August 2023

QIAsymphony® RNA Handbook

For purification of total RNA from animal and human cells and tissues using the QIAsymphony SP

Sample to Insight

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Kit Contents

QIAsymphony RNA Kit Catalog no. Number of preps	(192) 931636 192
Buffer RLT Plus*	2 x 45 mL
Reagent Cartridge*†	2
Enzyme Rack	2
Piercing Lid	2
Reuse Seal Set‡	2
RNase-Free Water	10 mL
DNase I, RNase Free	2 x 1500 Kunitz units
Quick-Start Protocol	1

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

† Contains sodium azide as a preservative.

‡ A Reuse Seal Set contains 8 Reuse Seal Strips.

Storage

The QIAsymphony RNA Kit should be stored at room temperature (15–25°C), except for the RNase-Free DNase I, which should be stored immediately upon receipt at 2–8°C. When stored at 2–8°C and handled correctly, the lyophilized enzyme can be kept for at least 9 months without showing any reduction in performance.

When stored properly, the kit is stable until the expiration date on the kit box.

Partially used reagent cartridges can be stored for a maximum of 2 weeks, enabling cost-efficient use of reagents and more flexible sample processing. If a reagent cartridge is partially used, replace the cover of the trough containing the magnetic particles, seal the buffer troughs with the provided Reuse Seal Strips, and close the enzyme tubes with screw caps immediately at the end of the protocol run to avoid evaporation. Store the reagent cartridge at room temperature. Do not store used reagent cartridges at temperatures below 15°C. Once prepared, the enzyme rack with solubilized DNase should be stored with enzyme tubes closed with screw caps at 2–8°C for up to 6 weeks.

To avoid evaporation, the reagent cartridge should be open for a maximum of 15 hours (including run times) at a maximum environmental temperature of 30°C.

Running batches with low sample numbers (<24) will increase both the time that the reagent cartridge is open and the required buffer volumes, potentially reducing the total number of sample preparations possible per cartridge.

Avoid exposure of the reagent cartridges to UV light (e.g., used for decontamination) as exposure may cause accelerated aging of the reagent cartridges and buffers.

Intended Use

The QIAsymphony RNA Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN® products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments or to other applicable guidelines.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at **www.qiagen.com/safety** where you can find, view, and print the SDS for each QIAGEN kit and kit component.

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

Buffer RLT Plus contains guanidine thiocyanate and buffers in the reagent cartridge contain guanidine salts, which can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

Quality Control

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

Introduction

The QIAsymphony RNA Kit is designed for fully automated purification of:

- Total RNA from animal and human cells and tissues
- Total RNA (including miRNA) from cells and tissues*
- Total RNA from formalin-fixed, paraffin-embedded (FFPE) sections and cores*

Proven, performance-leading magnetic-particle technology provides high-quality RNA, which is suitable for direct use in any downstream applications, such as the following:

- RT-PCR
- Quantitative, real-time RT-PCR
- Differential display
- cDNA synthesis
- Northern, dot, and slot blot analyses
- Primer extension
- Poly A+ RNA selection
- RNase/S1 nuclease protection
- Microarrays

The QIAsymphony SP performs all steps of the sample preparation procedure, except for tissue disruption and homogenization, and the procedure can be scaled up or down, allowing purification from varying amounts of starting material. A single run processes 1–96 samples.

* Additional components must be purchased for these protocols.

Principle and procedure

QIAsymphony technology combines the speed and efficiency of silica-based RNA purification with the convenient handling of magnetic particles (Figure 1). RNA is purified from lysates in one step through its binding to the silica surface of the particles in the presence of a chaotropic salt. The particles are separated from the lysates using a magnet, and DNA is removed by treatment with RNase-free DNase. The purification procedure comprises 4 steps: lyse, bind, wash, and elute (see flowchart on page 11).

The magnetic particles are efficiently washed, and RNA is eluted in RNase-free water.

With the QIAsymphony RNA procedure, RNA molecules longer than 200 nucleotides are primarily purified. The procedure provides enrichment for mRNA since most RNAs <200 nucleotides (such as 5.8S rRNA, 5S rRNA, tRNAs, and other small RNAs, which together comprise 15–20% of total RNA) are largely excluded. Information about kits and protocols for purification of small RNA can be accessed online by searching **miRNA kit** on **www.qiagen.com**.

The QIAsymphony miRNA CT 400 protocol recovers total RNA including miRNA (tRNA may not be recovered efficiently). The RNA FFPE 130 protocol will also recover small RNA fragments, but yields of RNA smaller than 100 nt may be reduced.

Description of protocols

The following protocols are available for purifying RNA on the QIAsymphony SP:

- "RNA CT 400" for processing standard-volume (400 µL) samples containing ≤3 x 10^6 cultured cells or ≤20 mg easy-to-lyse tissue
- "RNA CT 800" for processing large-volume (800 µL) samples containing 3 x 10^6 to 1 x 10^7 cultured cells or ≤50 mg easy-to-lyse tissue

- "RNA FT 400" for processing ≤20 mg fibrous or fatty tissues
- "RNA FFPE 130" for processing 1–2 sections of FFPE tissue (≤10 µm thick)
- "miRNA CT 400" for isolation of total RNA, including miRNA, from samples containing ≤3 x 10^6 cultured cells or ≤20 mg tissue.

