March 2021

# QIAzol® Handbook

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

Sample to Insight

QIAGEN

# Contents

# Kit Contents

| **QIAzol Lysis Reagent**<br>**Catalog no.** | **(200 ml)**<br>**79306** |
|---|---|
| QIAzol Lysis Reagent* | 200 ml |
| Quick-Start Protocol | 1 |

* Contains a guanidine salt. Not compatible with disinfectants containing bleach. See page 4 for Safety Information.

# Shipping and Storage

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

# Intended Use

The 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.

# 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 |

The 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.

# 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.

# Introduction

The 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 the 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, 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 the 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 safety data sheets (SDSs), available from the product supplier.

- Chloroform
- Isopropanol
- Ethanol, 75%
- RNase-free water
- Refrigerated laboratory centrifuge or microcentrifuge (capable of 12,000 x *g*)
- For stabilization of RNA in tissues (see page 7): RNAprotect® Tissue Reagent or Allprotect Tissue Reagent (see Ordering Information, page 20) or liquid nitrogen
- Equipment for tissue disruption and homogenization (see page 8). We recommend one of the following:
  - TissueRuptor® II with TissueRuptor Disposable Probes
  - TissueLyser II with the following accessories: TissueLyser Adapter Set 2 x 24; TissueLyser Single-Bead Dispenser, 5 mm; and Stainless Steel Beads, 5 mm
  - TissueLyser II 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); and 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 20.

# Important Notes

## Handling and storing starting material

RNA in harvested tissue is not protected until the sample is treated with RNAprotect Tissue 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 RNAprotect Tissue Reagent at room temperature. An alternative to RNAprotect Tissue Reagent is Allprotect Tissue Reagent, which provides immediate stabilization of DNA, RNA and protein in tissue samples at room temperature.

**Note**: RNAprotect Tissue 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 II (for processing samples individually) or the TissueLyser II (for processing multiple samples simultaneously). Disruption and 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

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 II 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 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. 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 microtubes 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.

# Protocol: Lysis and Homogenization of Fatty Tissues Using the TissueRuptor II

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 II 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 7).
- Fresh, frozen or RNAprotect Tissue Reagent- or 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.

* RNAprotect Tissue 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.

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 RNAprotect Tissue Reagent 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 in harvested tissues is not protected until the tissues are treated with RNAprotect Tissue Reagent 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 II at full speed until the tissue lysate is uniformly homogeneous (usually 20–40 s).

   **Note**: To avoid damage to the TissueRuptor II 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 II or TissueLyser II 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 II

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 II 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 7).
- Fresh, frozen or RNAprotect Tissue Reagent- or 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 the 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 hereafter, ▲ 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).

* RNAprotect Tissue 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.

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 RNAprotect Tissue Reagent 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 RNAprotect Tissue Reagent 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 in harvested tissues is not protected until the tissues are treated with RNAprotect Tissue Reagent 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 II for 2 min at 20 Hz. 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.

   ● 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 II for 2 min at 20 Hz. 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.

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 II or TissueRuptor II 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. |

| | | Comments and suggestions |
|---|---|---|
| **Low RNA yield** | | |
| a) | Insufficient disruption and homogenization | See "Disrupting and homogenizing starting material" (page 8) for details on disruption and homogenization methods.<br>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" in the previous page. |
| **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" in the previous page. |
| 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. |
| **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.<br>See "Handling and storing starting material" (page 7). |

* 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.

| | | Comments and suggestions |
|---|---|---|
| **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. |

# Appendix A: General Remarks on Handling RNA

## Handling RNA

RNases are very stable and active enzymes that generally do not require cofactors to function. Because 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. 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. To remove RNase contamination from bench surfaces, nondisposable plasticware, and laboratory equipment (e.g., pipets and electrophoresis tanks), use general laboratory reagents. To decontaminate plasticware, rinse with 0.1 M NaOH, 1 mM EDTA,* followed by RNase-free water (see "Solutions", page 21), or rinse with chloroform* if the plasticware is chloroform resistant. To decontaminate electrophoresis tanks, clean with detergent (e.g., 0.5% SDS),* rinse with RNase-free water, then rinse with ethanol (if the tanks are ethanol resistant), and allow 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.

## 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.* 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.

## 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 diethyl pyrocarbonate (DEPC)*, as described in "Solutions" below.

## 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 has 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 temperatures between –70 and –15°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 the amounts photometrically. Small amounts of RNA can be quantified using quantitative RT-PCR or fluorometric quantification.

