July 2023

PAXgene® Tissue DNA Kit Handbook

For isolation and purification of genomic DNA from tissue samples fixed in PAXgene Tissue FIX Containers

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

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

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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 DNA Kit for any particular use since 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.

Materials Provided

Kit contents

PAXgene Tissue DNA Kit Catalog no. Number of preps		(50) 767134 50
Component name	**Description**	**Quantity**
Buffer TD1	Lysis Buffer	10 mL
Buffer TD2	Binding Buffer*	12 mL
Buffer TD3	Wash Buffer 1 concentrate*†	19 mL
Buffer TD4	Wash Buffer 2 concentrate‡	13 mL
Buffer TD5	Elution Buffer	25 mL
Proteinase K	Green lid	1.4 mL
PAXgene DNA Spin Columns with Processing Tubes	–	5 × 10
Processing Tubes	2 mL	3 × 50
Safe-lock Microcentrifuge Tubes	1.5 mL	50
Microcentrifuge Tubes	1.5 mL	50
Carrier RNA	Red lid	310 µg
Handbook	–	1

* Contains a guanidine salt. See page 7 for Safety Information.

† Buffer TD3 is supplied as concentrate. Before using for the first time, add 25 mL of ethanol (96–100%, purity grade p.a.) as indicated on the bottle to obtain a working solution.

‡ Buffer TD4 is supplied as a concentrate. Before using for the first time, add 30 mL of ethanol (96–100%, purity grade p.a.) as indicated on the bottle to obtain a working solution.

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 or PreAnalytiX kit and kit component.

CAUTION

DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

Buffer TD2 and Buffer TD3 contain guanidine hydrochloride, which can form highly reactive compounds when combined with bleach. If liquid containing this buffer 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 DNA Kit is tested against predetermined specifications to ensure consistent product quality.

Storage and Handling

The PAXgene Tissue DNA Kit is shipped at ambient temperature.

The PAXgene DNA spin columns should be stored upon receipt at 2–8°C. All buffers can be stored at room temperature (15–25°C).

The Reconstituted Wash Buffer 1 (TD3) and reconstituted Wash Buffer 2 (TD4) are stable for 1 year when stored at room temperature (15–25°C).

Proteinase K is stable for at least 1 year after delivery when stored at room temperature (15–25°C). 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.

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 cause inhibition in sensitive downstream applications, such as RT-PCR, qPCR, or next-generation sequencing. 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 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 reagent 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 prefilled 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 purification of high-quality nucleic acids for molecular analysis.

Sample collection and stabilization with PAXgene Tissue FIX Containers

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

When fixed tissue is stored in PAXgene Tissue STABILIZER, 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 tissue samples.

Tissue samples can be stored in PAXgene Tissue STABILIZER 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 RNA/miRNA Kit Handbook* for information about RNA isolation, including miRNA, or the PAXgene Tissue supplementary protocols at **www.preanalytix.com** for protein purification and other applications.

DNA purification

The PAXgene Tissue DNA Kit enables purification of genomic DNA from tissues fixed and stabilized with the PAXgene Tissue System or from sections of PFPE tissues (see "Protocol: Purification of Genomic DNA from PAXgene Tissue-Fixed Samples", page 20, and "Protocol: Purification of Genomic DNA from Sections of PFPE Tissue", page 24). The purification of genomic DNA from sections of PFPE tissues can be automated on QIAGEN's QIAcube® Connect or classic QIAcube instruments (see "Automated purification of genomic DNA on QIAcube instruments", page 13).

Lysis of the tissue sample performed is in the lysis buffer, Buffer TD1, with digestion using Proteinase K. Buffering conditions are adjusted with binding Buffer TD2 and ethanol to provide optimal DNA-binding conditions, and the lysate is loaded onto the PAXgene DNA spin column. During centrifugation, DNA is selectively bound to the silica membrane and contaminants pass through. Remaining contaminants and enzyme inhibitors are removed in 2 efficient wash steps with wash buffers TD3 and TD4, and DNA is then eluted in low-salt elution Buffer TD5, ready for use.

