

PAXgene[®] Tissue Container Product Circular

For fixation and stabilization of tissue specimens

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

April 2013

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Kit Contents

PAXgene Tissue Container	(10)
Catalog no.	765112
PAXgene Tissue Container*	10
Product circular	1

* Contains methanol. See page 5 for safety information.

Symbols



Use by



Lot number



Material number



Upper limit of temperature



Legal manufacturer

Shipping and Storage

The PAXgene Tissue Container is shipped at ambient temperature.

PAXgene Tissue Container can be stored upon receipt at ambient or refrigerated temperatures (2–25°C).

Do not use containers after their expiration date.

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 Container for any particular use, since the performance characteristics of these Containers have not been validated for any specific application. 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 PreAnalytiX Kit and kit component.

The following risk and safety phrases apply to components of the PAXgene Tissue Container.

PAXgene Tissue FIX



Contains methanol, acetic acid: toxic, irritant, highly flammable. Risk and safety phrases:* R11–23/24/25–36/38–39/23/24/25, S7–16–36/37/39–45

PAXgene Tissue STABILIZER



Contains ethanol: highly flammable. Risk and safety phrases:* R11, S7–16

24-hour emergency information

Emergency medical information in English, French, and German can be obtained 24 hours a day from:

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

Quality Control

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

* R11: Highly flammable; R23/24/25: Toxic by inhalation, in contact with skin and if swallowed; R36/38: Irritating to eyes and skin; R39/23/24/25: Toxic: danger of very serious irreversible effects through inhalation, in contact with skin and if swallowed; S7: Keep container tightly closed; S16: Keep away from sources of ignition – No smoking; S36/37/39: Wear suitable protective clothing, gloves and eye/face protection; S45: In case of accident or if you feel unwell seek medical advice immediately (show the label where possible).

Introduction

The methods for tissue fixation currently used in traditional histology are of limited use for molecular analysis. Fixatives that contain formaldehyde cross-link biomolecules and modify nucleic acids and proteins. Such cross-links 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 downstream applications, such as quantitative PCR or RT-PCR. To enable both molecular and traditional pathology testing from the same specimen, a method is needed to stabilize molecular content and preserve morphology.

PreAnalytiX has developed the PAXgene Tissue System to meet this need. The system consists of a fixation reagent (PAXgene Tissue FIX), a stabilization reagent (PAXgene Tissue STABILIZER), prefilled containers for tissue collection, storage, and transportation, and kits for purification of RNA, 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 minutes.* In research applications, the reagent preserves morphology and biomolecules without the destructive cross-linking and degradation found with formalin-fixed tissues. The fixation process is stopped by transferring the tissue specimen into PAXgene Tissue STABILIZER.

After fixation, tissues can be stored directly in PAXgene Tissue STABILIZER for the 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 RNA, 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 kit (QIAGEN).

PAXgene Tissue reagents in prefilled containers 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 research analysis.

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

Description of protocols

Sample collection and stabilization with the PAXgene Tissue Container

PAXgene Tissue Containers are dual-chamber containers prefilled with 2 reagents. Chamber 1 contains PAXgene Tissue FIX and chamber 2 PAXgene Tissue STABILIZER. For fixation, tissue samples with a maximum size of 4 x 15 x 15 mm must be placed into a standard tissue cassette.

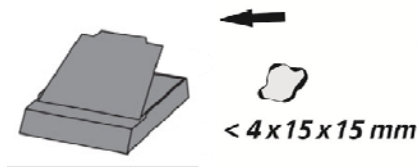
Fixation is performed in chamber 1 (Figure 1). PAXgene Tissue FIX rapidly penetrates and fixes the tissue. After fixation, the tissue cassette is removed from chamber 1 and transferred to PAXgene Tissue STABILIZER in the second chamber of the same container (Figure 2). When the tissue is stored in PAXgene Tissue Stabilizer, nucleic acids, proteins and morphology of the tissue sample are stable for a minimum of 7 days at room temperature (15–25°C) or for a minimum of 4 weeks at 2–8°C, depending on the type of tissue.*

Tissue samples can be stored in the PAXgene Tissue STABILIZER for longer periods at –20 to –80°C without negative effects on the morphology of the tissue or the integrity of the nucleic acids.†

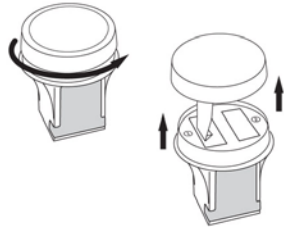
Stabilized samples can be embedded in paraffin for histological studies. Nucleic acids and proteins can be isolated from the stabilized samples before or after embedding in paraffin (Figure 3). See the *PAXgene Tissue Kit Handbooks* for information about DNA, RNA, or miRNA, and the PAXgene Tissue supplementary protocols (www.preanalytix.com) for protein purification and other applications.

