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PAXgene® Tissue RNA/miRNA Kit Handbook

For isolation and purification of total RNA, including miRNA, from tissue samples fixed in PAXgene Tissue FIX Containers

Important: To be used only in conjunction with the PAXgene Tissue FIX Containers and the PAXgene Tissue STABILIZER reagent.

For research use only. Not for use in diagnostic procedures.

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Kit Contents

PAXgene Tissue RNA/miRNA Kit	(50)
Catalog no.	766134
No. of preps	400
Buffer TM1 (Binding Buffer)*	18 mL
Buffer TM2 (Wash Buffer 1 concentrate)*†	12 mL
Buffer TM3 (Wash Buffer 2 concentrate)*†	11 mL
Buffer TM4 (Elution Buffer)	5 mL
RNase-Free Water (bottle)	125 mL
Proteinase K (green lid)	1.4 mL
PAXgene RNA MinElute® Spin Columns (red) with Processing Tubes	5 × 10
PAXgene Shredder Spin Columns (lilac) with Processing Tubes	5 × 10
Processing Tubes (2 mL)	5 × 50
Microcentrifuge Tubes (1.5 mL)	3 × 50, 1 × 10
DNase I, RNase-Free (lyophilized, 1500 Kunitz units‡)	1 glass vial
Buffer RDD (white lid)	2 × 2 mL
RNase-Free Water (tube, lilac lid) (DNase resuspension buffer)	2 mL
Carrier RNA (red lid)§	310 µg
Important Note	1

* Contains a guanidine salt. See page 8 for Safety Information.

† Buffer TM2 and TM3 are supplied as concentrate. Before using for the first time, add 3 volumes of isopropanol as indicated on the bottle to TM2 (96–100%, purity grade p.a.) and 4 volumes of ethanol (96–100%, purity grade p.a.) to TM3 to obtain working solutions.

‡ Kunitz units are the commonly used units for measuring DNase I; see page 22 for definition.

§ Carrier RNA is not required for the protocols in this handbook.

Shipping and Storage

The PAXgene Tissue RNA/miRNA Kit is shipped at ambient temperature.

PAXgene RNA MinElute spin columns and the RNase-Free DNase Set in the PAXgene Tissue RNA/miRNA Kit should be stored upon receipt at 2–8°C. All other components of the PAXgene Tissue RNA/miRNA Kit can be stored dry at room temperature (15–25°C). Under these conditions, the kit is stable for at least 9 months.

Proteinase K is stable for at least 1 year after delivery when stored at room temperature. For longer storage or if ambient temperatures often exceed 25°C, we recommend storing Proteinase K at 2–8°C.

Under these conditions, the components are stable as described without showing any reduction in performance and quality, unless otherwise indicated on the label.

Intended Use

For Research Use Only. Not for use in diagnostics procedures. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

It is the user's responsibility to validate the performance of the PAXgene Tissue RNA/miRNA Kit for any particular use because the performance characteristics of these kits have not been validated for any specific organism. The performance characteristics of this product have not been fully established.

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.

Buffer TM1 and Buffer TM2 contain guanidine thiocyanate, 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 PAXgene Tissue RNA/miRNA Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

The methods for tissue fixation currently used in traditional histology are of limited use for molecular analysis. Fixatives that contain formaldehyde crosslink biomolecules and modify nucleic acids and proteins. Such crosslinks lead to nucleic acid degradation during tissue fixation, storage, and processing. Since they cannot be removed completely, the resulting chemical modifications can lead to inhibition in sensitive downstream applications, such as RT-PCR, qPCR, or next-generation sequencing. In order to enable both molecular and traditional pathology testing from the same specimen, a method is needed to stabilize molecular content and preserve tissue morphology.

PreAnalytiX has developed the PAXgene Tissue System to meet this need. The system consists of a fixation reagent (PAXgene Tissue FIX) prefilled into containers for tissue collection, storage, and transport, along with a stabilization reagent (PAXgene Tissue STABILIZER) and kits for purification of DNA or total RNA, including miRNA. In addition, supplementary protocols for protein purification and other applications are available at **www.preanalytix.com.**

Principle and procedure

PAXgene Tissue FIX rapidly penetrates and fixes tissue, with a fixation rate of approximately 1 mm in 30 min.* PAXgene Tissue Containers provide tissue fixation for histopathology studies and enable purification of high-quality nucleic acids from the same sample for molecular analysis. The reagent preserves morphology and biomolecules without the destructive crosslinking and degradation found with formalin-fixed tissues.

* Tissue penetration and fixation rates may vary depending on tissue type and size.

After fixation, tissues can be stored in PAXgene Tissue STABILIZER for short or long term, used for extraction of nucleic acids or proteins, or processed and embedded in paraffin for further analysis. Sections of PAXgene Tissue-fixed, paraffin-embedded (PFPE) tissue can be used for histological studies or extraction of nucleic acids or proteins. Purification of total RNA, including miRNA, or DNA from PAXgene Tissue-fixed and stabilized tissue samples requires the use of one of the PAXgene Tissue Kits for RNA/miRNA or DNA. Purification of protein requires the Qproteome® FFPE Tissue Kit (QIAGEN).

PAXgene Tissue FIX in pre-filled container, PAXgene Tissue STABILIZER concentrate in a bottle, and PAXgene Tissue kits provide a complete preanalytical solution for collection, fixation, and stabilization of tissue, and for purification of high-quality nucleic acids for molecular analysis.

Sample collection and stabilization with PAXgene Tissue FIX Containers

PAXgene Tissue FIX Containers are single-chamber containers prefilled with 50 mL of the fixation reagent PAXgene Tissue FIX rapidly penetrates and fixes the tissue.* After fixation, PAXgene Tissue FIX is removed and replaced by PAXgene Tissue STABILIZER reagent.

When fixed tissue is stored in PAXgene Tissue STABILIZER reagent, the nucleic acids, proteins, and morphology of the tissue sample are stable for up to 7 days at room temperature or for up to 4 weeks at 2–8°C, depending on tissue type.†

* Fixation rates and stabilization times depend on type and size of tissue.

† Storage at 2–8°C for more than 4 weeks must be validated for each tissue type. Specifications for tissue size, fixation, and storage conditions using PAXgene Tissue FIX and PAXgene Tissue STABILIZER were determined using animal tissues samples.

Tissue samples can be stored in PAXgene Tissue STABILIZER reagent for longer periods at –20°C (–15°C to –30°C) or –80°C (–65°C to –90°C) without negative effects on the morphology of the tissue or the integrity of the nucleic acids. For the latest results on long-term storage, see the relevant technical notes and scientific posters at **www.preanalytix.com**.