Total DNA purified using the PAXgene Tissue DNA Kit is highly pure. DNA has A_{260}/A_{280} ratios of 1.7:1.9, and absorbance scans show a symmetrical peak at 260 nm, confirming the high purity of genomic DNA. Contamination is minimized, and purified DNA is ready to use in downstream applications with no detectable PCR inhibition.

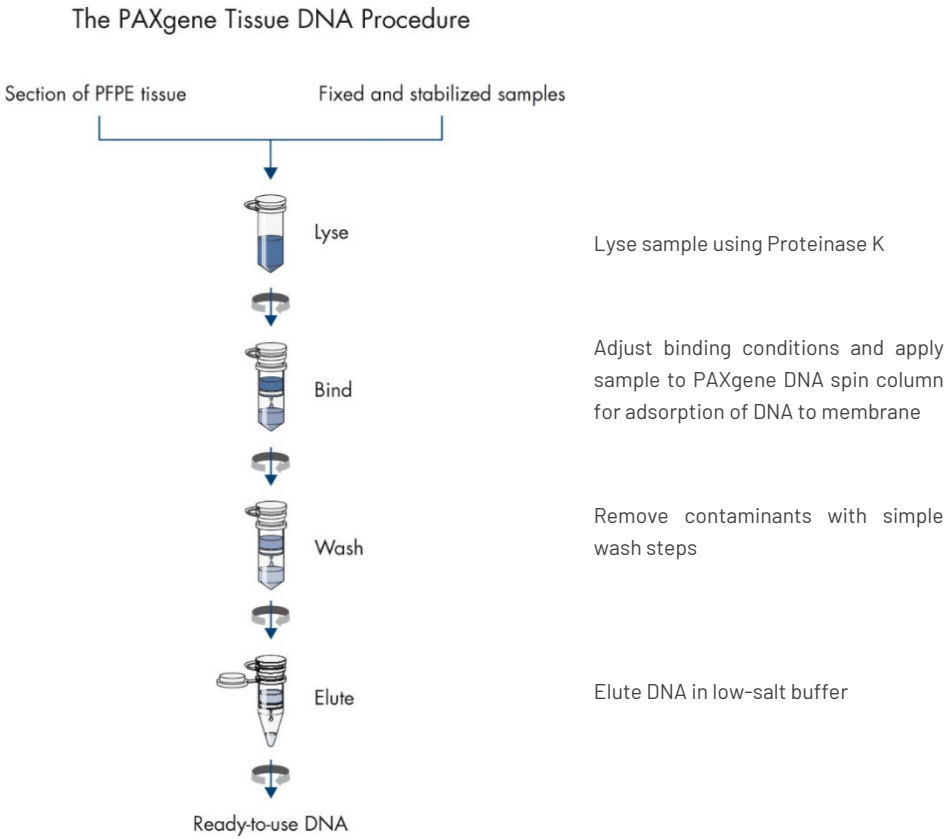

Figure 1. The PAXgene Tissue DNA procedure generates high yields of genomic DNA from tissue samples.

Automated purification of genomic DNA on QIAcube instruments

Purification of genomic DNA can be fully 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 prep 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 DNA Kit for purification of genomic DNA.

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 free of charge at **www.qiagen.com/qiacubeprotocols**.

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-DNA**).