* Fixation rates and stabilization times depend on type and size of tissue. Specifications for tissues size, fixation, and storage conditions in PAXgene Tissue FIX and PAXgene Tissue STABILIZER were determined using animal tissues samples.

† For the latest results on long-term storage, see technical notes at www.preanalytix.com.



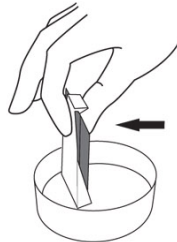
Excise and cut tissue to size; place into a standard tissue cassette.



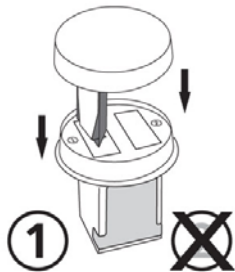
Remove screw cap/rack assembly.



Insert the lower edge of the tissue cassette into the bottom edge of the rack.



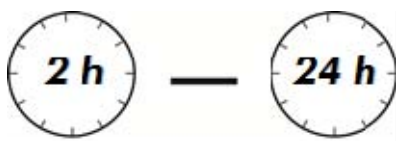
Attach tissue cassette to the rack.



Submerge the rack holding the tissue cassette into chamber 1 containing PAXgene Tissue FIX.

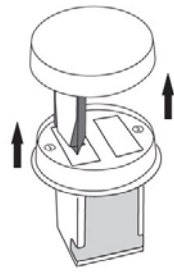


Screw the cap into place.

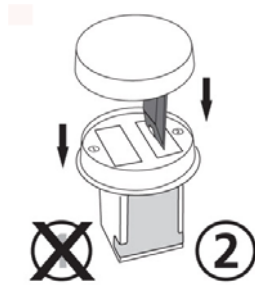


Allow fixation for 2 to 24 hours.

Figure 1. Fixation within Chamber 1 of the PAXgene Tissue Container.



After fixation, remove rack with tissue cassette from chamber 1.



Submerge rack holding tissue cassette into chamber 2 containing PAXgene Tissue STABILIZER.



Screw the cap into place; the container is ready for transport.



After processing and paraffin embedding, the block of PAXgene Tissue fixed, paraffin-embedded (PFPE) tissue is ready for sectioning.

Figure 2. Stabilization within Chamber 2 of the PAXgene Tissue Container.

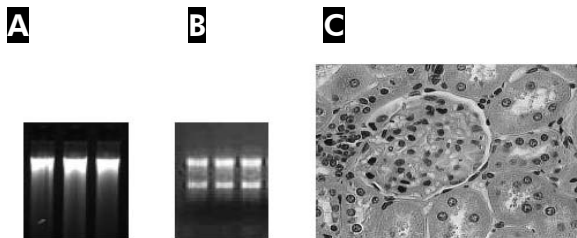


Figure 3. Preservation of nucleic acids and morphology. Subsequent analysis of fixed and stabilized material shows stable preservation of DNA **A** and RNA **B**. Microscope examination shows stable preservation of morphology **C**. For further information about applications using the PAXgene Tissue System, visit www.preanalytix.com.

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.

- Standard tissue cassette (available through EMS, VWR, Thermo Scientific, and others)*
- Ethanol, purity grade a.d. (96–100%) or denatured with methanol, isopropanol (e.g., histological-grade alcohol composed of 90 parts ethyl alcohol, 5 parts methyl alcohol and 5 parts isopropyl alcohol), or methyl ethyl ketone (i.e., 99 parts ethanol and 1 part methyl ethyl ketone)
- Xylene (or xylene substitute)
- Paraffin with a melting point of 54–58°C (e.g., Paraplast X-tra[®], Thermo Fisher Scientific, cat. no. 503002; or VWR (US), cat. no. 100504-164)*
- Optional for storage at –20°C (–15°C to –30°C) or –80°C (–65°C to –90°C): cryogenic vial with screw closure (e.g., Thermo Fisher Scientific, cat. no. 5005-0015)*
- Scalpel
- Heated water bath

* This is not a complete list of suppliers and does not include many important vendors of biological supplies.