Fixed and stabilized samples can be embedded in paraffin for histological studies. Nucleic acids and proteins can be isolated from the fixed and stabilized samples before or after embedding in paraffin. See the *PAXgene Tissue DNA Kit Handbook* for information about DNA isolation, or the PAXgene Tissue supplementary protocols at **www.preanalytix.com** for protein purification and other applications.

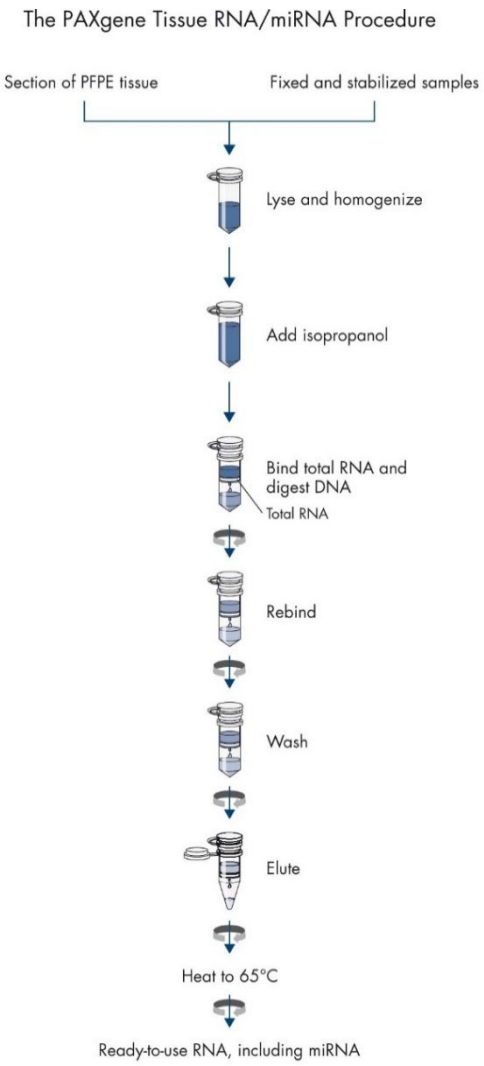
RNA purification

The PAXgene Tissue RNA/miRNA Kit enables purification of total RNA, including RNA molecules smaller than 200 nt, such as 5.8S rRNA, 5S rRNA, tRNAs, and miRNAs, from tissues fixed and stabilized with the PAXgene Tissue System or from sections of PFPE tissues. Optimized binding and washing conditions ensure the purification of RNA molecules as small as 18 nt. As a prerequisite, the tissue must be fixed and stabilized with PAXgene Tissue reagents (see "Description of protocols", page 17).

Disruption and homogenization of the tissue sample is performed in the binding buffer, Buffer TM1 (see "Disrupting and homogenizing starting materials", page 18). After the centrifugation step to remove residual cell debris, isopropanol is added to the lysate to provide appropriate binding conditions for all RNA molecules of 18 nt and longer. The sample is then applied to a PAXgene RNA MinElute spin column where the total RNA binds to the membrane and contaminants are efficiently washed away. Between the first and second wash steps, the membrane is treated with DNase I to remove trace amounts of bound DNA. After the wash steps, RNA including miRNA is eluted in a low-salt elution buffer.

Total RNA isolated using the PAXgene Tissue RNA/miRNA Kit is of high purity. Genomic DNA contamination is minimized, and purified RNA is ready to use in downstream applications with no detectable PCR inhibition. All RNA molecules longer than 18 nt are purified.

RNA isolation from PFPE tissue sections, without co-isolation of miRNA, can be automated on QIAGEN's QIAcube® Connect (see also page 14).



Disrupt sample and lyse with guanidine-containing buffer (Binding Buffer TM1)

Add isopropanol to adjust binding conditions

Apply sample to PAXgene RNA MinElute spin column for adsorption of RNA to membrane. DNase digest on column and centrifuge.

Add Buffer TM2 to flow-through; transfer mixture to RNA MinElute spin column and centrifuge.

Remove contaminants with simple wash steps.

Elute RNA, including miRNAs, in low-salt buffer.

Figure 1. The PAXgene Tissue RNA/miRNA procedure maximizes isolation of total RNA, down to 18 nt in length

Automated purification of RNA on QIAcube instruments

Purification of RNA, without co-isolation of miRNA, can be automated on the QIAcube Connect or the classic QIAcube. The innovative QIAcube instruments use advanced technology to process QIAGEN spin columns, enabling seamless integration of automated, low-throughput sample preparation into your laboratory workflow. Sample preparation using QIAcube instruments follows the same steps as the manual procedure (i.e., lyse, bind, wash, and elute), enabling you to continue using the PAXgene Tissue RNA/miRNA Kit for purification of high-quality RNA.

QIAcube instruments are preinstalled with protocols for purification of plasmid DNA, genomic DNA, RNA, viral nucleic acids, and proteins, plus DNA and RNA cleanup. The range of protocols available is continually expanding, and additional QIAGEN protocols can be downloaded at **www.qiagen.com/qiacubeprotocols** free of charge.



QIAcube Connect.

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 QIAGEN website (**www.qiagen.com/PAXgene-Tissue-RNAmiRNA**).

For both protocols

- PAXgene Tissue FIX Container
- PAXgene Tissue STABILIZER Concentrate
- Ethanol (96–100%, purity grade p.a.)
- Isopropanol
- 14.3 M β-mercaptoethanol (β-ME) (commercially available solutions are usually 14.3 M)
- Pipettes* (10 µL – 1 mL)
- Sterile, aerosol-barrier, ribonuclease (RNase)-free pipette tips†
- Graduated cylinder‡
- Variable-speed microcentrifuge* capable of attaining 1000–8000 × *g* and equipped with a rotor for 2 mL microcentrifuge tubes
- Shaker–incubator* capable of incubating at 45°C and 65°C and shaking at ≥400 rpm, not exceeding 1400 rpm (e.g., Eppendorf® Thermomixer Compact, **www.eppendorf.com**, or equivalent)§
- Vortex mixer*
- Crushed ice

* Ensure that instruments have been checked and calibrated according to the manufacturer's recommendations.
† Ensure that you are familiar with the guidelines on handling RNA (Appendix A, page 47).
‡ For the addition of isopropanol to Buffer TM2 and ethanol to Buffer TM3 concentrate.
§ This is not a complete list of suppliers and does not include many important vendors of biological supplies.

For PAXgene Tissue-fixed samples

- Equipment for tissue disruption and homogenization (see "Disrupting and homogenizing starting materials", page 18). We recommend either the TissueRuptor II* with TissueRuptor Disposable Probes or the TissueLyser* systems (see Ordering Information, page 46).
- Round-bottomed microcentrifuge tubes, 2 mL

For sections of PFPE tissue

- Microtome

* Ensure that instruments have been checked and calibrated regularly according to the manufacturer's recommendations.

