October 2019

RNAprotect® Handbook

RNAprotect Tissue Tubes
For collection of harvested animal tissues with immediate stabilization of the gene expression profile, and subsequent transport and storage

RNAprotect Tissue Reagent
For immediate stabilization of the gene expression profile in harvested animal tissues

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Kit Contents

RNAprotect Tissue Tubes	**(50 x 1.5 ml)**	**(20 x 5 ml)**
Catalog no.	**76154**	**76163**
RNAprotect Tissue Reagent (1.5 ml)	50	–
RNAprotect Tissue Reagent (5 ml)	–	20
Quick-Start Protocol	1	1

RNAprotect Tissue Reagent	**(50 ml)**	**(250 ml)**
Catalog no.	**76104**	**76106**
RNAprotect Tissue Reagent	50 ml	250 ml
Quick-Start Protocol	1	1

Intended Use

RNAprotect Tissue Tubes and RNAprotect Tissue Reagent are 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.

Storage

RNAprotect Tissue Tubes and RNAprotect Tissue Reagent should be stored dry at room temperature (15–25°C) and are stable for at least 12 months under these conditions, if not otherwise stated on the label.

Storage of RNAprotect Tissue Reagent at lower temperatures may cause precipitation. Before using the reagent, redissolve the precipitate by heating to 37°C with agitation.

Quality Control

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

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.

Introduction

RNA stabilization is an absolute prerequisite for reliable gene expression analysis. Immediate stabilization of RNA in biological samples is necessary because, directly after harvesting the samples, changes in the gene expression pattern occur due to specific and nonspecific RNA degradation as well as to transcriptional induction. Such changes need to be avoided for all reliable quantitative gene expression analyses, such as microarray analysis, quantitative RT-PCR, such as TaqMan® and LightCycler® technology, and other nucleic acid-based technologies.

RNAprotect Tissue Reagent is a novel technology for immediate preservation of the gene expression pattern in animal tissues, enabling reliable gene expression analysis. After harvesting, tissues are immediately submerged in RNAprotect Tissue Reagent, which rapidly permeates the tissues to stabilize and protect cellular RNA in situ. The reagent preserves RNA for up to 1 day at 37°C, 7 days at 15–25°C, or 4 weeks at 2–8°C, allowing transportation, storage, and shipping of samples without ice or dry ice. Alternatively, the samples can be archived at –30 to –15°C or –90 to –65°C. During storage or transport in RNAprotect Tissue Reagent, even at elevated temperatures (e.g., room temperature or 37°C), the cellular RNA remains intact and undegraded. RNAprotect technology allows large numbers of samples to be easily processed and replaces inconvenient, dangerous, and equipment-intensive methods, such as snap-freezing of samples in liquid nitrogen, storage at –90 to –65°C, cutting and weighing on dry ice, or immediate processing of harvested samples.

Note: RNAprotect Tissue Reagent is not suitable for stabilization of RNA in animal cells, whole blood, plasma, or serum. The reagent also cannot be used to stabilize RNA in adipose tissue due to the high abundance of fat (however, RNA stabilization of other fatty tissues such as brain is possible).

This handbook provides a detailed protocol for stabilization of RNA in harvested animal tissues. Purification of RNA from the stabilized tissues can then be performed using QIAGEN kits (see Table 1).