Important Notes

Storage and archiving of PFPE (PAXgene Tissue fixed, paraffin-embedded) tissue samples

For short-term storage or transport, blocks of PFPE tissue can be stored at room temperature (15–25°C) or refrigerated temperature (2–25°C). However, biomolecules within the paraffin blocks will undergo slow chemical degradation. For best preservation of morphology and maintenance of biomolecule integrity within the paraffin-embedded tissue, store PFPE blocks at –20°C (–15°C to –30°C).

Labeling of PAXgene Tissue Container

To allow calculation of the exact length of fixation time, record the date and time that tissue is placed in the PAXgene Tissue Container in the “Fixation Date/Time” field on the Container label (Figure 4). After transferring the specimen from PAXgene Tissue FIX into PAXgene Tissue STABILIZER, record the date and time of the transfer in the “Transfer Date/Time” field on the cap label.

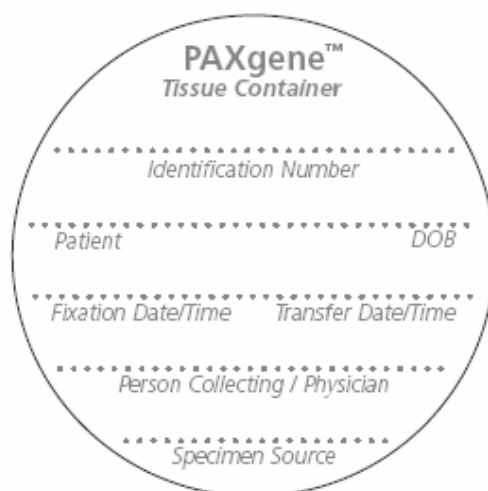


Figure 4. Cap label of a PAXgene Tissue Container.

Protocol: Sample Fixation and Stabilization

Starting material

Starting material for tissue fixation should be a sample cut to a maximum size of 4 x 15 x 15 mm.

Important points before starting

- **Do not use tissue samples larger than 4 x 15 x 15 mm as this will significantly reduce the quality of tissue morphology as well as the integrity of nucleic acids.**
- Ensure that the container boxes are intact and undamaged, and that reagents have not leaked. Do not use a container that has been damaged.
- To avoid transferring samples to the wrong container, ensure that the containers as well as the tissue cassettes are properly labeled.

Procedure

1. Excise tissue in preparation for fixation.

Note: Within 30 min of tissue excision, sample should be fixed in PAXgene Tissue FIX of chamber 1.

2. If necessary, cut tissue to a maximum size of 4 x 15 x 15 mm.

Note: Smaller tissue samples or biopsies can be fixed without cutting.

3. Place the tissue specimen into a standard tissue cassette (not provided).

4. Unscrew the cap of the PAXgene Tissue Container, and lift up the screw cap–rack assembly.

5. Attach the tissue cassette to the rack by inserting the lower edge of the tissue cassette into the bottom edge of the rack (grasp the cassette so that the slanted/angled end faces upwards and toward you), and press to secure it under the rack upper release tab (see flowchart, page 8).

6. Carefully submerge the rack holding the tissue cassette into chamber 1 containing PAXgene Tissue FIX. Screw the cap into place.

Note: Make sure that the cap is correctly seated on the rim of the PAXgene Tissue Container before tightening the cap.

7. Incubate tissue specimen at room temperature (15–25°C) for a minimum of 2 h, but preferably 3–24 h.

Note: Longer fixation periods may lead to degradation of biomolecules.

Note: For biopsies with a thickness of 1 mm or less, fixation time can be reduced to 30–60 min.

- 8. After fixation, unscrew the cap and lift up the screw cap–rack assembly with tissue cassette from chamber 1.**
- 9. Position the rack–tissue cassette directly above chamber 2 containing PAXgene Tissue STABILIZER, and carefully submerge. Tighten the screw cap.**

Note: Make sure that the cap is correctly seated on the rim of the PAXgene Tissue Container before tightening the cap.

