PAXgene®

Tissue DNA Kit Handbook

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

Important: To be used only in conjunction with PAXgene Tissue Containers

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

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Kit Contents

PAXgene Tissue DNA Kit	**(50)**
Catalog no.	**767134**
Number of preps	**50**
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 x 10
Processing Tubes (2 ml)	3 x 50
Microcentrifuge-Safelock 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 6 for safety information.

† Buffer TD3 is supplied as a 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.

Shipping and Storage

The PAXgene Tissue DNA Kit is shipped at ambient temperature.

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

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.

Product Use Limitations

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.

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).

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 miRNA 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 call one of the QIAGEN Technical Service Departments or local distributors (see last page or visit www.qiagen.com).

Quality Control

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

Safety Information

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

Introduction

Current tissue fixation methods used in traditional histology are of limited use for molecular analysis. Fixatives that contain formaldehyde cross-link biomolecules and modify nucleic acids and proteins. During tissue fixation, storage, and processing, cross-links lead to degradation of nucleic acids. Since cross-links cannot be removed completely, the resulting chemical modifications can cause inhibition in sensitive downstream applications such as quantitative PCR or RT-PCR. In order to enable both molecular and traditional pathology testing from the same specimen, a method is needed for stabilization of molecular content and preservation of morphology.

Principle and procedure

The PAXgene Tissue system consists of a tissue collection device and kits for purification of total RNA, DNA, or miRNA. 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 fixation and stabilization method preserves tissue morphology and the integrity of nucleic acids without destructive cross-linking and degradation found in formalin-fixed tissues.

For isolation of genomic DNA, the system requires the use of PAXgene Tissue Containers for tissue collection and stabilization, followed by DNA isolation and purification using the PAXgene Tissue DNA Kit. Together the container and kit provides a complete preanalytical solution for collection, fixation, and stabilization of tissue, and purification of high-quality DNA for molecular analysis.

Sample collection and stabilization

PAXgene Tissue Containers are dual-chamber containers prefilled with 2 reagents. PAXgene Tissue Fix rapidly penetrates and fixes the tissue. After fixation, the tissue is removed from the PAXgene Tissue Fix and transferred to PAXgene Tissue Stabilizer in the second chamber of the same container. When the tissue is stored in PAXgene Tissue Stabilizer, nucleic acids and morphology of the tissue sample are stable for a minimum of 3 and a maximum of 7 days at room temperature or for a minimum of 2 and a maximum of 4 weeks at 2–8°C, depending on the type of tissue. Storage at –20°C is also possible for at least 3 months without any negative effects on the morphology of the tissue or the integrity of the nucleic acids.*

* Specifications for fixation and storage conditions in PAXgene Tissue Fix and PAXgene Tissue Stabilizer were determined using animal tissues.

Stabilized samples can be embedded in paraffin for histological studies. Nucleic acids can be isolated from the stabilized samples either before or after embedding in paraffin. See the *PAXgene Tissue Container Product Circular* for information about tissue fixation, stabilization, processing, and paraffin embedding.

DNA purification

The PAXgene Tissue DNA Kit provides 3 protocols for purification of genomic DNA from tissues fixed and stabilized in PAXgene Tissue Containers (see "Description of protocols", below and flowchart, page 10).

Lysis of the tissue sample is performed 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.

Description of protocols

Sections of PAXgene treated, paraffin-embedded tissue (page 14)

Starting material for DNA purification should be freshly cut sections from tissue samples that have been fixed and stabilized in PAXgene Tissue Containers, 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 prep. 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.

PAXgene treated tissue samples (page 17)

Starting material for DNA purification should be up to 20 mg of a tissue sample fixed and stabilized in a PAXgene Tissue Container. 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 tissue cassette. If necessary, the sample is cut into a 2 mm cube. 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,