Table 1. QIAGEN Kits for RNA Purification from Stabilized Tissues

RNA purified	Procedure	QIAGEN kit Total RNA
Total RNA	Manual	RNeasy® Micro Kit RNeasy Mini Kit or RNeasy Protect Mini Kit RNeasy Midi Kit* RNeasy Maxi Kit* RNeasy Plus Micro Kit RNeasy Plus Mini Kit RNeasy Plus Universal Mini Kit RNeasy Plus Universal Midi Kit RNeasy Fibrous Tissue Mini Kit RNeasy Lipid Tissue Mini Kit
Total RNA including miRNA	Manual	miRNeasy Micro Kit miRNeasy Tissue/Cells Advanced Mini Kit miRNeasy Mini Kit
Total RNA	Manual (high-throughput)	RNeasy 96 Universal Tissue Kit†
Total RNA	Automated (1–6 samples/run)	EZ1 RNA Tissue Mini Kit‡§
Total RNA	Automated (96 samples/run)	RNeasy 96 QIAcube® HT Kit RNeasy 96 Universal Tissue 8000 Kit¶
Total RNA including miRNA	Manual (high-throughput)	miRNeasy 96 Kit
Total RNA and Genomic DNA	Manual	AllPrep DNA/RNA Micro Kit AllPrep DNA/RNA Mini Kit AllPrep DNA/RNA Protein Mini Kit
mRNA	Manual	Oligotex® Direct mRNA Mini Kit Oligotex Direct mRNA Midi/Maxi Kit

* Requires a centrifuge capable of attaining 3000–5000 x *g* and equipped with a swing-out rotor for 15 ml (midi) or 50 ml (maxi) centrifuge tubes.

† Requires the QIAGEN 96-Well-Plate Centrifugation system and, optionally, the QIAvac 96 vacuum manifold.

‡ Requires the BioRobot® EZ1 and EZ1 RNA Card.

§ Can also be used to purify total RNA and genomic DNA in the same eluate.

¶ Requires the BioRobot Universal System and Application Pack, Gene Expression or BioRobot 8000.

The range of QIAGEN kits for RNA purification is continuously expanding. Visit **www.qiagen.com/RNA** to find out about the latest kits.

Important Notes

RNA stabilization

RNA in harvested animal tissue is not protected until the tissue is completely submerged in a sufficient volume of RNAprotect Tissue Reagent. After harvesting, the tissue should be **immediately** placed in **at least 10 volumes of the reagent (or approximately 10 µl reagent per 1 mg tissue)**. Larger volumes can be used if necessary or desired. Smaller volumes may lead to RNA degradation during storage. Storage containers should be wide enough so that the reagent covers the entire tissue. Storage containers or tubes with large diameters may require more reagent to completely cover the tissue. The procedures for tissue harvesting and RNA stabilization should be carried out as quickly as possible.

Tissue size is critical for successful RNA stabilization with RNAprotect Tissue Reagent. Immediately upon contact, the reagent diffuses into the surface layer and outer portions of solid tissues. To ensure rapid and reliable stabilization of RNA even in the inner parts of solid tissues, the sample must be cut into slices **less than 0.5 cm thick**. The slices can be any convenient size, provided one dimension of the sample is <0.5 cm. If the slices are thicker than 0.5 cm, the reagent will diffuse too slowly into the interior of the sample and RNA degradation will occur. Small organs such as rat kidney and spleen or most mouse organs (except liver) do not require slicing: the entire organ can be placed in RNAprotect Tissue Reagent.

The following guide may help you to determine the amount of RNAprotect Tissue Reagent required for RNA stabilization:

- A cube of rat kidney with a 5 mm edge length ($[5\ mm]^3 = 125\ mm^3 = 125\ µl$) weighs 150–175 mg and requires at least 1.5–1.75 ml of the reagent.
- A 3 mm cube ($[3\ mm]^3 = 27\ mm^3 = 27\ µl$) of most animal tissues weighs 30–35 mg and requires at least 300–350 µl of the reagent.

Although weighing tissues is generally more accurate, RNA in unstabilized tissues will degrade during weighing. In some cases, however, it may be more convenient to quickly estimate the weight of tissue pieces. Average weights of various entire adult mouse organs and the corresponding amounts of RNAprotect Tissue Reagent to use are given in Table 2.

RNA in tissues weighing up to 150 mg can be stabilized in 1.5 ml RNAprotect Tissue Tubes. For tissue pieces weighing more than 150 mg and less than 500 mg, 5 ml RNAprotect Tissue Tubes can be used.

