the lyophilized DNase I (1500 Kunitz units) in 550 µl of the RNase-free water provided. To avoid loss of DNase I, do not open the vial. Inject RNase-free water into the vial using an RNase-free needle and syringe. Mix gently by inverting the vial. Do not vortex.
- For long-term storage of DNase I, remove the stock solution from the glass vial, divide it into single-use aliquots, and store at –30 to –15°C for up to 9 months. Thawed aliquots can be stored at 2–8°C for up to 6 weeks. Do not refreeze the aliquots after thawing.

Procedure

Prepare and load samples onto the RNeasy Mini spin column as indicated in steps 1–18 of the protocol starting on page 16. Instead of performing step 19, follow steps 1–4 below.

1. Add 350 µl Buffer RW1 to the RNeasy Mini spin column. Close the lid gently and centrifuge for 15 s at 8000 x *g* (10,000 rpm) to wash the membrane. Discard the flow-through.*

   Reuse the collection tube in step 4.
2. Add 10 µl DNase I stock solution (see above) to 70 µl Buffer RDD. Mix by gently inverting the tube, and centrifuge briefly to collect residual liquid from the sides of the tube.

   Buffer RDD is supplied with the RNase-Free DNase Set.

   Note: DNase I is especially sensitive to physical denaturation. Mixing should only be carried out by gently inverting the tube. Do not vortex.
3. Add the DNase I incubation mix (80 µl) directly to the RNeasy Mini spin column membrane, and place on the benchtop (15–25°C) for 15 min.

   Note: Be sure to add the DNase I incubation mix directly to the RNeasy Mini spin column membrane. DNase digestion will be incomplete if part of the mix sticks to the walls or the O-ring of the spin column.

* Flow-through contains Buffer RW1 and is therefore not compatible with bleach. See page 6 for safety information.

4. Add 350 µl Buffer RW1 to the RNeasy Mini spin column. Close the lid gently and centrifuge for 15 s at 8000 x *g* (10,000 rpm). Discard the flow-through.* Continue with step 20 of the protocol on page 21.

* Flow-through contains Buffer RW1 and is therefore not compatible with bleach. See page 6 for safety information.

Ordering Information

Product	Contents	Cat. no.
RNeasy Lipid Tissue Mini Kit (50)	50 RNeasy Mini Spin Columns, Collection Tubes, QIAzol Lysis Reagent, RNase-Free Reagents and Buffers	74804
Accessories		
Allprotect Tissue Reagent (100 ml)	For stabilization of DNA, RNA and protein in 50 x 200 mg tissue samples: 100 ml Allprotect Tissue Reagent, Allprotect Reagent Pump	76405
RNA*later* RNA Stabilization Reagent (50 ml)	For stabilization of RNA in 25 x 200 mg tissue samples: 50 ml RNA*later* RNA Stabilization Reagent	76104
RNA*later* RNA Stabilization Reagent (250 ml)	For stabilization of RNA in 125 x 200 mg tissue samples: 250 ml RNA*later* RNA Stabilization Reagent	76106
RNA*later* TissueProtect Tubes (50 x 1.5 ml)	For stabilization of RNA in 50 x 150 mg tissue samples: 50 screw-top tubes containing 1.5 ml RNA*later* RNA Stabilization Reagent each	76154
RNA*later* TissueProtect Tubes (20 x 5 ml)	For stabilization of RNA in 20 x 500 mg tissue samples: 20 screw-top tubes containing 5 ml RNA*later* RNA Stabilization Reagent each	76163
QIAzol Lysis Reagent (200 ml)	200 ml QIAzol Lysis Reagent	79306

Product	Contents	Cat. no.
TissueRuptor II	Handheld rotor–stator homogenizer, 5 TissueRuptor Disposable Probes	9002755* 9002754† 9002756‡ 9002757§ 9002758¶
TissueRuptor Disposable Probes (25)	25 nonsterile plastic disposable probes for use with the TissueRuptor II	990890
TissueLyser II	Universal laboratory mixer-mill disruptor	85300
TissueLyser Adapter Set 2 x 24	2 sets of Adapter Plates and 2 racks for use with 2 ml microcentrifuge tubes on the TissueLyser II	69982
TissueLyser Adapter Set 2 x 96	2 sets of Adapter Plates for use with Collection Microtubes (racked) on the TissueLyser II	69984
TissueLyser Single-Bead Dispenser, 5 mm	For dispensing individual beads (5 mm diameter)	69965
Stainless Steel Beads, 5 mm (200)	Stainless Steel Beads, suitable for use with the TissueLyser system	69989
RNase-Free DNase Set (50)	For 50 RNA minipreps: 1500 units RNase-Free DNase I, RNase-Free Buffer RDD, and RNase-Free Water	79254

* 120 V, 60 Hz (for North America); † 100 V, 50/60 Hz and Japan); ‡ 235 V, 50/60 Hz (for Europe, excluding UK and Ireland); § 235 V, 50/60 Hz (for UK and Ireland); ¶ 235 V, 50/60 Hz (for Australia).

Product	Contents	Cat. no.
Collection Tubes (2 ml)	1000 x 2 ml Collection Tubes	19201
Collection Microtubes (racked, 10 x 96)	Nonsterile polypropylene tubes (1.2 ml), 960 in racks of 96	19560
Collection Microtube Caps (120 x 8)	Nonsterile polypropylene caps for collection microtubes (1.2 ml), 960 in strips of 8	19566

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

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