September 2019

PAXgene®

Tissue FIX Container (50 ml) Handbook

For fixation and stabilization of tissue specimens

Important: To be used in conjunction with PAXgene Tissue STABILIZER Concentrate

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

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Please see the last page for contact information for your local PreAnalytiX distributor.

Contents

Kit Contents

PAXgene Tissue FIX Container (50 ml)	**(10)**
Catalog no.	**765312**
PAXgene Tissue FIX Container (50 ml)*	10
Handbook	1

* Contains methanol. See page 6 for safety information.

Symbols

Use by

Lot number

Material number

Upper limit of temperature

Legal manufacturer

Shipping and Storage

The PAXgene Tissue FIX Container is shipped at ambient temperature.

The PAXgene Tissue FIX Container can be stored at room temperature or refrigerated temperature (2–25°C).

An expiration date is printed on the box label.

Intended Use

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

It is the user's responsibility to validate the performance of the PAXgene Tissue FIX Container for any particular use, since the performance characteristics of these containers have not been validated for any specific organism. The performance characteristics of this product have not been fully established.

Quality Control

In accordance with QIAGEN's ISO-certified Total Quality Management System, each lot of PAXgene Tissue FIX Container 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 PreAnalytiX kit and kit component.

Introduction

The methods for tissue fixation currently used in traditional histology, such as H&E staining, are of limited use for molecular analysis. Fixatives that contain formaldehyde crosslink biomolecules and modify nucleic acids and proteins. Such crosslinks lead to nucleic acid degradation during tissue fixation, storage and processing. Since they cannot be removed completely, the resulting chemical modifications can lead to limitations in downstream applications, such as RT-PCR, qPCR or next-generation sequencing. In order to enable both molecular and 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.* The reagent preserves morphology and biomolecules without the destructive crosslinking and degradation found with formalin-fixed tissues.

After fixation, tissues can be stored 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 total

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

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 reagents in pre-filled containers and PAXgene Tissue Kits provide a complete preanalytical solution for collection, fixation and stabilization of tissue, and subsequent purification of nucleic acids. The resulting nucleic acids are free of crosslinks and chemical modifications and thus suitable for demanding molecular applications such as RT-PCR, long-range PCR and next-generation sequencing.

Description of protocols

Sample collection and stabilization with PAXgene Tissue FIX Container

PAXgene Tissue FIX Containers are single-chamber containers prefilled with 50 ml of the fixation reagent PAXgene Tissue FIX. PAXgene Tissue FIX Containers can accommodate four standard tissue cassettes (not provided), which can hold tissue samples with a maximum size of 4 x 15 x 15 mm. PAXgene Tissue FIX Containers also offer the possibility for direct fixation (without tissue cassettes) of larger tissue samples with a maximum size of 20 x 20 x 20 mm.

PAXgene Tissue FIX 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, the nucleic acids, proteins, and morphology of the tissue sample are stable for up to 7 days at room temperature (15–25°C) or for up to 4 weeks at 2–8°C, depending on tissue type.†

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

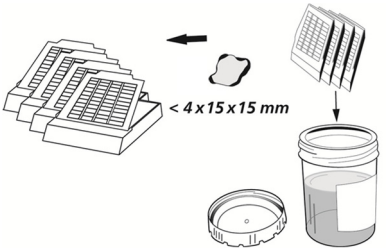
† Storage at 2–8°C for more than 4 weeks must be validated for each tissue type. Specifications for tissue size, fixation and storage conditions using PAXgene Tissue FIX and PAXgene Tissue STABILIZER were determined using animal tissues samples.

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

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

See Figure 1 on page 10 for an illustration of the steps in the fixation process. See Figure 2 on page 11 for an illustration of the steps in the stabilization process.