Table 2. Tissue Weights and Amounts of RNAprotect Tissue Reagent

Mouse organ	Weight (mg)	Minimum amount of RNAprotect Tissue Reagent (ml)	Appropriate RNAprotect Tissue Tube
Kidney	180–250	1.8–2.5	5 ml
Spleen	100–160	1–1.6	1.5 ml or 5 ml
Lung	190–210	1.9–2.1	5 ml
Heart	100–170	1–1.7	1.5 ml or 5 ml
Liver	1000–1800	10–18	Use other container

RNA purification

Before using a QIAGEN kit (see Table 1, page 6) to purify RNA from tissues stabilized with RNAprotect Tissue Reagent, carefully read the handbook supplied with the kit. The handbook provides guidelines on determining the amount of starting material and on choosing the appropriate method for disruption and homogenization of tissues. Optimal RNA yield and purity depend on using the correct amount of starting material and on efficient disruption and homogenization.

Weighing tissue is the most accurate way to quantify the amount of starting material. Storage in RNAprotect Tissue Reagent does not dissolve or disrupt the structure of tissue samples. Stabilized tissue can be removed from the reagent for weighing and cutting at room temperature. The tissue pieces can then be used for RNA purification or returned to the reagent for continued storage.

After storage in RNAprotect Tissue Reagent, tissues become slightly harder than fresh or thawed tissues. However, disruption and homogenization of this tissue is usually not a problem.

Tissues stored in RNAprotect Tissue Reagent at –30 to –15°C can be thawed prior to cutting and weighing at room temperature. RNA remains intact for up to 20 freeze–thaw cycles.

Protocol: Stabilization of RNA in Harvested Animal Tissues

This protocol describes how to stabilize and store human and animal tissues in RNAprotect Tissue Reagent. For RNA purification from the stabilized tissues using a QIAGEN kit (see Table 1, page 6), refer to the handbook supplied with the kit.

Important points before starting

- If using RNAprotect Tissue Tubes or RNAprotect Tissue Reagent for the first time, read "Important Notes" (page 7).
- RNAprotect Tissue Reagent may form a precipitate during storage below room temperature (15–25°C). Before using the reagent, redissolve the precipitate by heating to 37°C with agitation.
- RNAprotect Tissue Tubes are for single use only. Do not reuse.
- Only fresh, unfrozen tissues can be stabilized using RNAprotect Tissue Reagent. Previously frozen tissues thaw too slowly in the reagent, preventing the reagent from diffusing into the tissues quickly enough to prevent RNA degradation.

Procedure

1. Before excising the tissue sample, estimate the volume (or weight) of the sample to be stabilized in RNAprotect Tissue Reagent.
2. Determine the appropriate volume of RNAprotect Tissue Reagent for preserving the tissue. At least 10 volumes of the reagent (or approximately 10 µl reagent per 1 mg of tissue) is required. Pipet the correct amount of reagent into an appropriate collection vessel, or choose the appropriate sized RNAprotect Tissue Tube.

   Note: Be sure to completely submerge the tissue in RNAprotect Tissue Reagent. For details, see "Important Notes", page 7.

3. Excise the tissue sample from the animal and, if necessary, cut it into slices less than 0.5 cm thick. Perform this step as quickly as possible and proceed immediately to step 4.

   Note: For effective RNA stabilization, the tissue sample must be less than 0.5 cm thick. For details, see "Important Notes", page 7.

4. Completely submerge the tissue piece(s) in the collection vessel containing RNAprotect Tissue Reagent from step 2.

   Note: The tissue sample must be **immediately** submerged in RNAprotect Tissue Reagent to protect the RNA.

5. Store the tissue submerged in RNAprotect Tissue Reagent for up to 4 weeks at 2–8°C, up to 7 days at 15–25°C, or up to 1 day at 37°C.

   For archival storage at –30 to –15°C, first incubate the tissue overnight in the reagent at 2–8°C. Then transfer the tissue, in the reagent, to –30 to –15°C for storage.