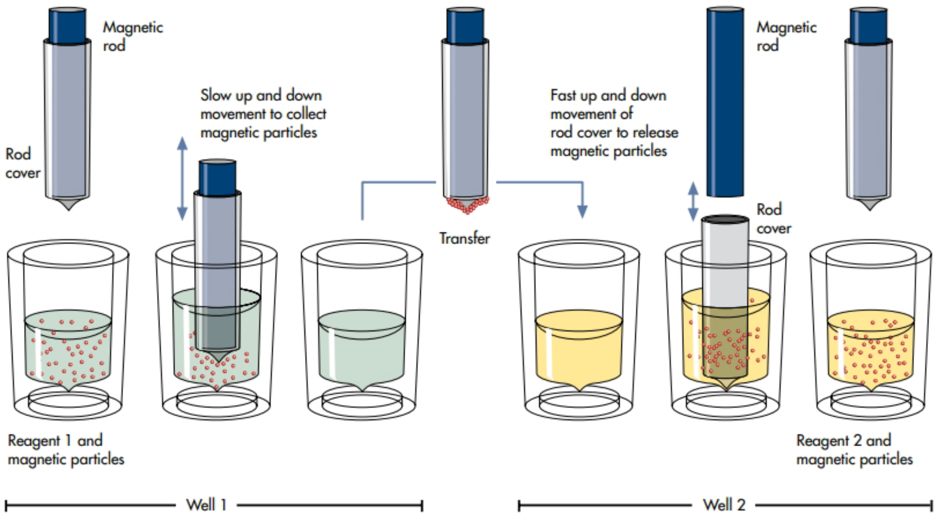
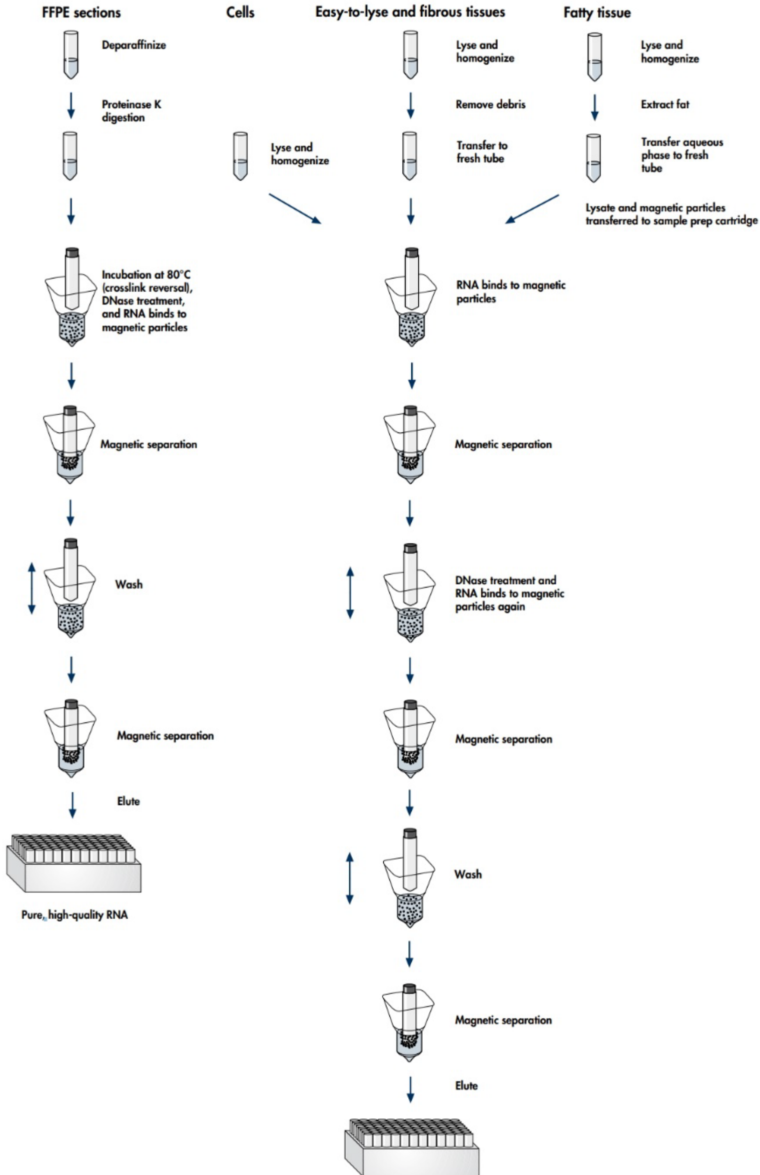


Figure 1. Schematic of the QIAsymphony SP principle. The QIAsymphony SP processes a sample containing magnetic particles as follows: A magnetic rod protected by a rod cover enters a well containing the sample and attracts the magnetic particles. The magnetic rod cover is positioned above another well and the magnetic particles are released. The QIAsymphony SP uses a magnetic head containing an array of 24 magnetic rods, and can therefore process up to 24 samples simultaneously. Steps 1 and 2 are repeated several times during sample processing.


QIAsymphony RNA Procedures
FFPE sections
Cells
Easy-to-lyse and fibrous tissues
Fatty tissue
Manual sample preparation
Deparaffinize
Proteinase K digestion
Lyse and homogenize
Lyse and homogenize
Remove debris
Transfer to fresh tube
Lyse and homogenize
Extract fat
Transfer aqueous phase to fresh tube
Lysate and magnetic particles transferred to sample prep cartridge
Incubation at 80°C (crosslink reversal), DNase treatment, and RNA binds to magnetic particles
RNA binds to magnetic particles
Magnetic separation
Magnetic separation
Wash
DNase treatment and RNA binds to magnetic particles again
Magnetic separation
Magnetic separation
Elute
Pure, high-quality RNA
Wash
Magnetic separation
Elute
Fully automated RNA purification

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.

For all protocols

- Sample Prep Cartridges, 8-well cartridges (cat. no. 997002)
- 8-Rod Covers (cat. no. 997004)
- Filter-Tips, 200 µL and 1500 µL (cat. nos. 990332 and 997024)
- Accessory Troughs (cat. no. 997012)
- Sample tubes or plates (e.g., QIAGEN tubes, conical, 2 mL, Qsym AS (500), QIAGEN cat. no. 997102, or 2 mL sample tubes with screw caps, Sarstedt® cat. no. 72.693, or without caps, Sarstedt cat. no. 72.608, or S-Blocks, QIAGEN cat. no. 19585). Compatible primary and secondary tube formats are listed under the **Resources** tab at **www.qiagen.com/QIAsymphony**.
- Elution tubes or plates (e.g., Elution Microtubes CL, cat. no. 19588). See the compatible elution tube formats under the **Resources** tab at **www.qiagen.com/QIAsymphony**.
- Pipettes, and sterile, RNase-free pipette tips with aerosol barriers
- Ethanol (96–100%)*
- 14.3 M b-mercaptoethanol (b-ME, commercially available solutions are usually 14.3 M) or dithiothreitol (DTT)
- Vortexer
- RNase-Free DNase Set (an extra set may be required for particularly DNA-rich samples, such as thymus or other lymphatic tissues; see ordering information, page 49)
- **Optional**: Reagent DX (antifoaming agent for Buffer RLT Plus, cat. no. 19088)

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

For tissue samples

- RNAprotect® Tissue Reagent, Allprotect® Tissue Reagent (see ordering information, page 49) or liquid nitrogen
- Equipment for sample disruption and homogenization. Depending on the method chosen, one or more of the following are required:
  - TissueLyser II system, comprising the TissueLyser II, the TissueLyser Adapter Set 2 x 24, Stainless Steel Beads, 5 mm, and (optional) the TissueLyser Single-Bead Dispenser, 5 mm (see ordering information, page 49)
  - TissueRuptor® II system, comprising the TissueRuptor II and TissueRuptor Disposable Probes (see ordering information, page 49)
  - Mortar and pestle

For fibrous tissues (e.g., muscle, skin) and for purification of total RNA including miRNA

- QIAGEN Proteinase K (cat. no. 19131)

For lipid-rich tissues (e.g., brain, breast)

- Chloroform

For FFPE tissue samples

- Deparaffinization Solution (cat. no. 19093)
- Buffer PKD (cat. no. 1034963)
- QIAGEN Proteinase K (cat. no. 19131)
- DNase Booster Buffer (cat. no. 1064143)

Important Notes

Automated purification on the QIAsymphony SP

The QIAsymphony SP makes automated sample preparation easy and convenient. Samples, reagents and consumables, and eluates are separated in different drawers. Simply load samples, reagents provided in special cartridges, and preracked consumables into the appropriate drawer before a run. Start the protocol and remove purified RNA from the "Eluate" drawer after processing. Refer to the *QIAsymphony SP/AS User Manual — Operating the QIAsymphony SP* for operating instructions.

Loading reagent cartridges into the "Reagents and Consumables" drawer

Reagents for purification of RNA are contained in an innovative reagent cartridge (Figure 2). Each trough of the reagent cartridge contains a particular reagent, such as magnetic particles, lysis buffer, wash buffer, or elution buffer. Partially used reagent cartridges can be reclosed with Reuse Seal Strips for later reuse, which avoids generation of waste due to leftover reagents at the end of the purification procedure.

Figure 2. QIAsymphony reagent cartridge. The reagent cartridge contains all reagents required for the protocol run.