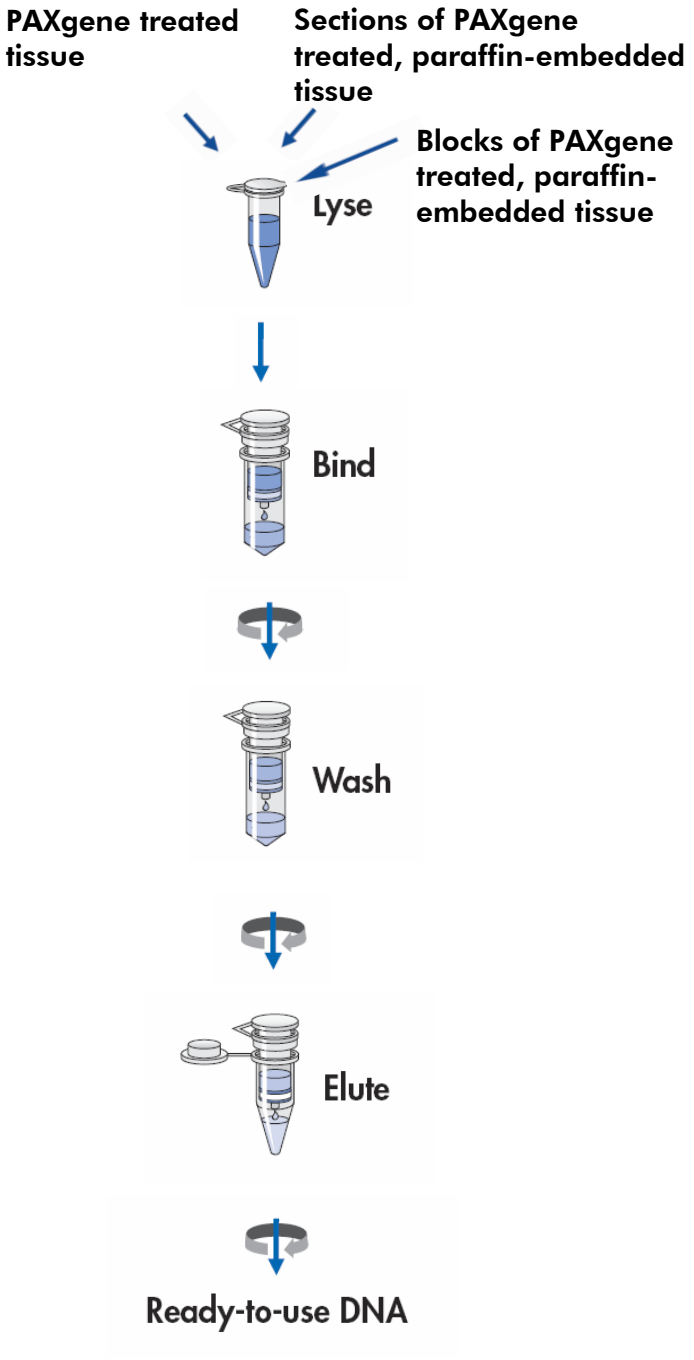
such as the TissueLyser, before proteinase K digestion (see “Disrupting and homogenizing using the TissueLyser”, page 12).

Blocks of PAXgene treated, paraffin-embedded tissue (page 20)

Starting material for DNA purification should be up to 20 mg of a block of tissue fixed and stabilized in a PAXgene Tissue Container, dehydrated, and embedded in paraffin. For samples with very high DNA contents (e.g., spleen, tonsils), no more than 10 mg of paraffin-embedded tissue should be used per sample prep.

The tissue sample is cut out of the paraffin block using a scalpel. After determining the amount of tissue by weight, paraffin is removed from the tissue by incubation in xylene, followed by addition of ethanol and centrifugation. Binding Buffer TD1 is added to the resulting pellet, and mechanical disruption and simultaneous homogenization is performed using the TissueLyser (see “Disrupting and homogenizing using the TissueLyser”, page 12).

The PAXgene Tissue DNA Procedure



Lyse sample using proteinase K

Adjust binding conditions

Apply sample to PAXgene DNA spin column for adsorption of DNA to membrane

Remove contaminants with simple wash steps

Elute DNA in low-salt buffer

Equipment and Reagents to Be Supplied by User

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

For all protocols

- PAXgene Tissue Container
- Xylene
- Ethanol (96–100%, purity grade p.a.)
- Pipets* (10 µl – 1 ml)
- Sterile, aerosol-barrier, RNase-free pipet tips
- Variable-speed microcentrifuge* capable of attaining 1000–20,000 x *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, or equivalent)
- Vortex mixer*
- Scalpel
- Crushed ice
- Optional: RNase A (100 mg/ml; cat. no. 19101)

For PAXgene treated tissue samples and PAXgene treated, paraffin-embedded blocks of tissue

- Equipment for tissue disruption and homogenization (see "Disrupting and homogenizing using the TissueLyser", page 12). We recommend the TissueLyser* system (see ordering information, page 30).
- 2 ml round-bottomed processing tubes

For sections of PAXgene treated, paraffin-embedded tissues

- Microtome

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

Important Notes

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 cells. The TissueLyser disrupts and homogenizes up to 48 tissue samples simultaneously when used in combination with the TissueLyser Adapter Set 2 x 24. The adapter set holds 48 x 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 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 dissolved carrier RNA 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 dissolved carrier RNA 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 (15–25°C) 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.