Multiple smaller tissue samples

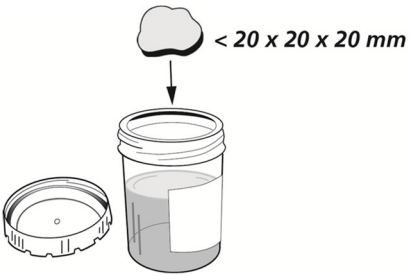


Resect and cut the tissue sample into max. 4 x 15 x 15 mm sections. Place in up to four (4) tissue cassettes.

Place tissue cassettes in PAXgene Tissue FIX Container containing PAXgene Tissue FIX reagent.

Fix tissue for 2 to 72 h, depending on tissue sample and size.

Single, large tissue sample

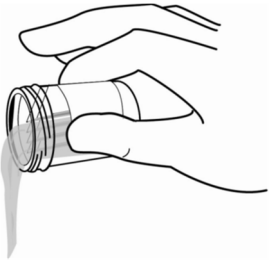


Tissue sample can have max. dimensions 20 x 20 x 20 mm.

Place tissue directly into PAXgene Tissue FIX Container containing PAXgene Tissue FIX reagent.

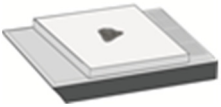
Fix tissue for 6 to 72 h, depending on tissue type and size.

Figure 1. Fixation. Resection and fixation of tissue samples using the PAXgene Tissue FIX Container is a straightforward process. It is suitable for multiple smaller tissue samples or a single, larger tissue sample.



Pour off PAXgene Tissue FIX.

Fill with PAXgene Tissue STABILIZER.



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

Figure 2. Stabilization. Tissue stabilization involves a single reagent change. Stabilized tissues can be stored, immediately used for nucleic acid and protein analysis or, as illustrated, processed and paraffin-embedded for sectioning.

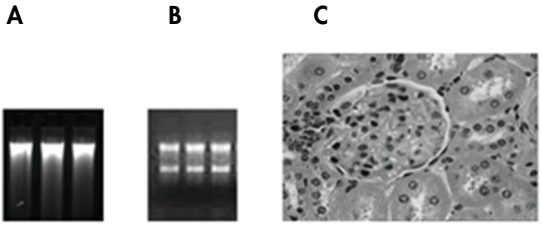


Figure 3. Extracted nucleic acids and tissue morphology. Analysis of fixed and stabilized material shows stable preservation of DNA (**A**), RNA (**B**) and tissue morphology (**C**).

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 tissue stabilization: PAXgene Tissue STABILIZER Concentrate (150 ml) (PreAnalytiX, cat. no. 765512)
- Standard tissue cassette (available from 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): cryovials 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

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 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).

Labeling of PAXgene Tissue FIX Container

In order to allow documentation of the exact length of fixation time, record the date and time that tissue is placed in the PAXgene Tissue FIX Container in the "Fixation Date/Time" field on the container label (Figure 4). In case PAXgene Tissue FIX was replaced with PAXgene Tissue STABILIZER, record the date and time of the exchange in the "Exchange with Tissue STABILIZER Date/Time" field and mark the checkbox beside the field, as shown in Figure 4.

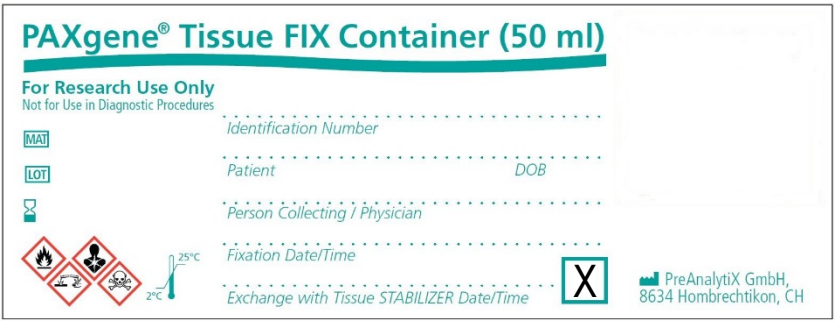


Figure 4. Label of PAXgene Tissue FIX Container.