- PAXgene Tissue FIX Container
- PAXgene Tissue STABILIZER Concentrate
- Xylene
- Ethanol (96–100%, purity grade p.a.)
- Pipettes* (10 µL – 1 mL)
- Sterile, aerosol-barrier, RNase-free pipette tips
- Graduated cylinder
- Variable-speed microcentrifuge* capable of attaining 1000–20,000 × *g*, and equipped with a rotor for 2 mL microcentrifuge tubes
- Shaker–incubator* capable of incubating at 56°C, 70°C, and 80°C, and shaking at ≥400 rpm, not exceeding 1400 rpm (e.g., Eppendorf© Thermomixer Compact, **www.eppendorf.com**, or equivalent)†
- Vortex mixer*
- Scalpel
- Crushed ice
- Optional: RNase A (100 mg/mL; QIAGEN, cat. no. 19101)

* Ensure that instruments have been checked and calibrated regularly according to the manufacturer's recommendations.
† 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 using the TissueLyser®", page 17). We recommend the TissueLyser* system (see Ordering Information, page 35).
- 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 20)

Starting material for DNA purification should be up to 20 mg of a tissue sample that has been fixed and stabilized with PAXgene Tissue reagents. For samples with very high DNA contents (e.g., spleen), no more than 10 mg of tissue should be used per sample prep.

The tissue sample is removed from the container. If necessary, the sample is cut into 2 mm cubes. A 2 mm cube of most tissues weighs approximately 8–12 mg. To enable efficient lysis, the tissue sample is further cut into smaller pieces and lysed in lysis Buffer TD1 by Proteinase K digestion. To reduce lysis time and to disrupt tissue efficiently, we strongly recommend using a bead mill, such as the TissueLyser before Proteinase K digestion (see "Disrupting and homogenizing using the TissueLyser", page 17).

Section of PFPE tissue (page 24)

Starting material for DNA purification is freshly cut sections from tissue samples that have been fixed and stabilized with PAXgene Tissue reagents and 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 tissue sections by incubation in xylene, followed by addition of ethanol, and centrifugation. The resulting pellet is resuspended in a lysis buffer and digested with Proteinase K.

Disrupting and homogenizing 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 disrupts and homogenizes up to 48 tissue samples simultaneously when used in combination with the TissueLyser Adapter Set 2 × 24. The adapter set holds 48 × 2 mL microcentrifuge tubes containing stainless steel beads of 5 mm mean diameter. For guidelines on using the TissueLyser, refer to the *TissueLyser Handbook*. For other bead mills, refer to suppliers' guidelines.

Carrier RNA

The kit is supplied with carrier RNA, which can be added to Buffer TD2 if required. Carrier RNA enhances binding of DNA to the PAXgene DNA spin column membrane if there are very few target molecules in the sample.

We recommend adding carrier RNA to Buffer TD2 for purification of DNA from very small samples, such as sections of paraffin-embedded tissue from biopsies or if the sample has less than 5 ng DNA (<10,000 copies).

If carrier RNA is used, eluates from PAXgene DNA spin columns contain both sample DNA and carrier RNA, with the amount of carrier RNA greatly exceeding the amount of DNA. Calculations of how much eluate to add to downstream amplifications should therefore be based on the amount of carrier RNA added to Buffer TD2. To obtain the highest levels of sensitivity in amplification reactions, it may be necessary to adjust the amount of carrier RNA added to Buffer TD2.

Adding carrier RNA to Buffer TD2

Add 310 µL Buffer TD5 to the tube containing 310 µg lyophilized carrier RNA to obtain a stock solution of 1 µg/µL. Dissolve the carrier RNA thoroughly, divide it into conveniently

sized aliquots, and store at –20°C. Do not freeze–thaw the aliquots of carrier RNA more than 3 times.

Add 1 µL of carrier RNA stock solution per 200 µL Buffer TD2 used in the procedure. Calculate the volume of Buffer TD2 and dissolved carrier RNA needed per batch of samples by multiplying by the number of samples to be simultaneously processed. To allow for pipetting errors, always prepare enough buffer for processing 2 extra samples. For example, when processing 8 samples, add 10 µL of dissolved carrier RNA to 2 mL Buffer TD2. Gently mix Buffer TD2 and carrier RNA stock solution by inverting the tube 10 times. To avoid foaming, do not vortex. Note that carrier RNA does not dissolve in Buffer TD2. It must first be dissolved in Buffer TD5 and then added to Buffer TD2. Buffer TD2 containing carrier RNA is stable at room temperature for up to 48 hours.