More than 200 µl should not be eluted into a 1.5 ml microcentrifuge tube because the spin column would 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 10 mM Tris·Cl, 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 (15–25°C). Heating Buffer TD5 before elution is not necessary.

Protocol: Purification of Genomic DNA from Sections of PAXgene Treated, Paraffin-Embedded Tissue

Starting material

Starting material for DNA purification should be a minimum of 2 and a maximum of 5 sections of tissue fixed and stabilized in PAXgene Tissue Containers, dehydrated, and embedded in paraffin (see the *PAXgene Tissue Container Product Circular* 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 pipet, 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 (15–25°C).

Things to do before starting

- Tissue specimens must be fixed, stabilized, processed, and embedded in paraffin according to the *PAXgene Tissue Container Product Circular*.
- 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 paraffin-embedded 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 microcentrifuge-safelock tube.**
3. **Add 650 µl xylene to the sample. Vortex vigorously for 20 s, and incubate for 3 min on the benchtop (15–25°C).**
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 (but do not exceed 20,000 x *g*).**

   To prevent damage to processing tubes, do not exceed 20,000 x *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; however, 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 (15–25°C) 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. After incubation, set the temperature of the shaker–incubator to 80°C for use in step 11.**
10. **Briefly centrifuge the microcentrifuge-safelock 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 (15–25°C). Proceed immediately to step 11.

11. **Incubate for 60 min at 80°C at 1400 rpm.**
12. **Briefly centrifuge the 1.5 ml microcentrifuge-safelock tube to remove drops from the inside of the lid.**

13. **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 12), add 1 µg dissolved carrier RNA 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.

14. **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 obtain a homogeneous solution.

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

15. **Briefly centrifuge the 1.5 ml tube to remove drops from the inside of the lid.**

16. **Pipet 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 x *g*. Place the spin column in a new 2 ml processing tube, and discard the old processing tube containing flow-through.***

17. **Pipet 500 µl Buffer TD3 into the PAXgene DNA spin column, and centrifuge for 1 min at 6000 x *g*. Place the spin column in a new 2 ml processing tube, and discard the old processing tube containing flow-through.***

18. **Pipet 500 µl Buffer TD4 into the PAXgene DNA spin column and centrifuge for 1 min at 6000 x *g*. Place the spin column in a new 2 ml processing tube, and discard the old processing tube containing flow-through.**

19. **Centrifuge for 3 min at maximum speed (but do not exceed 20,000 x *g*) to dry the membrane completely.**

    This step is necessary, since ethanol carryover into the eluate may interfere with some downstream applications.

20. **Discard the processing tube containing the flow-through. Place the PAXgene DNA spin column in a 1.5 ml microcentrifuge tube, and pipet 20–200 µl Buffer TD5 directly onto the PAXgene DNA spin column membrane. Centrifuge for 1 min at maximum speed (but do not exceed 20,000 x *g*) to elute the DNA.**

    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 6 for safety information.

Protocol: Purification of Genomic DNA from PAXgene Treated Tissue Samples

Starting material

Starting material for DNA purification should be up to 20 mg of a tissue sample fixed and stabilized in PAXgene Tissue Containers. 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 the PAXgene Tissue Container 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 pipet, 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 (15–25°C).

Things to do before starting

- Tissue specimens must be fixed, stabilized, processed, and embedded in paraffin according to the *PAXgene Tissue Container instructions for use*.
- 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 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 x 24.**

   Note: If no bead mill is available, alternatively, 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 since this can cause DNA fragmentation by 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 pipet the lysates into new 1.5 ml microcentrifuge-safelock tubes.**

   Do not reuse the stainless steel beads.

5. **Add 20 µl proteinase K. Mix by vortexing and 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 microcentrifuge-safelock 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 (15–25°C).

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 microcentrifuge-safelock tube to remove drops from the inside of the lid.**
11. **Pipet 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 x *g*. Place the spin column in a new 2 ml processing tube, and discard the old processing tube containing flow-through.***
12. **Pipet 500 µl Buffer TD3 into the PAXgene DNA spin column and centrifuge for 1 min at 6000 x *g*. Place the spin column in a new 2 ml processing tube, and discard the old processing tube containing flow-through.***
13. **Pipet 500 µl Buffer TD4 into the PAXgene DNA spin column and centrifuge for 1 min at 6000 x *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 (but do not exceed 20,000 x *g*) to dry the membrane completely.**

    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 pipet 50–200 µl Buffer TD5 directly onto the PAXgene DNA spin column membrane. Centrifuge for 1 min at maximum speed (but do not exceed 20,000 x *g*) to elute the DNA.**

    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 6 for safety information.