Important Notes

Description of protocols

PAXgene Tissue-fixed samples (page 21)

Starting material for RNA purification is up to 10 mg of a tissue sample that has been fixed and stabilized with PAXgene Tissue reagents.

The tissue sample is removed from the container. If necessary, the sample is cut into 2 mm cubes. Binding Buffer TM1 is added and mechanical disruption and simultaneous homogenization is performed using the QIAGEN TissueRuptor® II or TissueLyser® systems (see "Disrupting and homogenizing starting materials", page 18).

Sections of PFPE tissue (page 30)

Starting material for RNA purification is freshly cut sections from tissue samples that have been fixed and stabilized with PAXgene Tissue reagents, dehydrated, and embedded in paraffin.

A minimum of 2 and a maximum of 5 sections, each with a thickness of 5–10 µm and a tissue surface area of up to 100 mm^2, can be combined in one sample preparation. Paraffin is removed from the tissue sections by incubation in xylene, followed by addition of ethanol and centrifugation. The resulting pellet is resuspended in a lysis buffer, diluted, and treated with Proteinase K. Centrifugation through the PAXgene Shredder spin column homogenizes the cell lysate and removes residual cell debris.

Disrupting and homogenizing starting materials

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 RNA contained in the sample. Incomplete disruption results in significantly reduced RNA yields.
- **Homogenization**: Homogenization is necessary to reduce the viscosity of the lysates produced by disruption. Homogenization shears high-molecular-weight genomic DNA and other high-molecular-weight cellular components to create a homogeneous lysate. Incomplete homogenization results in inefficient binding of RNA to the PAXgene RNA MinElute spin column membrane and therefore significantly reduces RNA yields.

Disruption and homogenization of tissue samples can be carried out rapidly and efficiently using either the TissueRuptor II (for processing samples individually) or the TissueLyser (for processing multiple samples simultaneously).* Table 1 gives an overview of different disruption and homogenization methods used for the different starting materials.

* The TissueRuptor II, TissueLyser II, and TissueLyser LT simultaneously disrupt and homogenize individual samples. If the TissueRuptor II, TissueLyser II, TissueLyser LT, or other similar instrument is not available, contact QIAGEN Technical Services for an alternative method of disruption and homogenization.

Table 1. Disruption and homogenization

Sample	Disruption method	Homogenization method
Sections of PFPE tissue	Sectioning; no additional disruption required	PAXgene Shredder spin column
Tissue taken directly from PAXgene Tissue FIX Container	TissueRuptor II* TissueLyser II* TissueLyser LT*	TissueRuptor* TissueLyser II* TissueLyser LT*

* The TissueRuptor and TissueLyser II simultaneously disrupt and homogenize individual samples. If the TissueRuptor, TissueLyser II, or other similar instrument is not available, contact QIAGEN Technical Services for an alternative method of disruption and homogenization.

Disruption and homogenization using the TissueRuptor

The TissueRuptor is a rotor–stator homogenizer that thoroughly disrupts and simultaneously homogenizes single-tissue samples in the presence of lysis buffer in 15–90 s, depending on the toughness and size of the sample. The blade of the TissueRuptor disposable probe rotates at a very high speed, causing the sample to be disrupted and homogenized by a combination of turbulence and mechanical shearing. For guidelines on using the TissueRuptor, refer to the *TissueRuptor Handbook*. For other rotor–stator homogenizers, refer to suppliers' guidelines.

Disruption and homogenization using the TissueLyser

In bead-milling, tissues can be disrupted by rapid agitation in the presence of beads and lysis buffer. Disruption and simultaneous homogenization occur by the shearing and crushing action of the beads as they collide with the tissue cells.

The TissueLyser LT disrupts and homogenizes up to 12 tissue samples simultaneously. The TissueLyser II disrupts and homogenizes up to 48 tissue samples simultaneously when used in combination with the TissueLyser Adapter Set 2 × 24, which holds 48 × 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 × 96, which holds 192 × 1.2 mL microtubes containing stainless steel beads of 5 mm mean diameter. For guidelines on using the TissueLyser II systems, refer to the TissueLyser Handbooks. If using other bead mills for sample disruption and homogenization, refer to suppliers' guidelines.

Note: Tungsten carbide beads react with Buffer TM1 and must not be used to disrupt and homogenize tissues.

Homogenization using PAXgene Shredder spin columns

PAXgene Shredder spin columns provide a fast and efficient way to homogenize cell and tissue lysates without cross-contamination of samples. Up to 700 µL lysate is loaded onto a PAXgene Shredder spin column placed in a 2 mL collection tube, and centrifuged for 2 min at maximum speed in a microcentrifuge. The lysate is homogenized as it passes through the spin column.

Protocol: Purification of Total RNA, Including miRNA, from PAXgene Tissue-Fixed Samples

Starting material

Starting material for RNA purification is up to 10 mg of a tissue sample fixed and stabilized with PAXgene Tissue reagents.

It is essential to use the correct amount of starting material to obtain optimal RNA yield and purity. A maximum amount of 10 mg tissue fixed and stabilized using the PAXgene Tissue reagents can generally be processed. For most tissues, the RNA binding capacity of the PAXgene RNA MinElute spin column and the lysing capacity of Buffer TM1 will not be exceeded by these amounts.

Weighing tissue is the most accurate way to quantify the amount of starting material. As a guide, a 2 mm cube (8 mm^3) of most tissues weighs 8–12 mg.

Important things before starting

- Do not overload the PAXgene RNA MinElute spin column, as this will significantly reduce RNA yield and quality.
- If working with RNA for the first time, read "Appendix A: General Remarks on Handling RNA", page 40.
- Ensure that the kit boxes are intact and undamaged and that buffers have not leaked. Do not use a kit that has been damaged.
- Ensure that the pipette is set to the correct volume and that liquid is carefully and completely aspirated and dispensed.
- To avoid transferring samples to the wrong tube or spin column, ensure that all tubes and spin columns are properly labeled. Label the lid and the body of each tube. For spin columns, label the body of its processing tube.
- Close each tube or spin column after liquid is transferred to it.

- Spilling samples and buffers during the procedure may reduce the yield and purity of RNA.
- Unless otherwise indicated, all steps of this protocol, including centrifugation steps, should be carried out at room temperature.