## Spectrophotometric quantification of RNA

**Using the QIAxpert® UV/VIS Spectrophotometer for microvolume analysis**

To determine the concentration of your RNA sample purified with RNeasy QIAGEN kit, use the corresponding RNeasy App on the QIAxpert, which allows analysis at the level of microvolumes. For more information, see the QIAxpert product page (**www.qiagen.com/qiaxpert-system**).

**Using a standard spectrophotometer**

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 milliliter ($A_{260}$ = 1 → 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 24), the ratio between the absorbance values at 260 and 280 nm gives an estimate of RNA purity. When measuring RNA samples, be certain that cuvettes are RNase-free, especially if the RNA is to be recovered after measurement. 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 21). Use the buffer in which the RNA is diluted to blank the spectrophotometer. An example of the calculation involved in RNA quantification is shown below:

| | |
|---|---|
| Volume of RNA sample | = 100 µl |
| Dilution | = 10 µl of RNA sample + 490 µl of 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<br>= 44 µg/ml x 0.2 x 50<br>= 440 µg/ml |

| | |
|---|---|
| Total amount | = concentration x volume in milliliters<br>= 440 µg/ml x 0.1 ml<br>= 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

When using the QIAxpert with the corresponding RNeasy App. The assessment of RNA purity will be performed routinely See the QIAxpert user manual for more information (**www.qiagen.com/qiaxpert-system/user-manual**).

For standard photometric measurements, the ratio of the readings at 260 and 280 nm ($A_{260}/A_{280}$) provides an estimate of the purity of RNA with respect to contaminants, such as protein, that absorb in the UV spectrum. 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 when using pure water. 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 22).

## 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. 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.

* 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.

To prevent any interference by DNA in RT-PCR applications, such as with Applied Biosystems® and Rotor-Gene® 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 for reverse transcription. The kit integrates fast cDNA synthesis with rapid removal of genomic DNA contamination (see Ordering Information, page 26).

### Integrity of RNA

The integrity and size distribution of total RNA purified with RNeasy Plus Universal Kits can be checked by denaturing agarose gel electrophoresis and ethidium bromide* staining or by using the QIAxcel® system or Agilent® 2100 Bioanalyzer. 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. As a useful measure of RNA integrity, the QIAxcel Advanced system and the Agilent 2100 Bioanalyzer provide an RNA integrity score (RIS) and an RNA integrity number (RIN), respectively. Ideally, the value should be close to 10, but in many cases (particularly with tissue samples), RNA quality is greatly influenced by how well the original sample was preserved.

* 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.

# 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 |
| RNAprotect Tissue Reagent (50 ml) | 50 ml RNAprotect Tissue Reagent for stabilization of RNA in 25 x 200 mg tissue samples | 76104 |
| RNAprotect Tissue Reagent (250 ml) | 250 ml RNAprotect Tissue Reagent for stabilization of RNA in 125 x 200 mg tissue samples | 76106 |
| RNAprotect Tissue Tubes (50 x 1.5 ml) | For stabilization of RNA in 50 x 50 mg tissue samples: 50 screw-top tubes containing 1.5 ml RNAprotect Tissue Reagent each | 76154 |
| RNAprotect Tissue Tubes (20 x 5 ml) | For stabilization of RNA in 20 x 500 mg tissue samples: 20 screw-top tubes containing 5 ml RNAprotect Tissue Reagent each | 76163 |
| TissueLyser II | Universal laboratory mixer-mill | Various |
| 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 |

| Product | Contents | Cat. no. |
|---|---|---|
| TissueLyser Adapter Set 2 x 96 | 2 sets of Adapter Plates for use with Collection Microtubes (racked) on the TissueLyser II | 69984 |
| Collection Microtubes (racked) | 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 |
| 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 II | Handheld rotor–stator homogenizer, 5 TissueRuptor Disposable Probes | Various |
| TissueRuptor Disposable | 25 nonsterile plastic disposable probes for use with the TissueRuptor II | 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 |

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

| Product | Contents | Cat. no. |
|---|---|---|
| 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 |
| **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 |

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.

Visit **www.qiagen.com/geneXpression** to find out more about standardized solutions for gene expression analysis — from RNA preparation to real-time RT-PCR.

# Document Revision History

| Date | Changes |
|---|---|
| 01/2014 | Initial release |
| 03/2021 | Updated the Kit Contents section, Ordering Information section, and the branding of RNA protection products. Added Appendices A and B. |

## Notes

**Limited License Agreement for QIAzol Kit**

Use of this product signifies the agreement of any purchaser or user of QIAzol Lysis Reagent to the following terms:

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QIAzol Lysis Reagent is a subject of US Patent No. 5,346,994 and foreign equivalents.