Protocol: Purification of Genomic DNA from PAXgene Treated, Paraffin-Embedded Blocks of Tissue

Starting material

Starting material for DNA purification should be up to 20 mg of a block of tissue fixed and stabilized in PAXgene Tissue Containers, dehydrated, and embedded in paraffin (see the *PAXgene Tissue Container Product Circular* for information about tissue fixation, stabilization, and paraffin embedding). 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 embedded into paraffin 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 pipet, 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 (15–25°C).

Things to do before starting

- Tissue specimens must be fixed, stabilized, processed, and embedded in paraffin according to the *PAXgene Tissue Container Product Circular*.

- 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 15.

Procedure

1. **Cut out a tissue sample from a paraffin block using a scalpel and weigh the sample. Do not use more than 20 mg tissue (up to 10 mg spleen or tonsil).**

   Weighing tissue is the most accurate way to determine the amount.
2. **Cut the block into smaller pieces and place them into a 2 ml round-bottomed processing tube (not supplied).**
3. **Add 1 ml xylene to the sample. Vortex vigorously for 20 s, and incubate for 3 min on the benchtop (15–25°C).**
4. **Centrifuge at maximum speed for 3 min (but do not exceed 20,000 x *g*).**

   To prevent damage to processing tubes, do not exceed 20,000 x g.
5. **Remove the supernatant by pipetting. Do not remove any of the pellet.**
6. **Add 1 ml of ethanol (96–100%, purity grade p.a.) to the pellet, and mix by vortexing for 20 s.**
7. **Centrifuge at maximum speed for 3 min (but not to exceed 20,000 x *g*).**
8. **Remove the supernatant by pipetting. Do not remove any of the pellet.**
9. **Open the tube and incubate at room temperature (15–25°C) or up to 37°C. Incubate for 10 min or until all residual alcohol has evaporated.**
10. **Resuspend the pellet in 180 µl Buffer TD1.**
11. **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 x 24.**

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

12. **Operate the TissueLyser for 20 s at 15 Hz.**

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

13. **Carefully pipet the lysates into new 1.5 ml microcentrifuge-safelock tubes (supplied).**

    Do not reuse the stainless steel beads.

14. **Add 20 µl proteinase K, and mix by pulse-vortexing for 15 s.**
15. **Incubate for 1 h at 56°C using a shaker–incubator at 1400 rpm. After incubation, set the temperature of the shaker–incubator to 80°C for use in step 17.**
16. **Briefly centrifuge the microcentrifuge-safelock 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 (15–25°C).

17. **Incubate for 60 min at 80°C at 1400 rpm.**
18. **Briefly centrifuge the 1.5 ml microcentrifuge-safelock tube to remove drops from the inside of the lid.**
19. **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.

20. **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.

21. **Briefly centrifuge the 1.5 ml microcentrifuge-safelock tube to remove drops from the inside of the lid.**
22. **Pipet 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 x *g*. Place the spin column in a new 2 ml processing tube, and discard the old processing tube containing flow-through.***

23. **Pipet 500 µl Buffer TD3 into the PAXgene DNA spin column, and centrifuge for 1 min at 6000 x *g*. Place the spin column in a new 2 ml processing tube, and discard the old processing tube containing flow-through.***

24. **Pipet 500 µl Buffer TD4 into the PAXgene DNA spin column and centrifuge for 1 min at 6000 x *g*. Place the spin column in a new 2 ml processing tube, and discard the old processing tube containing flow-through.**

25. **Centrifuge for 3 min at maximum speed (but do not exceed 20,000 x *g*) to dry the membrane completely.**

    This step is necessary, since ethanol carryover into the eluate may interfere with some downstream applications.

26. **Discard the processing tube containing the flow-through. Place the PAXgene DNA spin column in a 1.5 ml microcentrifuge tube, and pipet 50–200 µl Buffer TD5 directly onto the PAXgene DNA spin column membrane. Centrifuge for 1 min at maximum speed (but do not exceed 20,000 x *g*) to elute the DNA.**

    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 6 for safety information.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: www.qiagen.com/FAQ/FAQList.aspx. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or sample and assay technologies (for contact information, see last page or visit www.qiagen.com).

	Comments and suggestions
Low yield	
a) 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.
b) 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.
c) 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 14, 17, and 20).
d) **DNA from tissue samples or paraffin-embedded blocks**: Insufficient lysis	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 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).