Things to do before starting

- Before using the kit for the first time, prepare 80% ethanol by mixing 24 mL ethanol (96–100%, purity grade p.a.) and 6 mL RNase-free water (supplied).
- A shaker–incubator is required in steps 5 and 21. Set the temperature of the shaker–incubator to 45°C.
- Buffer TM1 and Buffer TM2 may form a precipitate upon storage. If necessary, warm to 37°C to dissolve.
- β-Mercaptoethanol (β-ME) must be added to Buffer TM1 before use. Add 10 µL β-ME per 1 mL Buffer TM1. Dispense in a fume hood and wear appropriate protective clothing. Buffer TM1 containing β-ME can be stored at room temperature for up to 1 month.
- Buffer TM2 and Buffer TM3 are supplied as concentrates. Before using for the first time, add 3 volumes of isopropanol to Buffer TM2 and 4 volumes of ethanol (96–100%, purity grade p.a.) to Buffer TM3 as indicated on the bottles to obtain working solutions.
- If using the RNase-Free DNase Set for the first time, prepare a DNase I stock solution. Dissolve the solid DNase I (1500 Kunitz units* in 550 µL DNase resuspension buffer (RNase-free water) provided with the set. Take care that no DNase I is lost when opening the vial. Do not vortex the reconstituted DNase I. DNase I is especially sensitive to physical denaturation. Mixing should only be carried out by gently inverting the tube.

* Kunitz units are the commonly used units for measuring DNase I, defined as the amount of DNase I that causes an increase in A_{260} of 0.001 per minute per milliliter at 25°C, pH 5.0, with highly polymerized DNA as the substrate (Kunitz, M. [1950] J. Gen. Physiol. **33**, 349 and 363).

- Current data indicate that reconstituted DNase I can be stored at 2–8°C for up to 6 weeks. For long-term storage of DNase I, remove the stock solution from the glass vial, divide it into single-use aliquots, and store at –15°C to –30°C for up to 6 months. Thawed aliquots can be stored at 2–8°C for up to 6 weeks. Do not refreeze the aliquots after thawing. Ongoing studies may cause us to modify these times. Contact QIAGEN Technical Services for current details.
- When reconstituting and aliquoting DNase I, ensure that you follow the guidelines for handling RNA (see "Appendix A: General Remarks on Handling RNA", page 40).

Procedure

1. If using the TissueLyser, add one stainless steel bead (5 mm mean diameter) to each 2 mL round-bottomed microcentrifuge tube (not provided).
2. Retrieve the tissue sample from the PAXgene Tissue STABILIZER reagent using forceps and weigh the sample. Do not use more than 10 mg. Proceed immediately to step 3.

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

   Note: Tissue outside of the PAXgene Tissue STABILIZER is no longer protected; therefore, all handling steps prior to adding Buffer TM1 should be carried out on ice and as quickly as possible.
3. Disrupt the tissue and homogenize the lysate using either the TissueRuptor II (follow step 3a) or TissueLyser (follow step 3b).

   See "Disrupting and homogenizing starting materials", page 18, for more details on disruption and homogenization.

   Note: Ensure that β-ME is added to Buffer TM1 before use (see "Things to do before starting", page 22).

   Note: Incomplete homogenization leads to significantly reduced RNA yields and can cause clogging of the PAXgene RNA MinElute spin column. Homogenization

with the TissueRuptor or TissueLyser generally results in higher RNA yields than with other methods.

a) Disruption and homogenization using the TissueRuptor II

- Place the tissue in a suitably sized processing tube. Add 250 µL Buffer TM1.
  Generally, round-bottomed tubes allow more efficient disruption and homogenization than conical-bottomed tubes.
- Place the tip of the disposable probe into the processing tube and operate the TissueRuptor at full speed until the lysate is uniformly homogeneous (usually 20–40 s). Proceed to step 4.

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

b) Disruption and homogenization using the TissueLyser II

- Place the tissues in the tubes prepared in step 1.
- Immediately add 250 µL Buffer TM1 per tube.
- Place the tubes in the TissueLyser Adapter Set 2 × 24.
- Operate the TissueLyser II for 2 min at 20 Hz.
  The time depends on the tissue being processed and can be extended until the tissue is completely homogenized. If working with fibrous tissues, cutting the tissue into smaller pieces before starting disruption will improve disruption efficiency.
- Rearrange the tubes so that the outermost tubes are innermost and the innermost tubes are outermost. Operate the TissueLyser II for another 2 min at 20 Hz.
  Rearranging the tubes allows even homogenization.
- Proceed to step 4.
  Do not reuse the stainless-steel beads.
  For information on tissue disruption and homogenization using the TissueLyser LT, see the *TissueLyser LT Handbook*.

4. Add 480 µL RNase-free water to cell lysate suspension. Then add 20 µL Proteinase K and mix by vortexing for 5 s.

   Note: Do not mix Buffer TM1 and Proteinase K together before adding them to the sample.

5. Incubate for 15 min at 45°C using a shaker–incubator at 1400 rpm. After incubation, set the temperature of the shaker–incubator to 65°C (for step 19).
6. Centrifuge the tissue lysate for 3 min at maximum speed (but do not exceed 20,000 × *g*). Carefully transfer the supernatant fraction to a new 2 mL microcentrifuge tube (not provided) without disturbing the pellet in the processing tube.
7. Add 1100 µL isopropanol. Mix by vortexing for 5 s, and centrifuge briefly (1–2 s at 500–1000 × *g*) to remove drops from the inside of the tube lid.

   Note: The length of the centrifugation must not exceed 1–2 s, as this may result in pelleting of nucleic acids and reduced yields of total RNA.

   Note: A precipitate may form after the addition of isopropanol, but this will not affect the PAXgene Tissue RNA/miRNA procedure.

8. Pipette up to 700 µL sample, including any precipitate that may have formed, into a PAXgene RNA MinElute spin column (red) placed in a 2 mL processing tube. Centrifuge for 1 min at 8000 × *g*. Discard the flow-through.*

   Reuse the collection tube in step 9.

9. Repeat step 8 until the entire sample has passed through the PAXgene RNA MinElute spin column. Place the spin column in a new 2 mL processing tube, and discard the old processing tube containing flow-through.*

   If the lysate has not completely passed through the membrane after centrifugation, centrifuge again at a higher speed until the PAXgene RNA MinElute spin column is empty.

* Flow-through contains Buffer TM1 or Buffer TM2 and is therefore not compatible with bleach. See page 7 for **Error! Reference source not found.**.

10. Pipette 350 µL Buffer TM2 into the PAXgene RNA MinElute spin column. Centrifuge for 20 s at 8000 × *g*. Place the spin column in a new 2 mL processing tube, and discard the old processing tube containing flow-through.*
11. Add 10 µL DNase I stock solution to 70 µL Buffer RDD in a 1.5 mL microcentrifuge tube. Mix by gently flicking the tube, and centrifuge briefly to collect residual liquid from the sides of the tube.

    For example, if processing 10 samples, add 100 µL DNase I stock solution to 700 µL DNA Buffer RDD. Use the 1.5 mL microcentrifuge tubes supplied with the kit.