Copurification of RNA

The PAXgene Tissue DNA procedure copurifies DNA and RNA when both are present in the sample. Transcriptionally active tissues such as liver and kidney contain high levels of RNA, which will be copurified. RNA may inhibit some downstream enzymatic reactions, although it does not affect PCR. If RNA-free genomic DNA is required, RNase A should be added to the sample before addition of Buffer TD2 to digest the RNA.

Elution of pure nucleic acids

For downstream applications that require small starting volumes (e.g., some PCR assays), a concentrated eluate may increase assay sensitivity. PAXgene DNA spin columns allow a minimum elution volume of 20 µL for concentrated nucleic acid eluates.

The volume of eluate recovered may be up to 5 µL less than the volume of Buffer TD5 applied to the PAXgene DNA spin column. For example, an elution volume of 20 µL results in 15 µL eluate.

For maximum DNA yield, elution is performed in 2 successive steps using 100–200 µL Buffer TD5 for each step. The number of elution steps depends on the amount of DNA bound to the PAXgene DNA membrane. For samples containing up to 10 µg DNA, a single elution step using 100–200 µL is sufficient. For samples containing more than 10 µg DNA, a second elution step with another 100–200 µL Buffer TD5 is recommended. Approximately 60–80% of the DNA will elute in the first elution.

The volume of eluate should not exceed 200 µL per 1.5 mL microcentrifuge tube as the spin column could come into contact with the eluate, leading to possible aerosol formation during centrifugation.

If the purified DNA is to be stored for up to 24 hours, we recommend storage at 2–8°C. For periods longer than 24 hours, we recommend storage at –20°C. Buffer TD5 is composed of 10 mM Tris.Cl and 0.5 mM EDTA, pH 9.0. Elution with Buffer TD5 guarantees optimal recovery and stability of eluted DNA. Buffer TD5 should be used at room temperature. Heating Buffer TD5 before elution is not necessary.

Protocol: Purification of Genomic DNA from PAXgene Tissue-Fixed Samples

Starting material

Starting material for DNA purification should be up to 20 mg of a tissue sample fixed and stabilized with PAXgene Tissue reagents. For samples with very high DNA content (e.g., spleen or tonsils), no more than 10 mg of tissue should be used.

It is essential to use the correct amount of starting material in order to obtain optimal DNA yield and purity. A maximum amount of 20 mg tissue fixed and stabilized using PAXgene Tissue reagents can generally be processed. For most tissues, the DNA binding capacity of the PAXgene DNA spin column 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 points before starting

- **Do not overload the PAXgene DNA spin column as this will significantly reduce DNA yield and quality.**
- Ensure that the kit boxes are intact and undamaged and that buffers have not leaked. Do not use a kit that has been damaged.
- When using a pipette, ensure that it 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.

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

Things to do before starting

- Buffer TD1 and Buffer TD2 may form precipitates upon storage. If necessary, warm to 56°C until the precipitates have fully dissolved.
- Buffer TD3 and Buffer TD4 are supplied as concentrates. Before using for the first time, add the appropriate amount of ethanol (96–100%, purity grade p.a.) as indicated on the bottle to obtain a working solution.
- Preheat a shaker–incubator, thermomixer, or shaking water bath to 56°C for use in step 5.

Procedure

1. Cut up to 20 mg tissue (up to 10 mg spleen or tonsil) from a PAXgene Tissue FIX Container into small pieces. Place into a 2 mL round-bottomed processing tube (not supplied), and add 180 µL Buffer TD1.

   Cut the tissue into small pieces to enable more efficient lysis.

2. Add one stainless steel bead (5 mm mean diameter) to each 2 mL processing tube, and place the tubes in the TissueLyser Adapter Set 2 × 24.