Before starting the procedure, ensure that the magnetic particles are fully resuspended. Remove the magnetic-particle trough from the reagent cartridge frame, vortex it vigorously for at least 3 minutes, and replace it in the reagent cartridge frame before the first use. Place the reagent cartridge into the reagent cartridge holder. Place the enzyme rack into the reagent cartridge holder. Before using a reagent cartridge for the first time, place the piercing lid on top of the reagent cartridge (Figure 3, next page).

> **Important**: The piercing lid is sharp. Take care when placing it onto the reagent cartridge. Make sure to place the piercing lid onto the reagent cartridge in the correct orientation. After the magnetic-particle trough cover is removed and the enzyme rack tubes are opened (screw caps can be stored in dedicated slots, see Figure 2, page 14), the reagent cartridge is subsequently loaded into the "Reagents and Consumables" drawer.

> **Important**: The piercing lid is sharp. Take care when placing it onto the reagent cartridge. Make sure to place the piercing lid onto the reagent cartridge in the correct orientation.

After the magnetic-particle trough cover is removed and the enzyme rack tubes are opened (screw caps can be stored in dedicated slots, see Figure 2, page 14), the reagent cartridge is subsequently loaded into the "Reagents and Consumables" drawer.

Figure 3. Easy worktable setup with reagent cartridges.

Loading plasticware into the "Reagents and Consumables" drawer

Sample prep cartridges, 8-Rod Covers (both preracked in unit boxes), disposable filter-tips (200 µL tips provided in blue racks, 1500 µL tips provided in black racks), and Accessory Troughs are loaded into the "Reagents and Consumables" drawer into tip rack slot 5 and, if required, slot 12 (see Figure 4 and Table 1, page 18).

See Table 1 (page 18) for the consumables required for RNA protocols. For plasticware, see ordering information, page 49.

Note: Both types of tips have filters to help prevent cross-contamination.

Tip rack slots on the QIAsymphony SP worktable can be filled with either type of tip rack. The QIAsymphony SP will identify the type of tips loaded during the inventory scan.

Note: Do not refill tip racks before starting another protocol run. The QIAsymphony SP can use partially used tip racks.

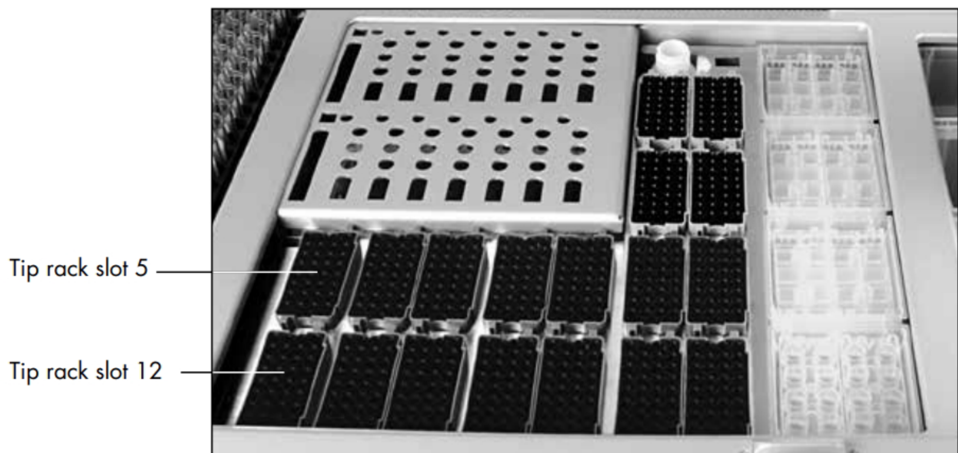


Figure 4. Setting up the QIAsymphony SP worktable setup – positions for accessory troughs.

Table 1. Consumables required for QIAsymphony RNA protocols

	Standard RNA CT 400		Large-volume RNA CT 800		Fibrous tissue RNA FT 400		miRNA CT 400		RNA FFPE 130	
Samples in one run	**24**	**96**	**24**	**48**	**24**	**96**	**24**	**72**	**24**	**96**
Reagent cartridges	1	1	1	1	1	1	1	1§	1	2¶
Sample prep cartridges*	21	84	21	42	21	84	21	63	15	45
8-Rod Covers†	3	12	3	6	3	12	3	9	3	9
1500 µL tips†	81	324	81	462	86	344	86	258	92	276
200 µL tips‡	24	96	24	48	24	96	24	72	24	96
Ethanol (mL)	70	2 x 140	140	2 x 140	70	2 x 140	140	2 x 140	140	2 x 140

* 28 sample cartridges/unit box.
† Twelve 8-Rod Covers/unit box.
‡ 32 tips/tip rack; the inventory scan requires additional tips (two 200 µL and seven 1500 µL tips).
§ 96 samples per reagent cartridge; 72 samples in one run.
¶ 72 samples per reagent cartridge.

Loading the "Waste" drawer

Sample prep cartridges and 8-Rod Covers used during a run are re-racked in empty unit boxes in the "Waste" drawer. Make sure that the "Waste" drawer contains sufficient empty unit boxes for plastic waste generated during the protocol run.

Note: Ensure that the covers of the unit boxes are removed before loading the unit boxes into the "Waste" drawer. If you are using 8-Rod Cover boxes for collecting used sample prep cartridges and 8-Rod Covers, ensure that the box spacer has been removed.

A bag for used filter-tips must be attached to the front side of the "Waste" drawer.

Note: The presence of a tip disposal bag is not checked by the system. Make sure that the tip disposal bag is properly attached before starting a protocol run. For more information, see the *QIAsymphony SP/AS User Manual — Operating the QIAsymphony SP.*

A waste container collects all liquid waste generated during the purification procedure. The "Waste" drawer can only be closed if the waste container is in place.

Loading the "Eluate" drawer

Load the required elution plate into the "Eluate" drawer. Do not load a 96-well plate onto "Elution slot 4". If eluates need to be cooled, use "Elution slot 1" with the corresponding cooling adapter. As long-term storage of eluates in the "Eluate" drawer may lead to evaporation of eluates, we strongly recommend using the cooling position.

Inventory scan

Before starting a run, the instrument checks that sufficient consumables for the queued batch(es) run have been loaded into the corresponding drawers (Table 1, page 18).

Determining the amount of starting material

The amount of starting material that can be used is limited by the binding capacity of the magnetic particles and by the amount of contaminants in the sample that may interfere with the purification procedure. Minimal elution volumes are determined by the amount of beads used in the respective protocol.

The QIAsymphony RNA Kit is optimized for RNA purification from up to 3 x 10^6 animal or human cultured cells. With the large-volume protocol, up to 1 x 10^7 cells can be processed. The QIAsymphony RNA Kit is optimized for RNA purification from up to 20 mg animal or human tissue, including fibrous and fatty tissue* or up to 50 mg easy-to-lyse tissue, such as kidney, using the large-volume protocol. The large-volume protocol uses twice the amount of reagents compared to the standard protocol (i.e., one reagent cartridge allows processing of 48 samples

* Processing fibrous tissue requires proteinase K (see ordering information page 54) and fatty tissue requires chloroform, both of which must be ordered separately.

using the large-volume protocol, instead of the usual 96 samples). Up to 10 mg of high-cell-density tissue, such as spleen, can be processed. If you use more than these amounts, RNA yields may decrease and/or the isolated RNA may be of lower purity. Samples containing more than 100 µg of RNA (200 µg for large-sample protocol) may result in lower yields than expected. The QIAsymphony protocol for FFPE samples allows processing of 1–2 microtome sections of 10 µm thickness or equivalent.

Table 2 provides a summary of the recommendations for the various processing options.