   For archival storage at –90 to –65°C, first incubate the tissue overnight in the reagent at 2–8°C. Then remove the tissue from the reagent, and transfer it to –90 to –65°C for storage.

   Note: Lower temperatures are recommended for longer storage (e.g., 2–8°C for up to 4 weeks instead of 37°C or room temperature; –30 to –15°C or –90 to –65°C for longer storage).

   Tissues stored in RNAprotect Tissue Reagent at –30 to –15°C may not freeze. The low temperature may cause the formation of crystals or a precipitate in the reagent. This will not affect subsequent RNA purification. There is no need to redissolve the precipitate. RNAprotect stabilized tissues stored at –30 to –15°C or –90 to –65°C can be thawed at room temperature and frozen again for up to 20 freeze–thaw cycles without affecting RNA quality or yield.

   If transporting tissue samples in RNAprotect Tissue Reagent, ensure that the tissues always remain submerged in the reagent. Either keep the tubes upright during transport or fill the tubes completely with RNAprotect Tissue Reagent.

6. After storage, purify RNA using a QIAGEN kit (see Table 1, page 6).

   Be sure to remove tissues from RNAprotect Tissue Reagent prior to disruption and homogenization in the RNA purification procedure. If tissues were stored at –30 to –15°C, remove any crystals that may have formed.

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**

	Comments and suggestions
RNA degraded	
a) Harvested animal tissue not immediately stabilized	Submerge the tissue in the appropriate volume of RNAprotect Tissue Reagent immediately after harvesting.
b) Too much animal tissue for proper stabilization	Reduce the amount of tissue, increase the amount of RNAprotect Tissue Reagent, or use 5 ml RNAprotect Tissue Tubes (see "Important Notes", page 7).
c) Animal tissue too thick for stabilization	Cut large samples into slices less than 0.5 cm thick for stabilization in RNAprotect Tissue Reagent.
d) Animal tissue not fully submerged in RNAprotect Tissue Reagent	Ensure that the tissue remains fully submerged in the RNAprotect Tissue Reagent. Smaller tissues may tend to stick to the lid or the side of the container.
e) Frozen animal tissue used for stabilization	Use only fresh, unfrozen tissue for stabilization in RNAprotect Tissue Reagent.
f) Storage duration in RNAprotect Tissue Reagent exceeded	RNAprotect stabilized tissue can be stored for up to 1 day at 37°C, up to 7 days at 15–25°C, or up to 4 weeks at 2–8°C, and can be archived at –30 to –15°C or –90 to –65°C. We recommend lower temperatures whenever possible.
g) RNA degradation during RNA purification	Although all QIAGEN buffers for RNA purification have been tested and are guaranteed RNase-free, RNases can be introduced during use. Be certain not to introduce any RNases during RNA purification or later handling.

Ordering Information

Product	Contents	Cat. no.
RNAprotect Tissue 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 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
RNAprotect Tissue Reagent (50 ml)	For stabilization of RNA in 25 x 200 mg tissue samples: 50 ml RNAprotect Tissue Reagent each	76104
RNAprotect Tissue Reagent (250 ml)	For stabilization of RNA in 125 x 200 mg tissue samples: 250 ml RNAprotect Tissue Reagent each	76106
RNeasy Micro Kit — for purification of concentrated total RNA from small amounts of tissue or small numbers of cells		
RNeasy Micro Kit (50)	50 RNeasy MinElute® Spin Columns, Collection Tubes, RNase-Free DNase I, Carrier RNA, RNase-Free Reagents and Buffers	74004
RNeasy Kits — for purification of total RNA from animal cells or tissues, yeast, or bacteria		
RNeasy Mini Kit (50)*	50 RNeasy Mini Spin Columns, Collection Tubes, RNase-Free Reagents and Buffers	74104
RNeasy Midi Kit (50)*	50 RNeasy Midi Spin Columns, Collection Tubes, RNase-Free Reagents and Buffers	75144
RNeasy Maxi Kit (12)	12 RNeasy Maxi Spin Columns, Collection Tubes, RNase-Free Reagents and Buffers	75162