Protocol: Sample Fixation and Stabilization

Starting material

Starting material can be up to 4 tissue samples with a maximum size of 4 x 15 x15 mm placed into standard tissue cassettes, or a single tissue sample with a maximum size of 20 x 20 x 20 mm.

Important things before starting

- Do not use tissue samples larger than 20 x 20 x 20 mm as this might significantly reduce the quality of tissue morphology and the integrity of nucleic acids.
- Do not reuse PAXgene Tissue FIX Containers as this will significantly reduce the quality of tissue morphology and the integrity of nucleic acids.
- PAXgene Tissue STABILIZER is not supplied with the PAXgene Tissue FIX Container and must be ordered separately (cat. no. 765512).
- 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 sample mix-up, ensure that the containers and the tissue cassettes are properly labeled.

Things to do before starting

- Dilute PAXgene Tissue STABILIZER Concentrate with ethanol as indicated on the bottle.

Procedure

1. Resect tissue in preparation for fixation.

   Note: After tissue resection, sample should be fixed in PAXgene Tissue FIX as soon as possible.

2. Cut tissue into appropriate sample size based on guidelines under "Starting material" above.

   Important: If using a larger tissue sample surrounded by fat (e.g., from a lymph node) or capsule (e.g., from kidney, liver, or spleen tissue), partially cut into the tissue every 5 mm (lamination) to enhance permeability of the fixative reagent.

3. If using smaller samples, place in standard tissue cassettes and place these into the prefilled PAXgene Tissue FIX Container such that each cassette is completely submerged. If using a larger sample, place it directly into the prefilled PAXgene Tissue FIX Container such that it is completely submerged.

   Note: If liquid flows over the edge of the container during submersion, the tissue specimen(s) were likely too large. Multiple containers should be used instead.

4. Depending on tissue size, incubate tissue specimen(s) at room temperature (15–25°C) for a minimum of 2 h for samples up to 4 x 15 x 15 mm, or a minimum of 6 h for samples up to 20 x 20 x 20 mm. Stop fixation by transfer into PAXgene Tissue STABIILIZER after a maximum of 72 h fixation.

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

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

5. Discard PAXgene Tissue FIX and replace with PAXgene Tissue STABILIZER.

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

   Note: Instead of exchanging PAXgene Tissue FIX with PAXgene Tissue STABILIZER, tissue can be removed from the container and transferred to a tissue processor filled with PAXgene Tissue STABILIZER at the first position. See Table 2, Appendix B on page 20 for an example of a processing protocol using PAXgene Tissue STABILIZER.

6. Process the samples into paraffin-embedded blocks, store or transport tissue samples in PAXgene Tissue STABILIZER.

   Note: When stored in PAXgene Tissue STABILIZER, standard storage conditions are up to 7 days at room temperature (15–25°C) or for up to 4 weeks at 2–8°C,* depending on tissue type. Samples can also be frozen at –20°C (–15°C to –30°C) or –80°C (–65°C to –90°C) for long-term storage. For freezing, we recommend transferring the tissue sample from the PAXgene Tissue FIX Container into a screw cap cryogenic vial filled with PAXgene Tissue STABILIZER.

* Storage at 2–8°C for more than 4 weeks must be validated for each tissue type.