Table 2. Amounts of starting material and elution volumes used in QIAsymphony RNA procedures

Amount of starting material	Lysate volume (µL)	QIAsymphony SP protocol	Assay Control Set*	Elution volume (µL)†
Cultured cells				
≤3 x 10^6 cells	400	Standard	RNA CT 400	50, 100, 200
≤3 x 10^6 cells	400	miRNA	miRNA CT 400	50, 100, 200
≤1 x 10^7 cells	400	Large-volume	RNA CT 800	100, 200
Easy-to-lyse tissue‡				
≤20 mg	400	Standard	RNA CT 400	50, 100, 200
≤20 mg	400	miRNA	miRNA CT 400	50, 100, 200
≤50 mg	800	Large-volume	RNA CT 800	100, 200
Thymus or spleen				
≤10 mg	400	miRNA	miRNA 400	50, 100, 200
≤10 mg§	400	Standard	RNA CT 400	50, 100, 200
Fibrous tissue¶				
≤20 mg	400	miRNA	miRNA 400	50, 100, 200
≤20 mg	400	Fibrous tissue	RNA FT 400	50, 100, 200
FFPE samples				
1–2 sections (up to 20 µm thick)	130	FFPE	RNA FFPE 130	50, 100, 200

* The term "Assay Control Set" is used in the user interface when choosing the protocol. For more information about Assay Control Sets, see the *QIAsymphony SP/AS User Manual — Operating the QIAsymphony SP*.

† Using the small elution volumes will result in higher RNA concentration but lower overall RNA yield and higher variability, particularly for the smallest option in each case. Actual eluate volumes may be smaller than the selected elution volume.

‡ For example, kidney, lung, and liver.

§ Preparing more concentrated DNase allows up to 20 mg of these tissue types to be processed. For more details about preparing DNase, see "Things to do before starting" section of the corresponding protocol.

¶ For example, skin and muscle.

Direct counting is the most accurate way to quantify the number of cells. However, as a guide, the number of HeLa cells obtained in various culture dishes after confluent growth is given in Table 3, next page.

Weighing is the most accurate way to quantify the amount of tissue. As a guide, a 2 mm cube (volume, approximately 8 mm^3) of most animal tissues weighs 3.5–4.5 mg.

Table 3. Growth area and number of HeLa cells in various culture dishes

Cell culture vessel	Growth area (cm^2)*	Number of cells†
Multiwell plates		
96-well	0.32–0.6	4–5 x 10^4
48-well	1	1 x 10^5
24-well	2	2.5 x 10^5
12-well	4	5 x 10^5
6-well	9.5	1 x 10^6
Dishes		
35 mm	8	1 x 10^6
Flasks		
40–50 mL	25	3 x 10^6

* Per well, if multiwell plates are used; varies slightly depending on the supplier.

† Cell numbers are given for HeLa cells (approximate length = 15 µm), assuming confluent growth. Numbers will vary for different kinds of animal cells, which vary in length from 10 to 30 µm

The average yield of total RNA varies depending on the type of tissue sample being processed. In addition, RNA yield can vary due to species, developmental stage, growth conditions, etc.

Nucleic acids in FFPE samples are usually heavily fragmented and chemically modified by formaldehyde. The degree of fragmentation depends on the type and age of the sample and on the conditions for fixation, embedding, and storage of the sample. While the QIAsymphony RNA protocol for FFPE is optimized to reverse as much formaldehyde modification as possible without further RNA degradation, nucleic acids purified from FFPE samples should not be used in downstream applications that require full-length RNA. Some applications may require modifications to allow the use of fragmented RNA (e.g., designing small amplicons for RT-PCR). For cDNA synthesis, either random or gene-specific primers should be used instead of oligo-dT primers.

When using the QIAsymphony RNA Kit to purify total RNA from 10 mg soft tissue, the average yield is 5–30 µg. Average yields from selected sources are listed in Table 4.

Handling and storage of starting material

RNA in tissues is not protected after harvesting until the sample is treated with RNAprotect Tissue Reagent, Allprotect Tissue Reagent, flash freezing, or disruption and homogenization in the presence of RNase-inhibiting or denaturing reagents. See the *RNAprotect Handbook* for information about RNAprotect Tissue Reagent and about stabilizing RNA in tissues and the *Allprotect Tissue Reagent Handbook* for information about Allprotect Tissue Reagent.

Table 4. RNA yields

Source	Average yield of total RNA (µg)*
Cell cultures	
(1 x 10^6 cells)	10–25
Mouse or rat tissues (10 mg)	
Kidney	20–30
Liver	40–60
Spleen	30–40
Thymus	40–50
Lung	10–20
Brain	5–20
Adipose	0.5–2.5
Muscle	5–10
Skin	2–8

* Amounts can vary due to factors such as species, developmental stage, and growth conditions.

After harvesting or excision, samples can be immediately flash frozen in liquid nitrogen and stored at –70°C. Frozen tissue should not be allowed to thaw during handling or weighing, but cell pellets can partially thaw enough to allow them to be dislodged by flicking. The relevant procedures should be carried out as quickly as possible.

After thorough disruption and homogenization in lysis buffer (Buffer RLT Plus), samples are stable for at least 4–5 hours at room temperature (15–25°C). Homogenates can also be stored at –70°C. Frozen samples are stable for months.

Starting material: FFPE

Standard formalin-fixation and paraffin-embedding procedures always result in significant fragmentation and crosslinking of nucleic acids. To limit the extent of nucleic acid fragmentation and crosslinking, be sure to:

- Use tissue samples less than 5 mm thick to allow complete penetration by formalin
- Fix tissue samples in 4–10% neutral-buffered formalin as quickly as possible after surgical removal
- Use a maximum fixation time of 24 hours (longer fixation times lead to over-fixation and more severe nucleic acid fragmentation, resulting in poor performance in downstream assays)
- Thoroughly dehydrate samples prior to embedding
- Use low-melting paraffin for embedding

The starting material for RNA purification should be freshly cut sections of FFPE tissue, each with a thickness of up to 20 µm. Thicker sections may result in lower nucleic acid yields, even after prolonged incubation with proteinase K. Up to 2 sections, each with a thickness of up to 10 µm and a surface area of up to 250 mm^2, can be combined in one preparation. More than 2 sections can be combined if the total sum of the thickness of the sections is 20 µm or less (e.g., four 5 µm thick sections), or if less than 30% of the surface area consists of tissue and the excess paraffin is removed using a scalpel prior to starting the protocol.

For microtome sections, no further disruption or homogenization is required. For recommendations about using FFPE material other than microtome sections (e.g., core punches), see **www.qiagen.com/RNeasyFFPE**.

Disruption and homogenization of starting material

Note: Recommendations in this section do not apply to FFPE sections. Efficient disruption and homogenization of the starting material is an absolute requirement at the start of QIAsymphony RNA procedures. Disruption and homogenization are 2 distinct steps, except for FFPE microtome sections (which are thin enough for lysis using a short proteinase K treatment).

Disruption

Complete disruption of plasma membranes of cells and organelles releases all the RNA contained in the sample. Incomplete disruption results in significantly reduced yields.

Homogenization

Homogenization reduces the viscosity of the cell lysates produced by disruption. Homogenization shears high-molecular-weight genomic DNA and other high-molecular-weight cellular components to create a homogeneous lysate. Incomplete homogenization results in inefficient binding of RNA to the magnetic particles and therefore significantly reduced yields.