   Note: If no bead mill is available, a rotor–stator homogenizer (e.g., the TissueRuptor®) can be used as an alternative. In this case, the homogenization time should be optimized and the sample should not be homogenized completely to avoid DNA shearing.

3. Operate the TissueLyser for 20 s at 15 Hz.

   Note: To avoid shearing of the genomic DNA do not exceed operating time or speed.

4. Carefully pipette the lysates into new 1.5 mL safe-lock microcentrifuge tubes.

   Do not reuse the stainless steel beads.

5. Add 20 µL Proteinase K and mix by vortexing. Incubate at 56°C for 1 h using a shaker–incubator at 1400 rpm. After incubation, set the temperature of the shaker–incubator to 70°C for use in step 8.

   Note: If tissue disruption was not carried out (e.g., using the TissueLyser), increase lysis time to 3 h or overnight until the tissue is completely lysed.

6. Briefly centrifuge the safe-lock microcentrifuge tube to remove drops from the inside of the lid.

   Note: If RNA-free genomic DNA is required, add 4 µL RNase A (100 mg/mL), mix by vortexing, and incubate for 2 min at room temperature.

7. Add 200 µL Buffer TD2, and mix by pulse-vortexing for 15 s.

   It is essential that the sample and Buffer TD2 are mixed thoroughly by vortexing or pipetting to yield a homogeneous solution.

8. Incubate at 70°C for 10 min.

9. Add 200 µL ethanol (96–100%) to the sample, and mix thoroughly by vortexing.

   It is important that the sample and the ethanol are mixed thoroughly to yield a homogeneous solution.

   A white precipitate may form on addition of ethanol. This precipitate does not interfere with the PAXgene Tissue DNA procedure.

10. Briefly centrifuge the safe-lock microcentrifuge tube to remove drops from the inside of the lid.

11. Pipette the sample, including any precipitate that may have formed, into the PAXgene DNA spin column (placed in a 2 mL processing tube), and centrifuge for 1 min at 6000 × *g*. Place the spin column in a new 2 mL processing tube, and discard the old processing tube containing flow-through.*

* Flow-through contains Buffer TD2 or Buffer TD3 and is therefore not compatible with bleach. See page 7 for Safety Information.

12. Pipette 500 µL Buffer TD3 into the PAXgene DNA spin column and centrifuge for 1 min at 6000 × *g*. Place the spin column in a new 2 mL processing tube, and discard the old processing tube containing flow-through.*
13. Pipette 500 µL Buffer TD4 into the PAXgene DNA spin column and centrifuge for 1 min at 6000 × *g*. Place the spin column in a new 2 mL processing tube, and discard the old processing tube containing flow-through.
14. Centrifuge for 3 min at maximum speed to dry the membrane completely.

    Note: Tubes may break if centrifugation speed exceeds 20,000 × *g*.

    This step is necessary, since ethanol carryover into the eluate may interfere with some downstream applications.
15. Discard the processing tube containing the flow-through. Place the PAXgene DNA spin column in a 1.5 mL microcentrifuge tube, and pipette 20–200 µL Buffer TD5 directly onto the PAXgene DNA spin column membrane. Centrifuge for 1 min at maximum speed to elute the DNA.

    Note: Tubes may break if centrifugation speed exceeds 20,000 × *g*.

    Incubating the PAXgene DNA spin column loaded with Buffer TD5 for 5 min at room temperature before centrifugation generally increases DNA yield.
16. Recommended: Repeat elution once as described in step 15.

    Omitting this step may result in reduced yields.

* Flow-through contains Buffer TD2 or Buffer TD3 and is therefore not compatible with bleach. See page 7 for Safety Information.