- 10. Store or transport tissue specimen in PAXgene Tissue STABILIZER.**

Note: Standard storage conditions are up to 7 days at room temperature or for up to 4 weeks at 2–8°C, depending on tissue type (see footnote on page 7). Samples can be frozen at –20°C (–15°C to –30°C) or at –80°C (–65°C to –90°C). For freezing we recommend transferring the tissue sample from the tissue cassette and placing it into a screw-cap cryovial (not supplied) filled with PAXgene Tissue STABILIZER.

Note: A minimum incubation time of 2 h in PAXgene Tissue Stabilizer is recommended before processing and embedding in paraffin.

Protocol: Sample Processing, Paraffin Embedding, and Sectioning

Important points before starting

- Refer to Appendices A and B for detailed processing protocols (pages 16–18).
- Do not begin tissue processing with either water or with ethanol at dilutions less than 80%.
- Denatured ethanol can be used for processing.
- Do not reuse processing reagents previously contaminated with formalin, even in trace amounts, as this can lead to significant reductions in DNA and RNA yield and quality.
- Tissue samples fixed and stabilized in PAXgene Tissue reagents can remain in the first station of the tissue processor in 80–99% ethanol for up to 12 h.
- For paraffin infiltration, the liquid paraffin should be held at 2°C above its melting point. Do not let the incubation temperature of the paraffin exceed 60°C, and do not incubate in liquid paraffin for longer than 3 h. Extensive incubation times above 60°C lead to degradation of RNA.

Procedure

- 1. Transfer the tissue cassette into the first station of a tissue processor filled with 80–99% ethanol to start dehydration.**

Note: Reagents used for processing must be free of formalin contamination.

Note: In case a tissue processor with formalin-free reagents is not available, specimens treated with the PAXgene Tissue System can be processed manually. A supplementary protocol for manual processing is available at the PAXgene Tissue Resources section of www.preanalytix.com.

- 2. Follow a protocol for paraffin embedding. See Appendix A on page 16 for a generic protocol, or Appendix B on page 17 for examples of 2 other successfully tested protocols.**
- 3. Immediately after the final paraffin incubation step, embed the tissue sample into a block of paraffin. For embedding, use the same low-melting paraffin used for infiltration.**
- 4. After hardening, store paraffin blocks in a dry, dark place.**

Note: The ideal storage temperature to preserve nucleic acid integrity and tissue morphology for paraffin-embedded tissue is –20°C (–15°C to –30°C).

5. **When ready for further analysis, cut paraffin blocks with a microtome and transfer the sections onto the surface of a water bath heated to 40°C for 1 min.**

IMPORTANT: Do not heat the water bath above 40°C. Stretching the sections on water with a temperature above 40°C overstretches morphological structures. Do not leave the sections in the water bath for longer than 1 min.

6. **Dry sections overnight at room temperature (15–25°C).**

Appendix A: Generic Processing Protocol for an Automated Tissue Processor That Supports Preservation of Biomolecules in Specimens Treated with the PAXgene Tissue System

Table 1. Generic processing protocol

Step	Media*	Alternatives
1	80 [†] –99% ethanol	PAXgene Tissue STABILIZER
2	90 [†] –99% ethanol	None, mandatory
3	95 [†] –99% ethanol	None, mandatory
4	99% ethanol	None, mandatory
5	99% ethanol	None, mandatory
6	99% ethanol	Isopropanol
7	99% ethanol	Isopropanol
8	Xylene	Xylene substitutes may be used, but do not use clearing agents based on D-limonene.
9	Xylene	Xylene substitutes may be used, but do not use clearing agents based on D-limonene.
10	Paraffin (with melting point 54–58°C)	1:1 mixture of paraffin and xylene
11	Paraffin (with melting point 54–58°C)	None, mandatory
12	Paraffin (with melting point 54–58°C)	If only 2 stations that can be heated are available, omit this step.

* When processing specimens fixed in PAXgene Tissue Containers, do not use reagents contaminated with formalin. Formalin contamination in the alcohol used for sample processing can lead to significant reduction of nucleic acid and protein yield.

[†] Use filtered or deionized water for preparation of 80, 90, or 95% ethanol.