    Note: DNase I is especially sensitive to physical denaturation. Mixing should only be carried out by gently flicking the tube. Do not vortex.
12. Pipette the DNase I incubation mix (80 µL) directly onto the PAXgene RNA MinElute spin column, and incubate for 15 min at room temperature.

    Note: Ensure that the DNase I incubation mix is placed directly onto the membrane. DNase digestion will be incomplete if part of the mix is applied to and remains on the walls or the O-ring of the spin column.
13. Centrifuge for 20 s at 8000 × *g*. Place the spin column in a new 2 mL processing tube. Keep the flow-through for rebinding in step 14.
14. Add 350 µL Buffer TM2 to the flow-through from step 13. Mix carefully by pipetting 5 times up and down.
15. Pipette the mixture from step 14 into the PAXgene RNA MinElute spin column, and centrifuge for 20 s at 8000 × *g*. Place the spin column in a new 2 mL processing tube, and discard the old processing tube containing flow-through.*
16. Pipette 500 µL Buffer TM3 into the PAXgene RNA MinElute spin column, and centrifuge for 20 s at 8000 × *g*. Discard the flow-through and reuse the 2 mL processing tube in step 17.

    Note: Buffer TM3 is supplied as a concentrate. Ensure that ethanol is added to Buffer TM3 before use (see "Things to do before starting", page 22).

17. Pipette 500 µL 80% ethanol into the PAXgene RNA MinElute spin column, and centrifuge for 2 min at 8000 × *g*.

    Prepare 80% ethanol with ethanol (96–100%, purity grade p.a.) and the RNase-free water supplied with the kit.

18. Discard the processing tube containing the flow-through, and place the PAXgene RNA MinElute spin column in a new 2 mL processing tube. Open the cap of the spin column, and centrifuge for 5 min at maximum speed (do not exceed 20,000 × *g*).

19. Discard the processing tube containing the flow-through. Place the PAXgene RNA MinElute spin column in a 1.5 mL microcentrifuge tube, and pipette 14–40 µL Buffer TM4 directly onto the PAXgene RNA MinElute spin column membrane. Centrifuge for 1 min at maximum speed to elute the RNA.

    Note: It is important to wet the entire membrane with Buffer TM4 to achieve maximum elution efficiency.

    Smaller volumes of Buffer TM4 can be used to obtain a higher total RNA concentration, but this will influence the overall yield.

    Approximately 2 µL remain the PAXgene RNA MinElute spin column; elution with 14 µL Buffer TM4 results in an eluate with a volume of 12 µL.

20. Recommended: Repeat elution step (step 19) as described, using 14–40 µL Buffer TM4.

    Omitting this step may result in reduced yields.

21. Incubate the eluate for 5 min at 65°C in the shaker–incubator without shaking. After incubation, chill immediately on ice.

    Note: Incubation at 65°C denatures the RNA for downstream applications. Do not exceed the incubation time or temperature.

22. If the RNA samples will not be used immediately, store at –20°C or –70°C. Since the RNA remains denatured after repeated freezing and thawing, it is not necessary to repeat the incubation at 65°C.

Note: For quantification in Tris buffer, use the relationship $A_{260} = 1 \Rightarrow 44$ µg/mL; see "Appendix A: General Remarks on Handling RNA", page 40.

Protocol: Purification of Total RNA, Including miRNA, from Sections of PFPE Tissue

Starting material

Starting material for purification of total RNA, including miRNA, is a minimum of 2 and a maximum of 5 sections of PFPE tissue. Before starting, the tissue sample must be fixed in PAXgene Tissue FIX and stabilized in PAXgene Tissue STABILIZER reagents, dehydrated, and embedded in paraffin (see the *PAXgene Tissue FIX Container Handbook* for information about tissue fixation, stabilization, processing, and paraffin embedding).

> **Note**: Each section should have a thickness of 5–10 µm and a tissue surface area of up to 100 mm^2. Thicker sections may result in lower RNA yields.

Important points before starting

- Do not overload the PAXgene RNA MinElute spin column, as this will significantly reduce RNA yield and quality.
- If working with RNA for the first time, read "Appendix A: General Remarks on Handling RNA", page 40.
- Ensure that the kit boxes are intact and undamaged and that buffers have not leaked. Do not use a kit that has been damaged.
- Ensure that the pipette is set to the correct volume and that liquid is carefully and completely aspirated and dispensed.
- To avoid transferring samples to the wrong tube or spin column, ensure that all tubes and spin columns are properly labeled. Label the lid and the body of each tube. For spin columns, label the body of its processing tube.
- Close each tube or spin column after liquid is transferred to it.

- Spilling samples and buffers during the procedure may reduce the yield and purity of RNA.
- Unless otherwise indicated, all steps of this protocol, including centrifugation steps, should be carried out at room temperature.

Things to do before starting

- Before using the kit for the first time, prepare 80% ethanol by mixing 24 mL ethanol (96–100%, purity grade p.a.) and 6 mL RNase-free water (supplied).
- A shaker–incubator is required in steps 9 and 25. Set the temperature of the shaker–incubator to 45°C.
- Buffer TM1 and Buffer TM2 may form a precipitate upon storage. If necessary, warm to 37°C to dissolve.
- β-Mercaptoethanol (β-ME) must be added to Buffer TM1 before use. Add 10 µL β-ME per 1 mL Buffer TM1. Dispense in a fume hood and wear appropriate protective clothing. Buffer TM1 containing β-ME can be stored at room temperature for up to 1 month.
- Buffer TM2 and Buffer TM3 are supplied as concentrates. Before using for the first time, add 3 volumes of isopropanol to Buffer TM2 and 4 volumes of ethanol (96–100%, purity grade p.a.) to Buffer TM3 as indicated on the bottles to obtain working solutions.
- If using the RNase-Free DNase Set for the first time, prepare a DNase I stock solution. Dissolve the solid DNase I (1500 Kunitz units)* in 550 µL DNase resuspension buffer (RNase-free water) provided with the set. Take care that no DNase I is lost when opening the vial. Do not vortex the reconstituted DNase I. DNase I is especially sensitive to physical denaturation. Mixing should only be carried out by gently inverting the tube.

* Kunitz units are the commonly used units for measuring DNase I, defined as the amount of DNase I that causes an increase in A_{260} of 0.001 per min per milliliter at 25°C, pH 5.0, with highly polymerized DNA as the substrate (Kunitz, M. [1950] J. Gen. Physiol. 33, 349 and 363).

