January 2009

QIAzol® Handbook

For efficient lysis of fatty tissues and all other types of tissue before RNA purification

QIAGEN®

Sample & Assay Technologies

QIAGEN Sample and Assay Technologies

QIAGEN is the leading provider of innovative sample and assay technologies, enabling the isolation and detection of contents of any biological sample. Our advanced, high-quality products and services ensure success from sample to result.

QIAGEN sets standards in:

- Purification of DNA, RNA, and proteins
- Nucleic acid and protein assays
- microRNA research and RNAi
- Automation of sample and assay technologies

Our mission is to enable you to achieve outstanding success and breakthroughs. For more information, visit www.qiagen.com.

Contents

Kit Contents

QIAzol Lysis Reagent	**(200 ml)**
Catalog no.	**79306**
QIAzol Lysis Reagent*	200 ml
Handbook	1

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

Shipping and Storage

QIAzol Lysis Reagent is shipped at ambient temperature. It can be stored at room temperature (15–25°C) or at 2–8°C. QIAzol Lysis Reagent is stable for at least 12 months under these conditions.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of QIAzol Lysis Reagent is tested against predetermined specifications to ensure consistent product quality.

Product Use Limitations

QIAzol Lysis Reagent is intended for molecular biology applications. This product is neither intended for the diagnosis, prevention, or treatment of a disease, nor has it been validated for such use either alone or in combination with other products. Therefore, the performance characteristics of the product for clinical use (i.e., diagnostic, prognostic, therapeutic, or blood banking) are unknown.

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.

Product Warranty and Satisfaction Guarantee

QIAGEN guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, QIAGEN will replace it free of charge or refund the purchase price. We reserve the right to change, alter, or modify any product to enhance its performance and design. If a QIAGEN product does not meet your expectations, simply call your local Technical Service Department or distributor. We will credit your account or exchange the product — as you wish. Separate conditions apply to QIAGEN scientific instruments, service products, and to products shipped on dry ice. Please inquire for more information.

A copy of QIAGEN terms and conditions can be obtained on request, and is also provided on the back of our invoices. If you have questions about product specifications or performance, please call QIAGEN Technical Services or your local distributor (see back cover or visit www.qiagen.com).

Technical Assistance

At QIAGEN, we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of QIAGEN products. If you have any questions or experience any difficulties regarding QIAzol Lysis Reagent or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, please see our Technical Support Center at www.qiagen.com/Support or call one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit www.qiagen.com).

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). These are available online in convenient and compact PDF format at www.qiagen.com/Support/MSDS.aspx where you can find, view, and print the MSDS 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, which can form highly reactive compounds when combined with bleach. If liquid containing this reagent 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.

The following risk and safety phrases apply to the components of QIAzol Lysis Reagent.

QIAzol Lysis Reagent

Contains phenol, guanidine thiocyanate: toxic, corrosive. Risk and safety phrases:* R23/24/25-32-34-48/20/21/22-68, S24/25-26-36/37/39-45

24-hour emergency information

Emergency medical information in English, French, and German can be obtained 24 hours a day from:

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

* R23/24/25: Toxic by inhalation, in contact with skin and if swallowed; R32: Contact with acids liberates very toxic gas; R34: Causes burns; R48/20/21/22: Harmful: danger of serious damage to health by prolonged exposure through inhalation, in contact with skin and if swallowed; R68: Possible risk of irreversible effects; S24/25: Avoid contact with skin and eyes; S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice; S36/37/39: Wear suitable protective clothing, gloves and eye/face protection; S45: In case of accident or if you feel unwell seek medical advice immediately (show the label where possible).

Introduction

QIAzol Lysis Reagent is a monophasic solution of phenol and guanidine thiocyanate, designed to facilitate lysis of fatty tissues and inhibit RNases. Tissue samples are disrupted and 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. RNA is precipitated from the aqueous phase by addition of isopropanol. The RNA is then pelleted and washed with ethanol before being redissolved in RNase-free water.

We recommend cleanup of the redissolved RNA using RNeasy® Kits, which are based on silica-membrane technology, in order to remove any contaminating phenol. The presence of residual phenol can result in overestimation of RNA yield and inhibition of enzymatic action in downstream applications. The removal of contaminants by RNA cleanup also improves the stability of the RNA during storage.

RNA purified using QIAzol Lysis Reagent may contain residual amounts of genomic DNA that can affect sensitive downstream applications such as real-time RT-PCR. Genomic DNA contamination in the RNA sample can be removed by adding DNase. After DNase digestion, the RNA sample can be cleaned up using RNeasy Kits to remove the DNase. Alternatively, DNase digestion can be carried out during RNA cleanup using RNeasy Kits. Details about DNase digestion are provided in the handbook supplied with RNeasy Kits.