Product	Contents	Cat. no.
RNeasy Plus Kits — for phenol-free total RNA extraction from cells/tissues with removal of genomic DNA contamination		
RNeasy Plus Micro Kit (50)	50 RNeasy MinElute Spin Columns, 50 gDNA Eliminator Spin Columns, Collection Tubes, Carrier RNA, RNase-Free Water and Buffers	74034
RNeasy Plus Mini Kit (50)*	50 RNeasy Mini Spin Columns, 50 gDNA Eliminator Mini Spin Columns, Collection Tubes, RNase-Free Reagents and Buffers	74134
RNeasy Plus Universal Kits — for purification of total RNA from all types of tissue using gDNA Eliminator Solution		
RNeasy Plus Universal Mini Kit (50)	50 RNeasy Mini Spin Columns, 50 gDNA Eliminator Solution, Collection Tubes, RNase-Free Water and Buffers	73404
RNeasy Plus Universal Midi Kit (10)	10 RNeasy Midi Spin Columns, 10 gDNA Eliminator Solution, Collection Tubes, RNase-Free Water and Buffers	73442
RNeasy Fibrous Tissue Kit — 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 Lipid Tissue Kit — for purification of total RNA from fatty tissues		
RNeasy Lipid Tissue Mini Kit (50)	50 RNeasy Mini Spin Columns, Collection Tubes, QIAzol® Lysis Reagent, RNase-Free Reagents and Buffers	74804

Product	Contents	Cat. no.
RNeasy Protect Mini Kit — for immediate stabilization of the gene expression profile in animal tissues and subsequent RNA purification		
RNeasy Protect Mini Kit (50)*	RNAprotect Tissue Reagent (50 ml), 50 RNeasy Mini Protect Midi Spin Columns, Collection Tubes, RNase-Free Reagents and Buffers	74124
miRNeasy Micro Kit — for purification of miRNA and total RNA from small amounts of cells and tissues		
miRNeasy Micro Kit (50)	50 RNeasy MinElute Spin Columns, Collection Tubes (1.5 ml and 2 ml), QIAzol Lysis Reagent, RNase-Free Reagents and Buffers	217084
miRNeasy Tissue/Cells Advanced Mini Kit – for purification of total RNA, including miRNA from tissue and cells		
miRNeasy Tissue/Cells Advanced Mini Kit (50)	For 50 total RNA preps: 50 RNeasy UCP MinElute Spin Columns, Collection Tubes (1.5 ml and 2 ml), RNase-free Reagents and Buffers	217604
miRNeasy Mini Kit — for purification of miRNA and total RNA from tissues and cells		
miRNeasy Mini Kit (50)	50 RNeasy Mini Spin Columns, Collection Tubes (1.5 ml and 2 ml), QIAzol Lysis Reagent, RNase-Free Reagents and Buffers	217004

Product	Contents	Cat. no.
RNeasy 96 Universal Tissue Kit — for high-throughput total RNA purification from any type of animal tissue		
RNeasy 96 Universal Tissue Kit (4)*†	For 4 x 96 total RNA preps: 4 RNeasy 96 Plates, Collection Microtubes, Elution Microtubes CL, Caps, S-Blocks, AirPore Tape Sheets, QIAzol Lysis Reagent, RNase-Free Reagents and Buffers	74881
EZ1 RNA Tissue Mini Kit — for automated total RNA purification from standard clinical tissues using the BioRobot EZ1		
EZ1 RNA Tissue Mini Kit (48)‡	For 48 RNA preps: Reagent Cartridges, Disposable Tips, Disposable Tip-Holders, Sample Tubes, Elution Tubes, Buffers, RNase-Free DNase Set	959034
RNeasy 96 QIAcube HT Kit — for automated high-throughput isolation of RNA from animal and human cells and tissue		
RNeasy 96 QIAcube HT Kit (5)	For 480 preps: RNeasy 96 plates, RNase-free water, buffers	74171
RNeasy 96 Universal Tissue 8000 Kit — for automated, high-throughput RNA purification from any type of animal tissue using the BioRobot 8000 or BioRobot Universal System		
RNeasy 96 Universal Tissue 8000 Kit (12)§	For 12 x 96 total RNA preps: 12 RNeasy 96 Plates, Collection Microtubes, Elution Microtubes CL, Caps, S-Blocks, QIAzol Lysis Reagent, RNase-Free Reagents and Buffers	967852