Some disruption methods simultaneously homogenize the sample while others require an additional homogenization step.

In QIAsymphony RNA procedures, disruption of cells is achieved by vortexing or mixing in Buffer RLT Plus. The method of homogenization depends on the cell count of the sample. If the cell count is 1×10^6 cells or fewer, sufficient homogenization is achieved by vortexing the sample for 1 minute. If the cell count is higher, homogenization must be performed using one of 3 methods:

- TissueLyser II or other bead mill

- TissueRuptor II or other rotor–stator homogenizer
- Syringe and needle

In QIAsymphony RNA procedures, disruption and homogenization of tissue can be performed using one of 3 methods:

- Disruption and homogenization using the TissueLyser II or other bead mill
- Disruption and homogenization using the TissueRuptor II or other rotor–stator homogenizer
- Disruption using a mortar and pestle, and homogenization using the TissueLyser II (or other bead mill) or the TissueRuptor II (or other rotor–stator homogenizer)

  Note: After storage in RNAprotect Tissue Reagent, tissues become slightly hardened compared with fresh or thawed tissues. Disruption and homogenization of this tissue, however, is usually not a problem.

The different disruption and homogenization methods are described in more detail below.

Disruption and homogenization using the TissueLyser II system

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. Disruption efficiency is influenced by:

- Size and composition of beads
- Ratio of buffer to beads
- Amount of starting material
- Speed and configuration of agitator
- Disintegration time

Stainless steel beads with a diameter of 5 mm are optimal for use with animal tissues in combination with the QIAsymphony RNA Kit. All other disruption parameters should be determined empirically for each application.

The protocol for purification of total RNA from tissues contains guidelines for disruption and homogenization of tissues using the TissueLyser II and stainless-steel beads. For other bead mills, please refer to suppliers' guidelines for further details.

Bead-milling can also be used to homogenize cell lysates. The optimal beads for use with animal or human cells are 3–7 mm diameter stainless steel beads. The protocol for purification of total RNA from cells contains guidelines for homogenization of cells using the TissueLyser II and stainless-steel beads. For other bead mills, please refer to suppliers' guidelines for further details.

Note: Tungsten carbide beads react with Buffer RLT Plus and must not be used to disrupt and homogenize tissues.

Disruption and homogenization using the TissueRuptor II

Using the TissueRuptor II, samples are simultaneously disrupted and homogenized by rapid rotation of the blade of the disposable probe. The transparent probe enables visual control of the sample disruption process. Cells and tissues are disrupted at room temperature (15–25°C) in lysis buffer.

The protocol for purification of total RNA from tissues contains guidelines for disruption and homogenization of tissues using the TissueRuptor II and disposable probes. For other rotor–stator homogenizers, please refer to suppliers' guidelines for further details.

Important: To prevent the rotor from becoming stuck in the stator tube, be sure to choose a suitably sized vessel for disruption. During homogenization and disruption, the tip of

the disposable probe must be submerged in lysis buffer to prevent damage to the instrument and probe. Use a minimum volume of 180 µL lysis buffer in a suitably sized vessel (e.g., 2 mL microcentrifuge tube).

Disruption using a mortar and pestle

For disruption using a mortar and pestle, freeze the sample immediately in liquid nitrogen and grind to a fine powder under liquid nitrogen. Transfer the suspension (tissue powder and liquid nitrogen) into an appropriately sized tube cooled with liquid nitrogen and allow the liquid nitrogen to evaporate without allowing the sample to thaw. Add lysis buffer and continue as quickly as possible with homogenization using either the TissueLyser II (or similar bead mill), the TissueRuptor II (or similar rotor–stator homogenizer), the QIAshredder homogenizer, or syringe and needle (see below).

Note: Grinding the sample using a mortar and pestle will disrupt the sample, but it will not homogenize it. Homogenization must be performed separately before continuing with QIAsymphony RNA procedures.

Homogenization using a syringe and needle

Cell lysates can be homogenized using a syringe and needle. High-molecular-weight DNA can be sheared by passing the lysate through a 20-gauge (0.9 mm) needle attached to a sterile plastic syringe at least 5–10 times or until a homogeneous lysate is achieved. Increasing the volume of lysis buffer may facilitate handling and minimize loss.

Quantification of RNA

Carryover of magnetic particles may affect the absorbance reading at 260 nm (A_{260}) of the purified RNA but should not affect downstream applications. The measured absorbance at 320 nm (A_{320}) should be subtracted from all absorbance readings. See Appendix B, page 39, for more information.

Setting up the enzyme rack

DNase I, proteinase K, and DNase Booster Buffer must be added to the enzyme rack according to the protocol being run.

Table 5. Enzyme rack setup

Protocol	Positions 1 and 2 DNase I	Positions 3 and 4 Proteinase K	Position 5 DNase Booster Buffer
RNA CT 400	1.4 mL	–	–
RNA CT 800	1.4 mL	–	–
RNA FT 400	1.4 mL	2 mL	–
miRNA CT 400	1.4 mL	2 mL	–
RNA FFPE 130	1.4 mL	–	2 mL

Excessive foaming of lysates

The QIAsymphony RNA Kit is supplied with Buffer RLT Plus lysis buffer. When processing samples lysed in Buffer RLT Plus, excessive foaming may occur. This foaming is substantially reduced by adding Reagent DX to Buffer RLT Plus at a final concentration of 0.5% (v/v) before lysing the samples. Reagent DX has been carefully tested with the QIAsymphony RNA Kit, and has no effect on RNA purity or on downstream applications, such as real-time RT-PCR. Buffer RLT Plus containing Reagent DX can be stored at room temperature (15–25°C) for at least 9 months. Reagent DX is supplied separately; see ordering information on page 49.

Protocol: General Purification Protocol

The following is a general protocol using the QIAsymphony RNA Kit. Detailed information for each protocol, including volumes and tubes, is provided in protocol sheets that can be downloaded at **www.qiagen.com/goto/QIAsymphony**. Click on the **Resources** tab.

Important points before starting

- Ensure that you are familiar with operating the QIAsymphony SP. Refer to the user manuals supplied with your instrument for operating instructions.
- Optional maintenance is not mandatory for instrument function, but is highly recommended to reduce risk of contamination.
- Before beginning the procedure, read "Important Notes" starting on page 14.
- Ensure that you are familiar with the protocol sheet corresponding to the procedure you want to use (available from the **Resources** tab at **www.qiagen.com/qiasymphonyRNAkit**).
- If working with RNA for the first time, read Appendix A (page 39).
- Try to avoid vigorous shaking of the reagent cartridge (RC), otherwise foam may be generated, which can lead to liquid-level detection problems.
- Buffer RLT Plus and buffers in the reagent cartridge contain a guanidine salt and are therefore not compatible with disinfecting reagents containing bleach. See page 6 for safety information. Take appropriate safety measures and wear gloves when handling.
- Lysates may exhibit excessive foaming during homogenization. If this happens, add Reagent DX to Buffer RLT Plus before starting the procedure. For details, see "Excessive foaming of lysates" (page 29).
- Unless otherwise indicated, perform all steps of the procedure at room temperature (15–25°C).