- Current data indicate that reconstituted DNase I can be stored at 2–8°C for up to 6 weeks. For long-term storage of DNase I, remove the stock solution from the glass vial, divide it into single-use aliquots (use the 1.5 mL microcentrifuge tubes supplied with the kit), and store at –15°C to –30°C for up to 6 months. Thawed aliquots can be stored at 2–8°C for up to 6 weeks. Do not refreeze the aliquots after thawing. Ongoing studies may cause us to modify these times. Contact QIAGEN Technical Services for current details.
- When reconstituting and aliquoting DNase I, ensure that you follow the guidelines for handling RNA (see "Appendix A: General Remarks on Handling RNA", page 40).

Procedure

1. Using a microtome, generate a minimum of 2 and a maximum of 5 tissue sections of 5–10 µm thickness from the PFPE tissue.

   Note: If the surface of the PPFE tissue block has been exposed to air, discard the first 2 or 3 sections.
2. Place sections in a 1.5 mL microcentrifuge tube.
3. Add 650 µL xylene to the sample. Vortex vigorously for 20 s, and incubate for 3 min at room temperature.
4. Add 650 µL ethanol (96–100%, purity grade p.a.), and mix by vortexing for 20 s.
5. Centrifuge at maximum speed for 5 min.

   To prevent damage to processing tubes, do not exceed 20,000 × *g*.
6. Remove the supernatant by pipetting. Do not remove any of the pellet. Proceed immediately to step 7.

   Note: In some cases the pellet may be loose. Remove the supernatant carefully.

   Note: The pellet might contain residual paraffin; however, the paraffin will dissolve during digestion with Proteinase K and will not affect the PAXgene Tissue RNA/miRNA procedure.

7. Add 150 µL Buffer TM1, and resuspend the pellet by vortexing for 20 s.
8. Add 290 µL RNase-free water to the resuspended pellet. Then add 10 µL Proteinase K and mix by vortexing for 5 s.

   Note: Do not mix Buffer TM1 and Proteinase K together before adding them to the sample.
9. Incubate for 15 min at 45°C using a shaker–incubator at 1400 rpm. After incubation, centrifuge briefly (1–2 s at 500–1000 × *g*) to remove drops from the inside of the tube lid. Set the temperature of the shaker–incubator to 65°C for use in step 25.
10. Pipette the lysate directly into a PAXgene Shredder spin column (lilac) placed in a 2 mL processing tube, and centrifuge for 3 min at maximum speed (do not exceed 20,000 × *g*).
11. Carefully transfer the entire supernatant of the flow-through fraction to a new 1.5 mL microcentrifuge tube without disturbing the pellet in the processing tube.
12. Add 675 µL isopropanol. Mix by vortexing for 5 s, and centrifuge briefly (1–2 s at 500–1000 × *g*) to remove drops from the inside of the tube lid.

    Note: The length of the centrifugation must not exceed 1–2 s, as this may result in pelleting of nucleic acids and reduced yields of total RNA.

    Note: A precipitate may form after the addition of isopropanol, but this will not affect the PAXgene Tissue RNA/miRNA procedure.
13. Pipette up to 700 µL sample, including any precipitate that may have formed, into a PAXgene RNA MinElute spin column (red) placed in a 2 mL processing tube. Centrifuge for 1 min at 8000 × *g*. Discard the flow-through.*

    Reuse the collection tube in step 14.

* Flow-through contains Buffer TM1 or Buffer TM2 and is therefore not compatible with bleach. See page 8 for Safety Information.

14. Repeat step 13 using the remainder of the sample. Place the spin column in a new 2 mL processing tube, and discard the old processing tube containing flow-through.*

    If the lysate has not completely passed through the membrane after centrifugation, centrifuge again at a higher speed until the PAXgene RNA MinElute spin column is empty.

15. Pipette 350 µL Buffer TM2 into the PAXgene RNA MinElute spin column. Centrifuge for 20 s at 8000 × *g*. Place the spin column in a new 2 mL processing tube, and discard the old processing tube containing flow-through.*

    Note: Buffer TM2 is supplied as a concentrate. Ensure that isopropanol is added to Buffer TM2 before use (see "Things to do before starting", page 31).

16. Add 10 µL DNase I stock solution to 70 µL Buffer RDD in a 1.5 mL microcentrifuge tube. Mix by gently flicking the tube, and centrifuge briefly to collect residual liquid from the sides of the tube.

    For example, if processing 10 samples, add 100 µL DNase I stock solution to 700 µL DNA Buffer RDD. Use the 1.5 mL microcentrifuge tubes supplied with the kit.

    Note: DNase I is especially sensitive to physical denaturation. Mixing should only be carried out by gently flicking the tube. Do not vortex.

17. Pipette the DNase I incubation mix (80 µL) directly onto the PAXgene RNA MinElute spin column, and incubate for 15 min at room temperature.

    Note: Ensure that the DNase I incubation mix is placed directly onto the membrane. DNase digestion will be incomplete if part of the mix is applied to and remains on the walls or the O-ring of the spin column.

18. Centrifuge for 20 s at 8000 × *g*. Place the spin column in a new 2 mL processing tube. Keep the flow-through for rebinding in step 19.

19. Add 350 µL Buffer TM2 to the flow-through from step 18. Mix carefully by pipetting up and down 5 times.

20. Pipette the mixture from step 19 into the PAXgene RNA MinElute spin column, and centrifuge for 20 s at 8000 × *g*. Place the spin column in a new 2 mL processing tube, and discard the old processing tube containing flow-through.*
21. Pipette 500 µL Buffer TM3 into the PAXgene RNA MinElute spin column, and centrifuge for 20 s at 8000 × *g*. Discard the flow-through and reuse the 2 mL processing tube in step 22.

    Note: Buffer TM3 is supplied as a concentrate. Ensure that ethanol is added to Buffer TM3 before use (see "Things to do before starting", page 30).
22. Pipette 500 µL 80% ethanol into the PAXgene RNA MinElute spin column, and centrifuge for 2 min at 8000 × *g*.
23. Discard the processing tube containing the flow-through, and place the PAXgene RNA MinElute spin column in a new 2 mL processing tube. Open the cap of the spin column, and centrifuge for 5 min at maximum speed (but do not exceed 20,000 × *g*).
24. Discard the processing tube containing the flow-through. Place the PAXgene RNA MinElute spin column in a 1.5 mL microcentrifuge tube, and pipette 14–40 µL Buffer TM4 directly onto the PAXgene RNA MinElute spin column membrane. Centrifuge for 1 min at maximum speed to elute the RNA.

    Note: It is important to wet the entire membrane with Buffer TM4 to achieve maximum elution efficiency.

    Smaller volumes of Buffer TM4 can be used to obtain a higher total RNA concentration, but this will influence the overall yield.

    Approximately 2 µL remain in the PAXgene RNA MinElute spin column; elution with 14 µL Buffer TM4 results in an eluate with a volume of 12 µL.

* Flow-through contains Buffer TM1 or Buffer TM2 and is therefore not compatible with bleach. See page 8 for Safety Information.