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 material safety data sheets (MSDSs), available from the product supplier.

- Chloroform
- Isopropanol
- 75% ethanol
- RNase-free water
- Refrigerated laboratory centrifuge or microcentrifuge (capable of 12,000 x *g*)
- For stabilization of RNA in tissues (see page 9): RNA*later*® RNA Stabilization Reagent or Allprotect Tissue Reagent (see ordering information, page 20) or liquid nitrogen
- Equipment for tissue disruption and homogenization (see page 9). We recommend one of the following:
  - TissueRuptor® with TissueRuptor Disposable Probes
  - TissueLyser with the following accessories: TissueLyser Adapter Set 2 x 24; TissueLyser Single-Bead Dispenser, 5 mm; Stainless Steel Beads, 5 mm
  - TissueLyser with the following accessories: TissueLyser Adapter Set 2 x 96; TissueLyser 5 mm Bead Dispenser, 96-well; Stainless Steel Beads, 5 mm; Collection Microtubes (racked); Collection Microtube Caps

  For ordering information, see page 20.
- Kit for RNA cleanup after the QIAzol procedure:
  - RNeasy MinElute® Cleanup Kit (for cleanup of up to 45 µg RNA)
  - RNeasy Mini Kit (for cleanup of up to 100 µg RNA)
  - RNeasy Midi Kit (for cleanup of up to 1 mg RNA)
  - RNeasy Maxi Kit (for cleanup of up to 6 mg RNA)

  For ordering information, see page 21.

Important Notes

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. Otherwise, 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 –70°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. After disruption and homogenization in QIAzol Lysis Reagent, samples can be stored at –70°C for at least 1 month.

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 2 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 significantly reduced RNA yields.

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

Disruption and homogenization using the TissueRuptor

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

Disruption and homogenization using the TissueLyser

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 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. The TissueLyser 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 microtubes containing stainless steel beads of 5 mm mean diameter. For guidelines on using the TissueLyser, 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.

Protocol: Lysis and Homogenization of Fatty Tissues Using the TissueRuptor

This protocol is intended for fatty tissues, but can also be used with all other types of tissue.

Important points before starting

- Ensure that you are familiar with operating the TissueRuptor by referring to the *TissueRuptor User Manual* and *TissueRuptor Handbook*. For other rotor–stator homogenizers, refer to suppliers' guidelines.
- If using QIAzol Lysis Reagent for the first time, read "Important Notes" (page 9).
- Fresh, frozen, or RNA*later*/Allprotect stabilized tissues can be used.* If freezing tissues, flash-freeze in liquid nitrogen and immediately transfer to –70°C, where they can be stored for several months. 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 –70°C for at least 1 month. Incubate frozen lysates at 37°C in a water bath until completely thawed and salts are dissolved before continuing with step 4. Avoid prolonged incubation, which may compromise RNA integrity.

Procedure

1. **Add QIAzol Lysis Reagent to an appropriate vessel for disruption and homogenization and subsequent centrifugation: 1 ml QIAzol Lysis Reagent per 100 mg tissue is required. The volume of tissue should not exceed 10% of the volume of QIAzol Lysis Reagent.**

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

2. **Excise the tissue sample from the animal or remove it from storage. Determine the amount of tissue and place it into the QIAzol Lysis Reagent. Proceed immediately to step 3.**

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

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

* RNA*later* RNA Stabilization Reagent cannot be used with adipose tissue due to the high abundance of fat, but can be used with other fatty tissues such as brain.

RNA in harvested tissues is not protected until the tissues are treated with RNA*later* or Allprotect 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. **Place the tip of the TissueRuptor disposable probe into the QIAzol Lysis Reagent, and operate the TissueRuptor at full speed until the tissue lysate is uniformly homogeneous (usually 20–40 s).**

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

   Note: Incomplete homogenization leads to significantly reduced RNA yields. Homogenization with the TissueRuptor or TissueLyser generally results in higher RNA yields than with other methods.

   Optional: For samples containing a relatively high content of fat, proteins, polysaccharides, or extracellular material, centrifuge the homogenate at 12,000 x *g* for 10 min at 4°C to remove insoluble material. Carefully transfer the supernatant to a new tube, and proceed to step 4.

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

   This step promotes dissociation of nucleoprotein complexes.

5. **Add 0.2 ml chloroform per 1 ml QIAzol Lysis Reagent pipetted in step 1. Securely cap the tube containing the homogenate, and shake it vigorously for 15 s.**

   Thorough mixing is important for subsequent phase separation.