Things to do before starting

- Before starting the procedure, ensure that the magnetic particles are fully resuspended. Vortex the trough containing the magnetic particles vigorously for at least 3 minutes before first use.
- Before loading the reagent cartridge, remove the cover from the trough containing the magnetic particles and open the enzyme tubes. Make sure that the enzyme has been equilibrated to room temperature (15–25°C). Make sure that the piercing lid is placed on the reagent cartridge or, if using a partially used reagent cartridge, make sure the Reuse Seal Strips have been removed.
- If samples are bar coded, orient samples in the tube carrier so that the bar codes face the bar code reader at the left side of the QIAsymphony SP.
- For information about sample tubes compatible with a certain protocol, see the corresponding protocol sheet (available from the **Resources** tab at **www.qiagen.com/qiasymphonyRNAkit**).
- For information about minimum sample volumes for samples in primary and secondary tubes for a certain protocol, see the corresponding protocol sheet (available from the **Resources** tab at **www.qiagen.com/qiasymphonyRNAkit**). This information also indicates which tubes can be used for different protocols.

- Prepare DNase I stock solution before using the reagent cartridge for the first time. Dissolve the lyophilized DNase I (1500 Kunitz units) first in 1.4 mL of the RNase-free water provided. (Note that the label on the DNase I vial indicates a different volume should be added. The label can be ignored.) To avoid loss of DNase I, do not open the vial. Inject RNase-free water into the vial using an RNase-free needle and syringe. Mix gently by inverting the vial. Do not vortex. Transfer 0.7 mL of DNase solution to each of the tubes in positions 1 and 2 of the enzyme rack on the reagent cartridge. Transfer additional 0.7 mL RNase-free water provided to each of the tubes in positions 1 and 2 of the enzyme rack on the reagent cartridge to prepare a total volume of 1.4 mL solubilized DNase I in each tube in positions 1 and 2 of the enzyme rack on the reagent cartridge. Close the tubes in the enzyme rack with the respective lids and mix solubilized DNase I gently by inverting the vial. Do not vortex.
  Note: **Do not use less than 1.4 mL of DNase with a new cartridge.** Once prepared, solubilized DNase should be stored in the enzyme rack with the enzyme tubes closed with screw caps at 2–8°C for up to 6 weeks or at –20°C for up to 9 months. The tubes can remain in the rack when processing with a protocol that does not require DNase. Do not refreeze after thawing.
- Place 1 Accessory Trough filled with 140 mL of 96–100% ethanol in the appropriate tip rack position (see Figure 3, page 16). When processing more than 24 samples (all protocols), use 2 Accessory Troughs with 140 mL each.

Procedure

1. Close all drawers and the hood.
2. Switch on the QIAsymphony SP, and wait until the "Sample Preparation" screen appears and the initialization procedure has finished.

   The power switch is located at the lower left corner of the QIAsymphony SP.
3. Log on to the instrument.

4. Ensure the "Waste" drawer is prepared properly, and perform an inventory scan of the "Waste" drawer, including the tip chute and liquid waste. Replace the tip disposal bag if necessary.
5. Load the required elution rack into the "Eluate" drawer.

   Do not load a 96-well plate onto "Elution slot 4". If eluates need to be cooled, use "Elution slot 1" with the corresponding cooling adapter.

   When using a 96-well plate, make sure that the plate is in the correct orientation, as incorrect placement may cause sample mix-up in downstream analysis.
6. Load the required reagent cartridge(s) and consumables into the "Reagents and Consumables" drawer.
7. Perform an inventory scan of the "Reagents and Consumables" drawer.
8. Place the samples into the appropriate sample carrier, and load them into the "Sample" drawer.
9. Using the touchscreen, enter the required information for each batch of samples to be processed.

   Enter the following information:

   - Sample information (depending on sample racks used)
   - Protocol to be run ("Assay Control Set")
   - Elution volume and output position

   Note: Small elution volumes will result in higher RNA concentration, but lower overall yield. Actual elution volumes may be smaller than the selected elution volume.
   After information about the batch has been entered, the status changes from "LOADED" to "QUEUED". As soon as one batch is queued the "Run" button appears.

   The Assay Control Set provides information about internal controls, if applicable.
10. Press the "Run" button to start the purification procedure.

    All processing steps are fully automated. At the end of the protocol run, the status of the batch changes from "RUNNING" to "COMPLETED".

11. Retrieve the elution rack containing the purified RNA from the “Eluate” drawer.

    The RNA is ready to use or can be stored at –20°C or –80°C.

    In general, magnetic particles are not carried over into eluates. If carryover does occur, magnetic particles in eluates will not affect most downstream applications.

    If magnetic particles need to be removed before performing downstream applications, tubes or plates containing eluates should first be placed in a suitable magnet and the eluates transferred to a clean tube.

    If the “Eluate” drawer is closed when a batch is running (e.g., if elution racks that contain eluates are removed), the run will be paused and an inventory scan of the “Eluate” drawer will be performed. A message appears during the scan and must be closed (by pressing the “Close” button) before the run can be restarted. Result files are generated for each elution plate.

12. If a reagent cartridge is only partially used, seal it with the provided Reuse Seal Strips and close tubes containing proteinase K with screw caps immediately after the end of the protocol run to avoid evaporation.

    Note: Partially used enzyme racks should be stored at 2–8°C. For more information, see “Storage”, page 5.

13. Discard used sample tubes, plates, and waste according to your local safety regulations. See page 6 for safety information.

14. Clean the QIAsymphony SP.

    Follow the maintenance instructions in the user manuals supplied with your instrument. Make sure to clean the tip guards regularly to minimize the risk of cross-contamination.

15. Close the instrument drawers, and switch off the QIAsymphony SP.

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
General handling		
a)	Error message displayed in the touchscreen	If an error message is displayed during a protocol run, refer to "Troubleshooting" in the *QIAsymphony SP/AS User Manual —* Operating the QIAsymphony SP
Precipitate in reagent trough of opened cartridge		
a)	Buffer evaporation	Excessive evaporation can lead to increased salt concentration in buffers. Discard reagent cartridge. Make sure to seal buffer troughs of a partially used reagent cartridge with Reuse Seal Strips when not being used for RNA purification.
b)	Storage of reagent cartridge	Storage of reagent cartridge under 15°C may lead to the formation of precipitates. If necessary, remove the trough containing Buffer QSW3 from the reagent cartridge and incubate for 30 min at 37°C with occasional shaking to dissolve precipitate. Make sure to replace the trough in the correct position. If the reagent cartridge is already pierced, make sure that the trough is reclosed with a Reuse Seal Strip and incubate the complete reagent cartridge for 30 min at 37°C with occasional shaking in a water bath.
Low RNA yield		
a)	Incomplete sample lysis	Before use, check that Buffer RLT Plus does not contain a precipitate by shaking the bottle. If necessary, remove the trough containing Buffer RLT Plus from the reagent cartridge and incubate for 30 min at 37°C with occasional shaking to dissolve precipitate. If the reagent cartridge is already pierced, make sure that the trough is reclosed with a Reuse Seal Strip, and incubate the complete reagent cartridge for 30 min at 37°C with occasional shaking in a water bath.