Protocol: Purification of Genomic DNA from Sections of PFPE Tissue

Starting material

Starting material for DNA purification should be a minimum of 2 and a maximum of 5 sections of PFPE tissue. Before starting, the tissue samples must be fixed and stabilized with PAXgene Tissue reagents, dehydrated, and embedded in paraffin (see the *PAXgene Tissue FIX Container Handbook* for information about tissue fixation, stabilization, and paraffin embedding). 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 DNA yields.

Important points before starting

- **Do not overload the PAXgene DNA spin column as this will significantly reduce DNA yield and quality.**
- Ensure that the kit boxes are intact and undamaged and that buffers have not leaked. Do not use a kit that has been damaged.
- When using a pipette, ensure that it 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.
- Spillages of samples and buffers during the procedure may reduce the yield and purity of DNA.
- Unless otherwise indicated, all steps of this protocol, including centrifugation steps, should be carried out at room temperature.

Things to do before starting

- Buffer TD1 and Buffer TD2 may form precipitates upon storage. If necessary, warm to 56°C until the precipitates have fully dissolved.
- Buffer TD3 and Buffer TD4 are supplied as concentrates. Before using for the first time, add the appropriate amount of ethanol (96–100%, purity grade p.a.) as indicated on the bottle to obtain a working solution.
- Preheat a shaker–incubator, thermomixer, or shaking water bath to 56°C for use in step 9.

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 sample surface has been exposed to air, discard the first 2 or 3 sections.
2. Place the sections in a 1.5 mL safe-lock 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.

   Note: 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.

   Note: In some cases, the pellet may be loose. Remove the supernatant carefully.
   Note: The pellet might contain residual paraffin. The paraffin will dissolve during digestion with Proteinase K and will not affect the PAXgene Tissue DNA procedure.
7. Open the tube and incubate at room temperature or up to 37°C. Incubate for 10 min or until all residual alcohol has evaporated.
8. Resuspend the pellet in 180 µL Buffer TD1. Add 20 µL Proteinase K, and mix by pulse-vortexing for 15 s.

9. Incubate for 1 h at 56°C using a shaker–incubator at 1400 rpm.
10. Briefly centrifuge the safe-lock microcentrifuge tube to remove drops from the inside of the lid.

    Note: If RNA-free genomic DNA is required, add 4 µL RNase A (100 mg/mL), mix by vortexing and incubate for 2 min at room temperature. Proceed immediately to step 11.
11. Briefly centrifuge the 1.5 mL safe-lock microcentrifuge tube to remove drops from the inside of the lid.
12. Add 200 µL Buffer TD2, and mix by pulse-vortexing for 15 s. It is essential that the sample and Buffer TD2 are mixed thoroughly by vortexing or pipetting to obtain a homogeneous solution.

    Note: If carrier RNA is required (see "Carrier RNA", page 17), add 1 µg carrier RNA stock solution to 200 µL Buffer TD2. Note that carrier RNA does not dissolve in Buffer TD2. It must first be dissolved in Buffer TD5 and then added to Buffer TD2.
13. Add 200 µL of ethanol (96–100%) to the sample, and mix thoroughly by vortexing. It is important that the sample and the ethanol are properly mixed to obtain a homogeneous solution. A white precipitate may form upon addition of ethanol. This precipitate does not interfere with the PAXgene Tissue DNA procedure.
14. Briefly centrifuge the 1.5 mL tube to remove drops from the inside of the lid.
15. Pipette the sample, including any precipitate that may have formed, into the PAXgene DNA spin column placed in a 2 mL processing tube, and centrifuge for 1 min at 6000 × *g*. Place the spin column in a new 2 mL processing tube, and discard the old processing tube containing flow-through.*

* Flow-through contains Buffer TD2 or Buffer TD3 and is therefore not compatible with bleach. See page 7 for Safety Information.