Comments and suggestions	
A_{260}/A_{280} ratio of purified DNA is low	
Water used instead of buffer to measure A_{260}/A_{280}	Use 10 mM Tris·Cl, pH 7.5* instead of water to dilute the sample before measuring purity. See "Appendix: Determination of Yield, Purity, and Length of DNA", page 27.
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	
a) Salt carryover	Ensure that Buffer TD4 has been used at room temperature (15–25°C). Ensure that Buffer TD3 and Buffer TD4 were added in the correct order.
b) 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 a further 1 min at maximum speed.
c) Too much DNA used	For PCR applications, a single-copy gene can typically be detected after 35 PCR cycles with 100 ng template 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.

	Comments and suggestions
DNA sheared	
a) **DNA from sections or blocks of paraffin-embedded tissue**: Storage of starting material	Store and archive PAXgene treated, paraffin-embedded blocks of tissue in a dry and dark place at 2–8°C or colder. The ideal storage temperature for preservation of nucleic acid is –20°C.
b) **DNA from tissue samples or paraffin-embedded blocks**: Inappropriate handling of TissueLyser	Homogenize tissue samples in the TissueLyser for 20 s at 15 Hz. Do not exceed operating time.

Appendix: Determination of Yield, Purity, and Length 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 220–330 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.† 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 of DNA 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 DNA sample	= 50 µg/ml x A_{260} x dilution factor
	= 50 µg/ml x 0.2 x 10
	= 100 µg/ml
Total amount	= concentration x volume of sample in milliliters
	= 100 µg/ml x 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.

† 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.

The ratio of the readings at 260 nm 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.

Determination of length

The precise length of genomic DNA should be determined by pulse-field gel electrophoresis (PFGE) through an agarose gel.* To prepare the sample for PFGE, the DNA should be concentrated by alcohol precipitation and the DNA pellet dried briefly at room temperature (15–25°C) for 5–10 minutes. Avoid drying the DNA pellet for more than 10 minutes since overdried genomic DNA is very difficult to redissolve. Redissolve in approximately 30 µl TE buffer, pH 8.0,* for at least 30 minutes at 60°C. Load 3–5 µg of DNA per well. Standard PFGE conditions are as follows:

1% agarose gel in 0.5 x TBE electrophoresis buffer;* switch intervals = 5–40 seconds; run time = 17 hours; voltage = 170 V

References

QIAGEN maintains a large, up-to-date online database of scientific publications utilizing QIAGEN and PreAnalytiX products. Comprehensive search options allow you to find the articles you need, either by a simple keyword search or by specifying the application, research area, title, etc.

For a complete list of references, visit the QIAGEN Reference Database online at www.qiagen.com/RefDB/search.asp or contact QIAGEN Technical Services or your local distributor.

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

Ordering Information

Product	Contents	Cat. no.
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 Containers	767134
Related products		
PAXgene Tissue Containers — for collection, fixation, and nucleic acid stabilization of human tissues		
PAXgene Tissue Containers (10)	For collection, fixation, and stabilization of 10 samples: 10 Prefilled Reagent Containers, containing PAXgene Tissue Fix and PAXgene Tissue Stabilizer	765112
PAXgene Tissue RNA Kit — for purification of total RNA from tissues fixed and stabilized in PAXgene Tissue Containers		
PAXgene Tissue RNA 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 Containers	765134
PAXgene Tissue miRNA Kit — for purification of microRNA and total RNA from tissues fixed and stabilized in PAXgene Tissue Containers		
PAXgene Tissue 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 Containers	766134

Product	Contents	Cat. no.
TissueLyser System — for high-throughput disruption of various sample types for molecular analysis		
TissueLyser	Universal laboratory mixer-mill disruptor	Varies*
TissueLyser Adapter Set 2 x 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, suitable for use with the TissueLyser system	69989
TissueLyser Single-Bead Dispenser, 5 mm	For dispensing individual beads (5 mm diameter)	69965
TissueRuptor System — for low-throughput sample disruption using disposable probes		
TissueRuptor	Handheld rotor–stator homogenizer, 5 TissueRuptor Disposable Probes	Varies*

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

PAXgene Tissue Containers, PAXgene Tissue DNA Kit, PAXgene Tissue RNA Kit, PAXgene Tissue miRNA Kit: For Research Use Only. Not for use in diagnostic procedures. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

The TissueRuptor and TissueLyser are intended for molecular biology applications. These products are neither intended for the diagnosis, prevention, or treatment of a disease, nor have they been validated for such use either alone or in combination with other products.

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