Appendix B: Examples of Automated Processing Protocols Successfully Used for Specimens Treated with the PAXgene Tissue System

Table 2. Processing protocol A

Step	Media	Time*	Temperature	Vacuum
1	PAXgene Tissue STABILIZER	Up to 7 days	18–22°C	–
2	99% ethanol	30–60 min	18–22°C	0.5 bar
3	99% ethanol	30–60 min	18–22°C	0.5 bar
4	99% ethanol	30–60 min	18–22°C	0.5 bar
5	99% ethanol	30–60 min	18–22°C	0.5 bar
6	99% ethanol	30–60 min	18–22°C	0.5 bar
7	Isopropanol	30–60 min	18–22°C	0.5 bar
8	Isopropanol	30–60 min	18–22°C	0.5 bar
9	Xylene	30–60 min	18–22°C	0.5 bar
10	Xylene	30–60 min	18–22°C	0.5 bar
11	Paraplast X-tra	30–60 min	56°C	0.5 bar
12	Paraplast X-tra	90 min	56°C	0.5 bar

* Optimal incubation times depend on the thickness of the tissue specimen.

Table 3. Processing protocol B

Step	Media	Time*	Temperature	Vacuum
1	80% ethanol	15 min – 12 h	15–25°C	–
2	90% ethanol	30 min	15–25°C	0.5 bar
3	95% ethanol	30–60 min	15–25°C	0.5 bar
4	99% ethanol	30–60 min	15–25°C	0.5 bar
5	99% ethanol	30–60 min	15–25°C	0.5 bar
6	Isopropanol	30–60 min	15–25°C	0.5 bar
7	Isopropanol	30–60 min	15–25°C	0.5 bar
8	Xylene	30–60 min	15–25°C	0.5 bar
9	Xylene	30–60 min	15–25°C	0.5 bar
10	1:1 mixture of Paraplast X-tra and Xylene	30–60 min	50°C	0.5 bar
11	Paraplast X-tra	30–60 min	56°C	0.5 bar
12	Paraplast X-tra	60 min	56°C	0.5 bar

* Optimal incubation times depend on the thickness of the tissue specimen.

Appendix C: Optimizing Immunohistochemistry (IHC) Assays with Sections of PFPE (PAXgene Tissue Fixed, Paraffin-Embedded) Tissue

In contrast to formalin or other fixation reagents containing aldehydes, the PAXgene Tissue System does not cause cross-linking of biomolecules. Therefore, it may not be necessary to unmask epitopes for immunohistochemistry assays either by heating or proteolytic digestion.

It is important to note, however, to note that since many antibodies used in immunohistochemical assays were developed for use with formalin-fixed tissue, it is often necessary to optimize antigen retrieval steps and/or to adjust antibody concentration in PFPE tissue to achieve optimal staining intensities.

For the latest information on IHC protocols and staining conditions see the tissue atlas at www.preanalytix.com/product-catalog/tissue/tissue-atlas.

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 at www.preanalytix.com or contact QIAGEN Technical Services or your local distributor.

Ordering Information

Product	Contents	Cat. no.
PAXgene Tissue Container (10)	For fixation and stabilization of tissue specimens: 10 Prefilled Reagent Containers, containing PAXgene Tissue FIX and PAXgene Tissue STABILIZER	765112
Related products		
PAXgene Tissue FIX Container — for fixation and stabilization of tissue specimens		
PAXgene Tissue FIX Container (50 ml)	10 Prefilled Reagent Containers, containing 50 ml of PAXgene Tissue FIX	765312
PAXgene Tissue STABILIZER — for stabilization of tissue samples treated with PAXgene Tissue		
PAXgene Tissue STABILIZER Concentrate (150 ml)	8 bottles of PAXgene Tissue STABILIZER concentrate, for 4 liters of PAXgene Tissue STABILIZER	765512
PAXgene Tissue RNA Kit — for purification of total RNA from tissues fixed and stabilized using the PAXgene Tissue System		
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 total RNA, including miRNA, from tissue samples fixed and stabilized using the PAXgene Tissue System		
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.
PAXgene Tissue DNA Kit — for purification of DNA from tissues samples fixed and stabilized using the PAXgene Tissue System		
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

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.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

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