16. Pipette 500 µL Buffer TD3 into the PAXgene DNA spin column, and centrifuge for 1 min at 6000 × *g*. Place the spin column in a new 2 mL processing tube, and discard the old processing tube containing flow-through.*
17. Pipette 500 µL Buffer TD4 into the PAXgene DNA spin column and centrifuge for 1 min at 6000 × *g*. Place the spin column in a new 2 mL processing tube, and discard the old processing tube containing flow-through.
18. Centrifuge for 3 min at maximum speed to dry the membrane completely.

    Note: Tubes may break if centrifugation speed exceeds 20,000 × *g*. This step is necessary, since ethanol carryover into the eluate may interfere with some downstream applications.
19. Discard the processing tube containing the flow-through. Place the PAXgene DNA spin column in a 1.5 mL microcentrifuge tube, and pipette 20–200 µL Buffer TD5 directly onto the PAXgene DNA spin column membrane. Centrifuge for 1 min at maximum speed to elute the DNA.

    Note: Tubes may break if centrifugation speed exceeds 20,000 × *g*.

    Incubating the PAXgene DNA spin column loaded with Buffer TD5 for 5 min at room temperature before centrifugation generally increases DNA yield.

* Flow-through contains Buffer TD2 or Buffer TD3 and is therefore not compatible with bleach. See page 7 for Safety Information.

Reference

1. 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.

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/or protocols in this handbook or sample and assay technologies (for contact information, visit **www.qiagen.com**).

	Comments and suggestions
Low Yield	
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.
Insufficient mixing of sample with Buffer TD2 and ethanol before binding	Mix sample first with Buffer TD2 and then with ethanol by pulse vortexing for 15 s each time before applying the sample to the PAXgene DNA spin column.
Buffer TD3 or Buffer TD4 prepared incorrectly	Make sure that ethanol has been added to Buffers TD3 and Buffer TD4 before use (see "Things to do before starting", pages 21 and 25).
Insufficient lysis of tissue samples	Cut tissue into smaller pieces to facilitate lysis. After lysis, vortex sample vigorously; this will not damage or reduce the size of the DNA. If a substantial gelatinous pellet remains after incubation and vortexing, extend incubation time at 56°C for Proteinase K digestion.
PAXgene Tissue DNA spin column clogged	
Too much starting material and/or insufficient lysis	Increase *g*-force and/or duration of centrifugation step. Reduce the amount of starting material used (see "Starting material" at the beginning of each protocol).
A_{260}/A_{280} ratio of purified DNA is low	
Water used instead of buffer to measure A_{260}/A_{280} ratio	Use 10 mM Tris·Cl, pH 7.5* instead of water to dilute the sample before measuring purity. See "Appendix A: Determination of Yield and Purity of DNA", page 32.

* 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
A_{260}/A_{280} ratio of purified DNA is high	
High level of residual RNA	Perform the optional RNase treatment in the protocol.
DNA does not perform well in downstream applications	
Salt carryover	Ensure that Buffer TD4 has been used at room temperature. Ensure that Buffer TD3 and Buffer TD4 were added in the correct order.
Ethanol carryover	Ensure that, when washing with Buffer TD4, the column is centrifuged for 3 min at maximum speed to dry the PAXgene DNA spin column membrane. Following the centrifugation step, remove the PAXgene DNA spin column carefully so that the column does not come into contact with the flow-through. If ethanol is visible in the PAXgene DNA spin column (as either drops or a film), discard the flow-through, keep the collection tube, and centrifuge for an additional 1 min at maximum speed.
Too much DNA used	For PCR applications, a single-copy gene can typically be detected after 35 PCR cycles with 100 ng template DNA.
DNA sheared	
DNA from sections of PFPE tissue: Storage of starting material	Tissue morphology is preserved in PFPE tissue when stored at room temperature. However, biomolecules within paraffin blocks will undergo slow chemical degradation. For best preservation of morphology and biomolecule integrity within the paraffin-embedded tissue, store PFPE blocks refrigerated at 5°C (2–8°C) or, ideally, frozen at –20°C (–15°C to –30°C).
DNA from PAXgene Tissue-fixed samples: Inappropriate handling of TissueLyser	Homogenize tissue samples in the TissueLyser for 20 s at 15 Hz. Do not exceed operating time.