		Comments and suggestions
b)	Magnetic particles were not completely resuspended	Before starting the procedure, ensure that the magnetic particles are fully resuspended. Vortex for at least 3 min before use.
c)	Insufficient disruption and homogenization	See "Disruption and homogenization of starting material" (starting on page 25) for a detailed description of homogenization methods. In subsequent preparations, reduce the amount of starting material (see "Determining the amount of starting material", page 19, and the protocol) and/or increase the volume of lysis buffer and the homogenization time.
d)	Too much starting material	In subsequent preparations, reduce the amount of starting material. It is essential to use the correct amount of starting material (see "Determining the amount of starting material", page 19 and the protocol). Use the large-volume protocol, if required.
e)	Incomplete removal of cell-culture medium or RNA stabilization reagent for tissue	When processing cultured cells, ensure complete removal of the cell-culture medium after harvesting cells (see protocol). For stabilized tissue samples, remove samples from the reagent and remove any excess reagent from the samples.
Low RNA yield from FFPE tissues		
a)	Poor quality starting material	Samples that were fixed for over 20 h or stored for very long periods may contain very little usable RNA. Sections that were mounted on microscope slides may yield very little usable RNA due to prolonged exposure to air or damage to RNA from staining procedures.
b)	Insufficient deparaffinization or sample contains too much paraffin	Trim off excess paraffin if possible. Increase volume of Deparaffinization Solution if the top phase turns waxy or solidifies upon cooling.
RNA does not perform well in downstream applications		
a)	Insufficient RNA used in downstream application	Quantify the purified RNA by spectrophotometric measurement of the absorbance at 260 nm, (see "Quantification of RNA", page 29).
b)	Excess RNA used in downstream application	Excess RNA can inhibit some enzymatic reactions Quantify the purified RNA by spectrophotometric measurement of the absorbance at 260 nm, (see "Quantification of RNA", page 29).

	Comments and suggestions
RNA from FFPE sections does not perform well in downstream assays	
RNA fragmented or blocked due to formaldehyde modification	Although the 80°C incubation on the QIAsymphony instrument removes some of the formaldehyde modifications, RNA purified from FFPE sections is not an optimal template for enzymatic reactions. We recommend using only random primers or gene-specific primers for cDNA synthesis. We also recommend keeping amplicons for PCR as short as possible (<500 nucleotides).
A_{260}/A_{280} ratio for purified RNA is low	
a) Absorbance reading at 320 nm was not subtracted from the absorbance readings at 260 nm and 280 nm	To correct for the presence of magnetic particles in the eluate, an absorbance reading at 320 nm should be taken and subtracted from the absorbance readings obtained at 260 nm and 280 nm (see “Quantification of RNA”, page 29).
b) Wrong buffer used for RNA dilution	Use 10 mM Tris·Cl, pH 7.5, not RNase-free water, to dilute the sample before measuring purity (see “Purity of RNA”, page 43).
RNA degraded	
a) Tissue sample not immediately stabilized	Submerge the sample in the appropriate volume of RNAprotect Tissue Reagent immediately after harvesting the material.
b) Too much tissue sample for proper stabilization	Reduce the amount of starting material or increase the amount of RNAprotect Tissue Reagent used for stabilization (see the RNAprotect *Handbook*).
c) Tissue sample too thick or stabilization	Cut large samples into slices less than 0.5 cm thick for stabilization in RNAprotect Tissue Reagent
d) Frozen tissue samples used for stabilization	Use only fresh, unfrozen material for stabilization.
e) Storage duration exceeded	Storage of RNAprotect- or Allprotect-stabilized material is possible for up to 1 day at 37°C, up to 7 days at 18–25°C, and up to 4 weeks (RNAprotect) or 6 months (Allprotect) at 2–8°C. Store at –20°C or –80°C for archival storage.
f) Sample inappropriately handled	For frozen cell pellets, ensure that they were flash-frozen immediately in liquid nitrogen and properly stored at –70°C. Perform the protocol quickly, especially the first few steps. See Appendix A (page 39). Ensure that tissue samples are properly stabilized and stored in RNAprotect or Allprotect reagent. For frozen tissue samples, ensure that they were flash-frozen immediately in liquid nitrogen and properly stored at –70°C. Perform the protocol quickly, especially the first few steps. See Appendix A (page 39).

		Comments and suggestions
g)	RNase contamination	Although all buffers have been tested and guaranteed RNase-free, RNases can be introduced during use. Be certain not to introduce any RNases during the procedure or later handling. See Appendix A (page 39). Do not put RNA samples into a vacuum dryer that has been used in DNA preparation where RNases may have been used.
h)	Tissue incubated in lysis buffer for too long before homogenization was started	After adding Buffer RLT Plus to tissue, proceed with homogenization immediately.
DNA contamination in downstream experiments		
a)	DNA content of sample too high for DNase treatment	Use the large-volume protocol and/or prepare DNase solution at higher concentration.
b)	Less than 1.4 mL DNase solution per enzyme tube used with a fresh cartridge	Make sure to transfer the recommended amount of DNase solution as described in the protocol.
Low reproducibility between samples		
a)	Incomplete homogenization	Some types of tissues are more difficult to homogenize, resulting in greater variability from sample to sample.
b)	Variability between tissue samples	RNA yields from tissue samples can vary more than, for example, cultured cells due to the heterogeneous nature of most tissues and donor-to-donor variability.

Appendix A: General Remarks on Handling RNA

Handling RNA

Ribonucleases (RNases) are very stable and active enzymes that generally do not require cofactors to function. Since RNases are difficult to inactivate and even minute amounts are sufficient to destroy RNA, do not use any plasticware or glassware without first eliminating possible RNase contamination. Great care should be taken to avoid inadvertently introducing RNases into the RNA sample during or after the purification procedure. 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., pipettes 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 40), or rinse with chloroform* if the plasticware is chloroform-resistant. To

* 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.

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.

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 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

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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.

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

Storage of RNA

Purified RNA may be stored at –70°C to –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 amounts photometrically. Small amounts of RNA can be quantified using quantitative RT-PCR or fluorometric quantification.

Spectrophotometric quantification of RNA

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 mL ($A_{260} = 1 \rightarrow 4$ 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 43), 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 spectrophotometry. This can be accomplished by washing

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cuvettes with 0.1 M NaOH, 1 mM EDTA,* followed by washing with RNase-free water (see "Solutions", page 40). Use the buffer in which the RNA is diluted to zero the spectrophotometer. An example of the calculation involved in RNA quantification is shown below:

Volume of RNA sample	= 100 µL
Dilution	= 10 µL 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
	= 44 µg/mL x 0.2 x 50
	= 440 µg/mL

Total amount	= concentration x volume in milliliters
	= 440 µg/mL x 0.1 mL
	= 44 µg of RNA

Purity of RNA

For standard photometric measurements, the ratio of the readings at 260 nm and 280 nm (A_{260}/A_{280}) provides an estimate of the purity of RNA with respect to contaminants, such as protein, that absorb in the UV spectrum. However, the A_{260}/A_{280} ratio is considerably influenced 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

* 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.

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 42).

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. RNeasy Kits will, however, remove the vast majority of cellular DNA. gDNA Eliminator Solution helps to further reduce genomic DNA contamination; however, trace amounts of genomic DNA may still remain, depending on the amount and nature of the sample. For analysis of very low abundance targets, any interference by residual DNA contamination can be detected by performing real-time RT-PCR control experiments in which no reverse transcriptase is added prior to the PCR step.

To prevent any interference by DNA in real-time RT-PCR applications, such as with Applied Biosystems®, Rotor-Gene® Q, and QIAquant 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 49).

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® Connect 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 Connect 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.

Appendix C: Protocol for Formaldehyde Agarose Gel Electrophoresis

The following protocol for formaldehyde agarose (FA) gel electrophoresis is routinely used at QIAGEN and gives enhanced sensitivity for gel and subsequent analysis (e.g., northern blotting). A key feature is the concentrated RNA loading buffer that allows a larger volume of RNA sample to be loaded onto the gel than conventional protocols (e.g., Sambrook, J. and Russell, D.W. [2001] *Molecular Cloning: A Laboratory Manual*, 3rd ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press).