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

7. **Centrifuge at 12,000 x *g* for 15 min at 4°C.**

   After centrifugation, the sample separates into 3 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 is approximately 60% of the volume of the QIAzol Lysis Reagent pipetted in step 1.

8. **Transfer the upper, aqueous phase to a new tube. Add 0.5 ml isopropanol per 1 ml QIAzol Lysis Reagent pipetted in step 1. Mix thoroughly by vortexing.**

9. **Place the tube on the benchtop at room temperature for 10 min.**

10. **Centrifuge at 12,000 x *g* for 10 min at 4°C.**

11. **Carefully aspirate and discard the supernatant.**

    The RNA pellet is often visible as a gel-like or white pellet at the bottom of the tube.

12. **Add at least 1 ml of 75% ethanol per 1 ml QIAzol Lysis Reagent pipetted in step 1. Centrifuge at 7500 x *g* for 5 min at 4°C.**

    If the RNA pellet floats or sticks to the side of the tube, bring it to the bottom of the tube by centrifuging at 12,000 x *g* for 5 min at 4°C.

13. **Remove the supernatant completely, and briefly air-dry the RNA pellet.**

    Do not dry the RNA using a vacuum.

14. **Redissolve the RNA in an appropriate volume of RNase-free water. Clean up the RNA using the RNeasy MinElute Cleanup Kit or RNeasy Mini, Midi, or Maxi Kit.**

    We recommend RNA cleanup to remove contaminating phenol. The RNeasy MinElute Cleanup Kit and RNeasy Mini, Midi, and Maxi Kits allow cleanup of up to 45 µg, 100 µg, 1 mg, and 6 mg total RNA, respectively. For details, refer to the RNA cleanup protocol in the handbook supplied with these kits.

Protocol: Lysis and Homogenization of Fatty Tissues Using the TissueLyser

This protocol is intended for fatty tissues, but can also be used with all other types of tissue.

Important points before starting

- Ensure that you are familiar with operating the TissueLyser by referring to the operating instructions and *TissueLyser Handbook*. For other bead mills, refer to suppliers' guidelines.
- If using QIAzol Lysis Reagent for the first time, read "Important Notes" (page 9).
- Fresh, frozen, or RNA*later*/Allprotect stabilized tissues can be used.* If freezing tissues, flash-freeze in liquid nitrogen and immediately transfer to –70°C, where they can be stored for several months. 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 –70°C for at least 1 month. Incubate frozen lysates at 37°C in a water bath until completely thawed and salts are dissolved before continuing with step 4. Avoid prolonged incubation, which may compromise RNA integrity.
- In the procedure below, ▲ refers to use of the TissueLyser Adapter Set 2 x 24 with 5 mm diameter stainless steel beads (for <100 mg tissue), and ● refers to use of the TissueLyser Adapter Set 2 x 96 with 5 mm diameter stainless steel beads (for <75 mg tissue).

Procedure

1. **Add ▲ one stainless steel bead (5 mm mean diameter) per 2 ml microcentrifuge tube, or ● one stainless steel bead (5 mm mean diameter) per collection microtube. Place the tubes on dry ice.**

   The tubes do not need to be placed on dry ice if the tissue samples are stabilized in RNA*later* or Allprotect Reagent.

2. **Excise the tissue samples from the animal or remove them from storage. Determine the amount of each tissue. Place each tissue into a tube from step 1.**

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

   If the tissue samples were stored in RNA*later* or Allprotect Reagent, remove them from the reagent using forceps and be sure to remove any excess reagent or crystals that may have formed.

* RNA*later* RNA Stabilization Reagent cannot be used with adipose tissue due to the high abundance of fat, but can be used with other fatty tissues such as brain.

RNA in harvested tissues is not protected until the tissues are treated with RNA*later* or Allprotect 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. **Remove the tubes from the dry ice. Add QIAzol Lysis Reagent to each tube: 1 ml QIAzol Lysis Reagent per 100 mg tissue is required. The volume of tissue should not exceed 10% of the volume of QIAzol Lysis Reagent.**

   ▲ Place the tubes in the TissueLyser Adapter Set 2 x 24. Operate the TissueLyser for 2 min at 20 Hz. Disassemble the adapter set, rotate the rack of tubes so that the tubes nearest to the TissueLyser are now outermost, and reassemble the adapter set. Operate the TissueLyser for another 2 min at 20 Hz.