Protocol: Sample Processing, Paraffin Embedding and Sectioning

Important points before starting

- Samples for processing must first be incubated in PAXgene Tissue FIX for an appropriate length of time (see Step 4 on page 18).
- Refer to Appendices A and B for detailed processing protocols (pages 19 and 20).
- Do not begin tissue processing with water or with ethanol at dilutions less than 80%.
- Denatured ethanol can be used for processing.
- Do not reuse alcohol previously contaminated with formalin as this can lead to significant reductions in DNA and RNA yield and quality. We recommend keeping alcohol for processing PAXgene Tissue-treated samples separate from alcohol used for processing formalin-fixed samples for at least the first 5 positions in the processing (see Table 1, page 19). With this precaution, it is possible to process PAXgene Tissue-fixed and formalin-fixed samples on the same instrument. For additional information, see the PAXgene Tissue supplementary protocols at **www.preanalytix.com**.
- 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.
- The first station of the tissue processor can be filled with PAXgene Tissue STABILIZER.
- For paraffin infiltration, the liquid paraffin should be held at temperatures 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.

Things to do before starting

- Cut larger samples to a size that will fit in a standard tissue cassette.
- Dilute PAXgene Tissue STABILIZER concentrate with ethanol as indicated on the bottle.

Procedure

1. Transfer the tissue cassette into the first position of a tissue processor filled with PAXgene Tissue STABILIZER or with 80–99% ethanol to start dehydration.
2. Follow a protocol for paraffin embedding. See Appendix A on page 19 for a generic protocol, or Appendix B on page 20 for examples of two other successfully tested protocols.
3. After the final paraffin incubation step, proceed with embedding the tissue sample into a block of paraffin. Use the same low melting paraffin as for infiltration.
4. After hardening, store paraffin blocks in a dry, dark place.

   Note: Tissue morphology is preserved in PFPE tissue when stored at room temperature. However, for best preservation of 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).
5. 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 to prepare slides, or into centrifuge tubes to isolate biomolecules.

   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 slide sections overnight at room temperature (15–25°C). Extract DNA, RNA or proteins from sections cut into centrifuge tubes, or store tubes at –20°C or –80°C for later extraction.

Appendix A: Generic Processing Steps that Support Preservation of Biomolecules in Specimens Treated with the PAXgene Tissue System

Table 1. Generic processing protocol

Step	Media	Alternatives
1	80*–99% ethanol (formalin-free)†	PAXgene Tissue STABILIZER
2	90*–99% ethanol (formalin-free)	None, mandatory
3	95*–99% ethanol (formalin-free)	None, mandatory
4	99% ethanol (formalin-free)	None, mandatory
5	99% ethanol (formalin-free)	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.

* Use filtered or deionized water for preparation of 80,90 or 95% ethanol.

† When processing specimens fixed in PAXgene Tissue FIX Containers, do not use alcohol that has been used for processing formalin-fixed samples in at least the first 5 positions (1–5). Formalin contamination in the alcohol can lead to significant reduction in DNA and RNA yield. Usually there is no need to keep xylene and paraffin separate for formalin and PAXgene Tissue-fixed sample processing because formalin contamination is typically limited at these positions due to dilution.

Appendix B: Processing Protocols Successfully Tested for Use with 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 by heating or proteolytic digestion.

However, it is important 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 in order to achieve optimal staining intensities.

For the latest information on IHC protocols and staining conditions **www.preanalytix.com**.

Ordering Information

Product	Contents	Cat. no.
PAXgene Tissue FIX Container (50 ml)	For fixation and stabilization of tissue specimens: 10 Prefilled Reagent Containers, containing 50 ml of PAXgene Tissue FIX	765312
Related Products		
PAXgene Tissue STABILIZER Concentrate (150 ml)	8 bottles of PAXgene Tissue STABILIZER concentrate, for 4 liters of PAXgene Tissue STABILIZER	765512
PAXgene Tissue RNA/miRNA Kit (50)	For 50 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
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
Qproteome FFPE Tissue Kit (20)	For 20 protein preparation from FFPE or PFPE tissue samples: Extraction Buffer, Collection Tubes, Collection Tube Sealing Clips	37623

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Handbook Revision History

Document	Changes	Date
HB-1477-001	Initial release	February 2013
HB-1477-002	Revisions throughout document to reflect discontinuation of related products; update of Safety Information; general update into new handbook template.	September 2019

Notes

Notes

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