Product	Contents	Cat. no.
miRNeasy 96 Kit — for 96-well purification of microRNA and total RNA from tissues and cells		
miRNeasy 96 Kit (4)	For 4 x 96 preps: 4 RNeasy 96 plates, Collection Microtubes (racked), Elution Microtubes CL, Caps, S-Blocks, AirPore Tape Sheets, QIAzol Lysis Reagent, RNase-Free Reagents and Buffers	217061
AllPrep DNA/RNA Micro Kit — for simultaneous purification of DNA and RNA from small cell and tissue samples		
AllPrep DNA/RNA Micro Kit (50)	50 AllPrep DNA Mini Spin Columns, 50 RNeasy MinElute Spin Columns, Collection Tubes, RNase-Free Reagents and Buffers	80284
AllPrep DNA/RNA Mini Kit — for simultaneous purification of genomic DNA and total RNA from the same cell or tissue sample		
AllPrep DNA/RNA Mini Kit (50)	50 AllPrep DNA Mini Spin Columns, 50 RNeasy Mini Spin Columns, Collection Tubes, RNase-Free Reagents and Buffers	80204
AllPrep DNA/RNA Protein Mini Kit —for simultaneous purification of DNA, RNA, and protein from cells and tissues		
AllPrep DNA/RNA Protein Mini Kit (50)	50 AllPrep DNA Spin Columns, 50 RNeasy MinElute Spin Columns, Collection Tubes, Carrier RNA, and RNase Free Water	80004

Product	Contents	Cat. no.
Oligotex Direct mRNA Kits — for purification of poly A+ mRNA directly from animal cells or tissues		
Oligotex Direct mRNA Mini Kit (12)	For 12 mRNA minipreps: 420 µl Oligotex Suspension, Small Spin Columns, Collection Tubes, RNase-Free Reagents and Buffers	72022
Oligotex Direct mRNA Midi/Maxi Kit (6/2)	For 6 mRNA midipreps or 2 mRNA maxipreps: 1 ml Oligotex Suspension, Large Spin Columns, Collection Tubes, RNase-Free Reagents and Buffers	72041

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

† Requires the QIAGEN 96-Well-Plate Centrifugation system and, optionally, the QIAvac 96 vacuum manifold. For ordering information, visit **www.qiagen.com/products/accessories**

‡ Requires the BioRobot EZ1 and EZ1 RNA Card. For ordering information and recommended warranty, visit **www.qiagen.com/automation**

§ Requires the BioRobot Universal System and Application Pack, Gene Expression or BioRobot 8000. For ordering information and recommended warranty, visit **www.qiagen.com/automation**

QIAGEN also provides products for stabilization and purification of RNA from:

- Human blood — PAXgene™ Blood RNA System
- Human saliva — RNeasy Protect Saliva Mini Kit
- Cultured cells — RNeasy Protect Cell Mini Kit
- Bacteria — RNeasy Protect Bacteria Kits

For details, visit **www.qiagen.com/RNA**.

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.

Document Revision History

Date	Changes
10/2019	Change in product names. Removed discontinued products and added related products in Table 1 and in Ordering Information. Clarification of temperature ranges for tissue storage. Updated safety information from “MSDS” to “SDS”. Updated to latest template.

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