   ● Close the collection microtubes using the collection microtube caps. Place the rack of tubes in the TissueLyser Adapter Set 2 x 96. Operate the TissueLyser for 2 min at 20 Hz. Disassemble the adapter set, rotate the rack of tubes so that the tubes nearest to the TissueLyser are now outermost, and reassemble the adapter set. Operate the TissueLyser for another 2 min at 20 Hz.

   The time and frequency depend on the tissue being processed and can be increased until the tissue is completely homogenized (e.g., up to 2 x 5 min at 25 Hz). Rearranging the tubes allows even homogenization. Do not reuse the stainless steel beads.

   Note: Incomplete homogenization leads to significantly reduced RNA yields. Homogenization with the TissueLyser or TissueRuptor generally results in higher RNA yields than with other methods.

   Optional: For samples containing a relatively high content of fat, proteins, polysaccharides, or extracellular material, centrifuge the homogenate at 12,000 x *g* for 10 min at 4°C to remove insoluble material. Carefully transfer the supernatant to a new tube, and proceed to step 4.

4. **Place the tubes containing the homogenates on the benchtop at room temperature (15–25°C) for 5 min.**

   This step promotes dissociation of nucleoprotein complexes.

   ● Centrifuge the rack of collection microtubes at 6000 x *g* for 1 min at 15–25°C to collect residual liquid from the caps of the tubes.

5. **Add 0.2 ml chloroform per 1 ml QIAzol Lysis Reagent pipetted in step 3. Securely cap the tubes containing the homogenates, and shake vigorously for 15 s.**

   Thorough mixing is important for subsequent phase separation.

6. **Place the tubes containing the homogenates on the benchtop at room temperature for 2–3 min.**

7. **Centrifuge at ▲ 12,000 x *g* or ● 6000 x *g* for 15 min at 4°C.**

   After centrifugation, the sample separates into 3 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 is approximately 60% of the volume of the QIAzol Lysis Reagent pipetted in step 3.

8. **Transfer the upper, aqueous phase to new tubes. Add 0.5 ml isopropanol per 1 ml QIAzol Lysis Reagent pipetted in step 3. Mix thoroughly by vortexing.**

9. **Place the tubes on the benchtop at room temperature for 10 min.**

10. **Centrifuge at 12,000 x *g* for 10 min at 4°C.**

11. **Carefully aspirate and discard the supernatants.**

    The RNA pellet is often visible as a gel-like or white pellet at the bottom of the tube.

12. **Add at least 1 ml of 75% ethanol per 1 ml QIAzol Lysis Reagent pipetted in step 3. Centrifuge at 7500 x *g* for 5 min at 4°C.**

    If the RNA pellet floats or sticks to the side of the tube, bring it to the bottom of the tube by centrifuging at 12,000 x *g* for 5 min at 4°C.

13. **Remove the supernatants completely, and briefly air-dry the RNA pellets.**

    Do not dry the RNA using a vacuum.

14. **Redissolve the RNA in an appropriate volume of RNase-free water. Clean up the RNA using the RNeasy Micro, Mini, Midi, or Maxi Kit.**

    We recommend RNA cleanup to remove contaminating phenol. The RNeasy MinElute Cleanup Kit and RNeasy Mini, Midi, and Maxi Kits allow cleanup of up to 45 µg, 100 µg, 1 mg, and 6 mg total RNA, respectively. For details, refer to the RNA cleanup protocol in the handbook supplied with these kits.

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 protocols in this handbook or sample and assay technologies (for contact information, see back cover or 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 5), 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 6 and 7.
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.
RNA difficult to dissolve		
a)	RNA pellet overdried	Air-dry RNA pellets instead of using a vacuum. If necessary, dissolve the RNA in a larger volume of RNase-free water, or allow more time for the RNA to dissolve.
b)	Too much isopropanol in RNA pellet	Be sure to wash the RNA pellet with 75% ethanol, as described in the protocol, to remove isopropanol. If necessary, dissolve the RNA in a larger volume of RNase-free water, or allow more time for the RNA to dissolve.
Low RNA yield		
a)	Insufficient disruption and homogenization	See "Disrupting and homogenizing starting material" (page 9) for details on disruption and homogenization methods.

		Comments and suggestions
		In subsequent preparations, reduce the amount of starting material and/or increase the volume of QIAzol Lysis Reagent and the homogenization time.
b)	RNA pellet incompletely dissolved	Check for residual pellet. Be sure to wash any RNA from the side of the tube, especially if the tube is made of glass. See also "RNA difficult to dissolve" above.
Low A_{260}/A_{280} value		
a)	Not enough 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)	Contamination of aqueous phase with phenol	When removing the aqueous phase, be sure not to carry over any of the other phases. After the QIAzol procedure, clean up the RNA by following an RNeasy RNA cleanup protocol.
c)	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. This step is important to promote dissociation of nucleoprotein complexes.
d)	RNA pellet incompletely dissolved	Check for residual pellet. Be sure to wash any RNA from the side of the tube, especially if the tube is made of glass. See also "RNA difficult to dissolve" above.
e)	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.