1.2% FA gel preparation

To prepare FA gel (1.2% agarose) of size 10 x 14 x 0.7 cm, mix:

1.2 g agarose*

10 mL 10x FA gel buffer (see composition below)

Add RNase-free water to 100 mL

If smaller or larger gels are needed, adjust the quantities of components proportionately.

Heat the mixture to melt agarose. Cool to 65°C in a water bath. Add 1.8 mL of 37% (12.3 M) formaldehyde* and 1 µL of a 10 mg/mL ethidium bromide* stock solution. Mix thoroughly and pour onto gel support. Before running the gel, equilibrate in 1x FA gel running buffer (see composition on next page) for at least 30 minutes.

* 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.

RNA sample preparation for FA gel electrophoresis

Add 1 volume of 5x loading buffer (see composition below) per 4 volumes of RNA sample (for example 10 µL of loading buffer and 40 µL of RNA) and mix. Incubate for 3–5 minutes at 65°C, chill on ice, and load onto the equilibrated FA gel.

Gel running conditions

Run gel at 5–7 V/cm in 1x FA gel running buffer

Composition of FA gel buffers

10x FA gel buffer

200 mM 3-[N-morpholino]propanesulfonic acid (MOPS) (free acid)*

50 mM sodium acetate*

10 mM EDTA*

pH to 7.0 with NaOH*

1x FA gel running buffer

100 mL 10x FA gel buffer

20 mL 37% (12.3 M) formaldehyde*

880 mL RNase-free water

* 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.

5x RNA loading buffer

16 µL saturated aqueous bromophenol blue solution*†

80 µL 500 mM EDTA, pH 8.0

720 µL 37% (12.3 M) formaldehyde

2 mL 100% glycerol*

3084 µL formamide*

4 mL 10x FA gel buffer

RNase-free water to 10 mL

Stability: approximately 3 months at 4°C

* 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.

† To make a saturated solution, add solid bromophenol blue to distilled water. Mix and continue to add more bromophenol blue until no more will dissolve. Centrifuge to pellet the undissolved powder, and carefully pipette the saturated supernatant.

Ordering Information

Product	Contents	Cat. no.
QIAsymphony RNA Kit	For as many as 192 preps, depending on protocol: includes 2 reagent cartridges and enzyme racks and accessories	931636
Related products		
Accessory Trough (10)	Accessory troughs for use with the QIAsymphony SP	997012
Reagent Cartridge Holder (2)	Reagent cartridge holder for use with the QIAsymphony SP	997008
Sample Carrier, plate, Qsym	Plate carrier for sample input; for use with the QIAsymphony SP	9017660
Tube Insert, 11 mm, Revision, sample carrier, Qsym (24)	Primary tube adapter (11 mm, with tube insert 2A) for use with the QIAsymphony SP tube carrier (all software versions)	9242057
Tube Insert, 13 mm, sample carrier, Qsym (24)	Primary tube adapter (13 mm, with tube insert 1A) for use with the QIAsymphony SP tube carrier (all software versions)	9242058
Insert, 2.0 mL v2, sample carrier, Qsym (24)	Secondary tube adapter (for 2 mL screw cap tubes) for use with the QIAsymphony SP tube carrier	9242083
Cooling Adapter, 2 mL, v2 Qsym	Cooling adapter for 2 mL screw cap tubes for use in the QIAsymphony SP/AS instruments (software version 3.1 or higher)	9020674

Product	Contents	Cat. no.
Cooling Adapter, MTP, RB, v2, Qsym	Cooling adapter for round bottom microplates (MTP) for use in the QIAsymphony SP/AS instruments (software version 3.1 or higher)	9020729
Adapter, tubes, 2 mL, v2, Qsym	Non-cooling adapter for 2 mL screw-cap tubes (new version; with bar code)	9021670
Elution Microtubes CL (24 x 96)	Nonsterile polypropylene tubes (0.85 mL maximum capacity, less than 0.7 mL storage capacity, 0.4 mL elution capacity); 2304 in racks of 96; includes cap strips	19588
Reagent DX (1 mL)	1 mL Antifoaming Reagent for QIAGEN Lysis Buffers	19088
Deparaffinization Solution (16 mL)	2 x 8 mL deparaffinization solution	19093
Buffer PKD (15/15)	15 mL digestion buffer for use with the QIAsymphony RNA Kit (FFPE protocol), RNeasy FFPE Kit, AllPrep FFPE DNA/RNA Kit or miRNeasy FFPE Kit	1034963
QIAGEN Proteinase K (2 mL)	2 mL (>600 mAU/mL, solution)	19131
DNase Booster Buffer (2 mL)	Concentrated DNase treatment buffer for use with the QIAsymphony RNA Kit (FFPE protocol), RNeasy FFPE Kit or miRNeasy FFPE Kit	1064143
Sample Prep Cartridges, 8-well (336)	8-well Sample Prep Cartridges for use with the QIAsymphony SP	997002
8-Rod Covers (144)	8-Rod Covers for use with the QIAsymphony SP	997004

Product	Contents	Cat. no.
Filter-Tips, 200 µL (1024)	Disposable Filter-Tips, racked; (8 x 128). For use with the QIAcube and the QIAsymphony SP/AS instruments	990332
Filter-Tips, 1500 µL, Qsym SP (1024)	Disposable Filter-Tips, racked; (8 x 128). For use with the QIAsymphony SP/AS instruments	997024
Tip Disposal Bags (15)	Tip disposal bags for use with the QIAsymphony SP/AS instruments	9013395
TissueLyser II	For medium- to high-throughput sample disruption for molecular analysis	Varies
TissueLyser Adapter Set 2 x 24	2 sets of Adapter Plates and 2 racks for use with 2.0 mL microcentrifuge tubes on the TissueLyser II	69982
Stainless Steel Beads, 5 mm (200)	200 stainless steel beads (5 mm diameter), suitable for use with TissueLyser systems	69989
TissueLyser Single-Bead Dispenser, 5 mm	For dispensing individual beads (5 mm diameter)	69965
TissueRuptor II	Handheld rotor–stator homogenizer, 5 TissueRuptor Disposable Probes	Varies
TissueRuptor Disposable Probes (25)	25 nonsterile plastic disposable probes for use with the TissueRuptor II (and the old TissueRuptor)	990890
RNase-Free DNase Set (50)	1500 units RNase-free DNase I, RNase-free Buffer RDD, and RNase-free water for 50 RNA minipreps	79254

Product	Contents	Cat. no.
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
Allprotect Tissue Reagent (100 mL)	100 mL Allprotect Tissue Reagent, Allprotect Reagent Pump	76405
12-Tube Magnet	Magnet for separating magnetic particles in 12 x 1.5 mL or 2 mL tubes	36912
96-Well Magnet Type A	Magnet for separating magnetic particles in wells of 96-well plates, 2 x 96-Well Microplates FB	36915
S-Blocks (24)	96-well blocks with 2.2 mL wells, 24 per case	19585
QIAxcel Connect System	Capillary electrophoresis device: includes computer, QIAxcel ScreenGel Software, and 1-year warranty on parts and labor	9003110

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Document Revision History

Date	Changes
November 2020	Updated format as well as branding of RNA protection products.
August 2023	Updated "Things to do before starting" section under General Purification Protocol for dissolving lyophilized DNase I for use within QIAsymphony RNA application with alternative DNase I vial.

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