25. Incubate the eluate for 5 min at 65°C in the shaker–incubator (from step 9) without shaking. After incubation, chill immediately on ice.

    Note: Incubation at 65°C denatures the RNA for downstream applications. Do not exceed the incubation time or temperature.

26. If the RNA samples will not be used immediately, store at –20°C or at –70°C. Since the RNA remains denatured after repeated freezing and thawing, it is not necessary to repeat the incubation at 65°C.

    Note: For quantification in Tris buffer, use the relationship $A_{260} = 1 \Rightarrow 44$ µg/mL; see "Appendix A: General Remarks on Handling RNA", page 40.

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 on the respective product page at **www.preanalytix.com** or our Technical Support Center at **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 or protocols in this handbook (for contact information, visit **support.qiagen.com**).

	Comments and suggestions
RNA degraded	
a) Tissue not immediately stabilized	Tissue specimen must be fixed after resection as soon as possible, ideally within 30 min after resection.
b) Tissue specimen too large	For efficient and even fixation, the tissue specimen must not be too large. A single sample should have a maximum size of 20 × 20 × 20 mm for fixation in PAXgene Tissue FIX Containers. See "Protocol: Sample Fixation and Stabilization" in the *PAXgene Tissue FIX Container Handbook*.
c) Fixation time exceeded	Do not fix for longer than 72 h in PAXgene Tissue Fix before transferring into PAXgene Tissue STABILIZER. Longer fixation periods may lead to RNA degradation. Fixation for 30 min per millimeter thickness of the sample is sufficient for most tissue types. See "Protocol: Sample Fixation and Stabilization" in the *PAXgene Tissue FIX Container Handbook*.
d) Sample inappropriately handled during processing	Incubating tissues fixed and stabilized with PAXgene Tissue reagents in water, formalin, alcoholic solutions with less than 80% ethanol, or in paraffin with a temperature above 60°C leads to degradation of RNA. Start processing with 80–100% ethanol first. Incubate specimen in liquid paraffin at 56°C for up to 3 h. Follow the protocol for "Sample Processing, Paraffin Embedding, and Sectioning" and see examples for processing protocols in the appendix "Processing Protocols Successfully Tested for Use with Specimens Treated with the PAXgene Tissue System" in the *PAXgene Tissue FIX Container Handbook*.
e) PFPE tissue stored inappropriately	Tissue morphology is preserved in PFPE tissue when stored at room temperature. However, biomolecules within paraffin will undergo slow chemical degradation. For best preservation of morphology and biomolecule integrity within the paraffin-embedded tissue, blocks of PFPE tissue can be stored at 2–25°C for short-term storage or transport, or at –15°C to –30°C for long-term storage or transport.

	Comments and suggestions
f) RNase contamination	Although all PAXgene buffers have been tested and are guaranteed RNase-free, RNases can be introduced during use. Be certain not to introduce any RNases during the PAXgene Tissue procedure or later handling. See Appendix A (page 40) for general remarks on handling RNA. Do not put RNA samples into a vacuum dryer or microcentrifuge that has been used in DNA preparations where RNases may have been used.
g) 80% ethanol not made with RNase-free water	The 80% ethanol used to wash the PAXgene RNA MinElute spin column membrane must be free of RNases. Be sure to prepare the 80% ethanol using ethanol (96–100%, purity grade p.a.) and the RNase-free water supplied with the kit, as described in "Things to do before starting" in each protocol.
Low RNA yield	
a) Reagents used for processing contaminated with formalin	Do not reuse alcohol contaminated with formalin to process PAXgene Tissue-fixed samples as this can lead to significant reduction in RNA yield and quality. We recommend separating alcohol used to process PAXgene Tissue-treated samples from alcohol used to process formalin-fixed samples. At least the first 5 positions in the processing should be formalin free. With this precaution, it is possible to process PAXgene Tissue-fixed and formalin-fixed samples on the same instrument. For more information refer to the *PAXgene Tissue FIX Container Handbook*.
b) Too much starting material	Reduce the amount of starting material used. Do not use more than the amount specified in "Starting material" at the beginning of each protocol.
c) RNA still bound to spin column membrane	Repeat RNA elution, but incubate the PAXgene RNA MinElute spin column on the benchtop for 10 min with Buffer TM4 before centrifuging.
d) Ethanol carryover	After the wash with 80% ethanol, be sure to centrifuge at full speed for 5 min to dry the PAXgene RNA MinElute spin column membrane. After centrifugation, carefully remove the PAXgene RNA MinElute spin column from the collection tube so that the column does not contact the flow-through. Otherwise, carryover of ethanol will occur.
e) RNA concentration measured in water	RNA concentration must be measured in 10 mM Tris·Cl,* pH 7.5, for accurate quantification (see Appendix B, page 42).
f) Centrifugation for more than 1–2 s after adding isopropanol to the lysate (step 7, page 26; step 13, page 33)	After adding ethanol to the lysate, the samples should only be centrifuged briefly: no more than 1–2 s. Longer centrifugation may result in pelleting of RNA and reduced yields.
g) Elution Buffer TM4 incorrectly dispensed	Pipette RNase-free water into the center of the PAXgene RNA MinElute spin column membrane to ensure that the membrane is completely covered.

* 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
h) Insufficient disruption and homogenization	See "Description of protocols", page 17, for details on disruption and homogenization methods. In subsequent preparations, reduce the amount of starting material (see the individual protocols).
PAXgene RNA MinElute spin column clogged	
a) Too much starting material and or insufficient lysis	Reduce the amount of starting material used (see "Starting material" at the beginning of each protocol). Increase *g*-force and/or duration of centrifugation steps.
b) Centrifugation temperature too low	The centrifugation temperature should be 20–25°C.
Low A_{260}/A_{280} ratio	
a) Too much starting material and or insufficient lysis	Reduce the amount of starting material used (see "Starting material" at the beginning of each protocol). Increase *g*-force and/or duration of centrifugation steps.
b) Centrifugation temperature too low	The centrifugation temperature should be 20–25°C.
Low miRNA yield or miRNA does not perform well in downstream applications	
a) Salt carryover during elution	Ensure that Buffer TM3 has been used at room temperature (15–25°C).
b) Ethanol carryover	After the wash with 80% ethanol, be sure to centrifuge at full speed for 5 min to dry the PAXgene RNA MinElute spin column membrane. After centrifugation, carefully remove the PAXgene RNA MinElute spin column from the collection tube so that the column does not contact the flow-through. Otherwise, carryover of ethanol will occur.

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, 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 carry bacteria and molds, and these 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.

Protocols for removing RNase-contamination from glassware and solutions can be found in general molecular biology guides, such as Sambrook and Russell (1).