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

	Comments and suggestions
RNA degraded	
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 QIAzol procedure quickly, especially the first few steps. See "Handling and storing starting material" (page 9).
DNA contamination in downstream experiments	
a) Phase separation performed at too high a temperature	The phase separation (step 7) should be performed at 4°C. Make sure that the centrifuge does not heat above 10°C during the centrifugation.
b) Interphase contamination of aqueous phase	Contamination of the aqueous phase with the interphase results in an increased DNA content in the purified RNA. Make sure to transfer the aqueous phase without interphase contamination.
c) Not enough 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	Treat the RNA sample with DNase and then clean up the RNA using an RNeasy Kit. Alternatively, carry out RNA cleanup and on-column DNase digestion using an RNeasy Kit. For details, see the handbook supplied with the RNeasy Kit.

Ordering Information

Product	Contents	Cat. no.
QIAzol Lysis Reagent (200 ml)	200 ml QIAzol Lysis Reagent	79306
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
TissueLyser II	Universal laboratory mixer-mill	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	69982
TissueLyser Adapter Set 2 x 96	2 sets of Adapter Plates for use with Collection Microtubes (racked) on the TissueLyser	69984
Collection Microtubes (racked)	Nonsterile polypropylene tubes (1.2 ml), 960 in racks of 96	19560

Ordering Information

Product	Contents	Cat. no.
Collection Microtube Caps (120 x 8)	Nonsterile polypropylene caps for collection microtubes (1.2 ml), 960 in strips of 8	19566
TissueLyser Single-Bead Dispenser, 5 mm	For dispensing individual beads (5 mm diameter)	69965
TissueLyser 5 mm Bead Dispenser, 96-Well	For dispensing 96 beads (5 mm diameter) in parallel	69975
Stainless Steel Beads, 5 mm (200)	Stainless Steel Beads, suitable for use with the TissueLyser system	69989
TissueRuptor	Handheld rotor–stator homogenizer, 5 TissueRuptor Disposable Probes	9001271* 9001272† 9001273‡ 9001274§
TissueRuptor Disposable Probes (25)	25 nonsterile plastic disposable probes for use with the TissueRuptor	990890
RNeasy MinElute Cleanup Kit (50)	50 RNeasy MinElute Spin Columns, Collection Tubes, RNase-Free Reagents and Buffers	74204
RNeasy Mini Kit (50)¶	50 RNeasy Mini Spin Columns, Collection Tubes, RNase-Free Reagents and Buffers	74104
RNeasy Midi Kit (10)¶	10 RNeasy Midi Spin Columns, Collection Tubes, RNase-Free Reagents and Buffers	75142
RNeasy Maxi Kit (12)	12 RNeasy Maxi Spin Columns, Collection Tubes, RNase-Free Reagents and Buffers	75162

* 120 V, 60 Hz (for North America 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)

¶ Larger kit size available; see www.qiagen.com/RNA.

Ordering Information

Product	Contents	Cat. no.
Related products		
RNeasy Lipid Tissue Kits — for purification of total RNA from fatty tissues and all other types of tissue		
RNeasy Lipid Tissue Mini Kit (50)	50 RNeasy Mini Spin Columns, Collection Tubes, QIAzol Lysis Reagent, RNase-Free Reagents and Buffers	74804
RNeasy Lipid Tissue Midi Kit (10)	10 RNeasy Midi Spin Columns, Collection Tubes, QIAzol Lysis Reagent, RNase-Free Reagents and Buffers	75842
RNeasy Fibrous Tissue Kits — for purification of total RNA from fiber-rich tissues		
RNeasy Fibrous Tissue Mini Kit (50)	50 RNeasy Mini Spin Columns, Collection Tubes, Proteinase K, RNase-Free DNase I, RNase-Free Reagents and Buffers	74704
RNeasy Fibrous Tissue Midi Kit (10)	10 RNeasy Midi Spin Columns, Collection Tubes, Proteinase K, RNase-Free DNase I, RNase-Free Reagents and Buffers	75742
RNeasy Plus Mini Kit — for purification of total RNA from cultured cells and tissues using gDNA Eliminator columns		
RNeasy Plus Mini Kit (50)	50 RNeasy Mini Spin Columns, 50 gDNA Eliminator Mini Spin Columns, Collection Tubes, RNase-Free Reagents and Buffers	74134

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