Technical Assistance

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

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

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

Appendix A: Determination of Yield and Purity of DNA

Determination of yield and purity

DNA yield is determined by measuring the concentration of DNA in the eluate by its absorbance at 260 nm. Absorbance readings at 260 nm should fall between 0.1 and 1.0 to be accurate. Sample dilution should be adjusted accordingly. Measure the absorbance at 260 nm or scan absorbance from 220 to 330 nm (a scan will show if there are other factors affecting absorbance at 260 nm; for instance, absorbance at 325 nm would indicate contamination by particulate matter or a dirty cuvette). An A_{260} value of 1 (with a 1 cm detection path) corresponds to 50 µg DNA per milliliter water.* Water should be used as diluent when measuring DNA concentration since the relationship between absorbance and concentration is based on extinction coefficients calculated for nucleic acids in water (1). Both DNA and RNA are measured with a spectrophotometer at 260 nm; to measure only DNA in a mixture of DNA and RNA, a fluorimeter must be used.

An example of the calculations involved in DNA quantification is shown below.

Volume of DNA sample	=	100 µL
Dilution	=	20 µL sample + 180 µL distilled water (1/10 dilution)
Measure absorbance of diluted sample in a 0.2 mL cuvette		
A_{260}	=	0.2
Concentration of sample	=	50 µg/mL × A_{260} × dilution factor
	=	50 µg/mL × 0.2 × 10
	=	100 µg/mL
Total amount	=	concentration × volume of sample in milliliters
	=	100 µg/mL × 0.1 mL
	=	10 µg DNA

* 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.

The ratio of the readings at 260 and 280 nm (A_{260}/A_{280}) provides an estimate of the purity of DNA with respect to contaminants that absorb UV, such as protein. However, the A_{260}/A_{280} ratio is influenced considerably by pH. Since water is not buffered, the pH and the resulting A_{260}/A_{280} ratio can vary greatly. 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,* in which pure DNA has an A_{260}/A_{280} ratio of 1.8:2.0. Always be sure to calibrate the spectrophotometer with the same solution.

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

Appendix B: 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**).

Ordering Information

Product	Contents	Cat. no.
PAXgene Tissue DNA Kit (50)	For 50 DNA preps: PAXgene DNA Mini Spin Columns, Processing Tubes, Microcentrifuge Tubes, Carrier RNA, and Buffers; to be used in conjunction with PAXgene Tissue FIX Containers	767134
Related Products		
PAXgene Tissue FIX Containers (50 mL)	For fixation and stabilization of tissue specimen: 10 prefilled Reagent Containers containing 50 mL of PAXgene Tissue FIX	765312
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
TissueLyser*	Universal laboratory mixer-mill disruptor	Varies*
TissueLyser Adapter Set 2 × 24	2 sets of Adapter Plates and 2 racks for use with 2 mL microcentrifuge tubes on the TissueLyser	69982
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)	69965

* Visit **www.qiagen.com/automation** to find out more about the TissueLyser and TissueRuptor and to order.

Product	Contents	Cat. no.
TissueRuptor*	Handheld rotor–stator homogenizer, 5 TissueRuptor Disposable Probes	Varies*
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 QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

* Visit **www.qiagen.com/automation** to find out more about the TissueLyser and TissueRuptor and to order.

† 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
06/2016	Changes to comply with GHS regulations throughout document
04/2021	Revisions throughout document to reflect discontinuation of related products (PAXgene Tissue Container and PAXgene Tissue RNA Kit), added information about QIAcube Connect, removed the protocol for PFPE tissue blocks, and general update into revised template.
07/2023	Street address of PreAnalytiX GmbH changed from "Feldbachstrasse" to "Garstligweg 8".

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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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