Avoiding cross-contamination

Because of the sensitivity of nucleic acid amplification technologies, the following precautions are necessary when handling samples to avoid cross-contamination:

- Carefully pipette the sample into the spin column without moistening the rim of the column.
- Always change pipette tips between liquid transfers. Use aerosol-barrier pipette tips.
- Avoid touching the spin column membrane with the pipette tip.
- After vortexing or heating a microcentrifuge tube, briefly centrifuge it to remove drops from the inside of the lid.
- Wear gloves throughout the entire procedure. In case of contact between gloves and sample, change gloves immediately.
- Close the spin column before placing it in the microcentrifuge. Centrifuge as described in the procedure.
- Open only one spin column at a time, and take care to avoid generating aerosols.
- For efficient parallel processing of multiple samples, fill a rack with processing tubes to which the spin columns can be transferred after centrifugation. Discard the used processing tubes containing flow-through, and place the new processing tubes containing spin columns directly in the microcentrifuge.

Appendix B: Quantification and Determination of Quality of Total RNA

Quantification of RNA

The concentration of RNA should be determined by measuring the absorbance at 260 nm (A_{260}) in a spectrophotometer. To ensure significance, readings should be in the linear range of the spectrophotometer. An absorbance of 1 unit at 260 nm corresponds to 44 µg of RNA per mL ($A_{260} = 1 \Rightarrow 44$ µg/mL). This relation is valid only for measurements in 10 mM Tris·Cl,* pH 7.5. Therefore, if it is necessary to dilute the RNA sample, this should be done in 10 mM Tris Cl. 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, ensure that cuvettes are RNase free. Use the buffer in which the RNA is diluted to zero the spectrophotometer, and make sure to add the same volume of Buffer TM4 as the volume of eluted RNA to be diluted.

* 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.

An example of the calculation involved in RNA quantification is shown below:

Volume of RNA sample = 120 µL
Dilution = 10 µL RNA sample + 140 µL 10 mM Tris·Cl, pH 7.5 (1/15 dilution)
Measure absorbance of diluted sample in a cuvette (RNase-free).
A_{260} = 0.2

Concentration of RNA sample	= 44 × A_{260} × dilution factor
	= 44 × 0.2 × 15
	= 132 µg/mL
Total yield	= concentration × volume of sample in milliliters
	= 132 µg/mL × 0.12 mL
	= 15.8 µg RNA

Purity of RNA

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 that absorb UV light, such as protein. However, the A_{260}/A_{280} ratio is influenced considerably by pH. Lower pH results in a lower A_{260}/A_{280} ratio and reduced sensitivity to protein contamination (2). 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 1.8–2.2 in 10 mM Tris·Cl,* pH 7.5. Use the buffer in which the RNA is diluted to zero the spectrophotometer, and make sure to add the same volume of Buffer TM4 as the volume of eluted RNA to be diluted. Buffer TM4 has high absorbance at 220 nm, which can lead to high background absorbance levels if the spectrophotometer is not properly zeroed.

* 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: Product Warranty and Satisfaction Guarantee

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

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

References

1. Sambrook, J. and Russell, D.W. (2001) Molecular Cloning: A Laboratory Manual. 3rd ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.
2. Wilfinger, W.W., Mackey, M., and Chomcynski, P. (1997) Effect of pH and ionic strength on the spectrophotometric assessment of nucleic acid purity. BioTechniques **22**, 474–476.

Ordering Information

Product	Contents	Cat. no.
PAXgene Tissue RNA/miRNA Kit (50)	For 50 RNA preps: PAXgene RNA MinElute Spin Columns, PAXgene Shredder Spin Columns, Processing Tubes, Microcentrifuge Tubes, Carrier RNA, RNase-Free DNase, and RNase-Free Buffers; to be used in conjunction with PAXgene Tissue FIX Containers	766134
Related products		
PAXgene Tissue FIX Containers (50 mL)	For collection, fixation, and stabilization of 10 samples: 10 Prefilled Reagent Containers, containing PAXgene Tissue FIX and PAXgene Tissue STABILIZER	765312
PAXgene Tissue STABILIZER Concentrate (150 mL)	For transport or storage of tissue specimens fixed with the PAXgene Tissue FIX Container	765512
PAXgene Tissue DNA Kit (50)	For 50 DNA preps: PAXgene DNA Mini Spin Columns, PAXgene Shredder Spin Columns, Processing Tubes, Microcentrifuge Tubes, Carrier RNA, and Buffers; to be used in conjunction with PAXgene Tissue Containers	767134
TissueRuptor II	Handheld rotor–stator homogenizer	Variable*
TissueRuptor Disposable Probes (25)	Nonsterile plastic disposable probes (25) for use with the TissueRuptor	990890
TissueLyser III	Bead mill, 100–120/220–240 V, 50/60 Hz; requires the TissueLyser Adapter Set 2 × 24 or TissueLyser Adapter Set 2 × 96 (available separately)	9003240
TissueLyser Adapter Set 2 × 24	Two sets of Adapter Plates and 2 racks for use with 2 mL microcentrifuge tubes on the TissueLyser	69982

* Contact your QIAGEN sales team or local distributor for the instrument available in your country.

Product	Contents	Cat. no.
TissueLyser Adapter Set 2 × 96	Two sets of Adapter Plates for use with Collection Microtubes (racked) on the TissueLyser II	69984
Stainless Steel Beads, 5 mm (200)	Stainless Steel Beads (5 mm diameter), suitable for use with the TissueLyser system	69989
TissueLyser Single-Bead Dispenser, 5 mm	For dispensing individual beads (5 mm diameter)	
QIAcube Connect*	Instrument, connectivity package, 1-year warranty on parts and labor	9002864
Starter Pack, QIAcube	Reagent bottle racks (3); 200 µL filter-tips (1024); 1000 µL filter-tips (1024); 30 mL reagent bottles (12); rotor adapters (240); rotor adapter holder	990395

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

* All QIAcube Connect instruments are provided with a region-specific connectivity package, including tablet and equipment necessary to connect to the local network. Furthermore, QIAGEN offers comprehensive instrument service products, including service agreements, installation, introductory training, and preventive subscription. Contact your local sales representative to learn about your options.

Document Revision History

Date	Changes
05/2021	Revisions throughout document to reflect discontinuation of related products (PAXgene Tissue Container and PAXgene Tissue RNA Kit), change of kit name and removal of the protocol for PFPE tissue blocks, addition of information about QIAcube Connect, and general update into revised template.
06/2021	Corrected the numbering of protocol steps of the section "Protocol: Purification of Total RNA, Including miRNA, from Sections of PFPE Tissue".
02/2026	Updated the street address of PreAnalytiX GmbH changed from "Feldbachstrasse" to "Garstligweg 8".



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

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