

PAXgene[®]

**Tissue FIX Container (50 ml)
Product Circular**

For fixation and stabilization of tissue specimens

Important: To be used in conjunction with PAXgene
Tissue STABILIZER

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

February 2013

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Contents

Kit Contents	4
Symbols	4
Shipping and Storage	4
Intended Use	4
Safety Information	5
Quality Control	5
Introduction	6
Principle and procedure	6
Description of protocols	7
Equipment and Reagents to Be Supplied by User	10
Important Notes	11
Storage and archiving of PFPE (PAXgene Tissue-fixed, paraffin-embedded) tissue samples	11
Labeling of PAXgene Tissue FIX Container	11
Protocol: Sample Fixation and Stabilization	12
Protocol: Sample Processing, Paraffin Embedding, and Sectioning	14
Appendix A: Generic Processing Protocol That Supports Preservation of Biomolecules in Specimens Treated with the PAXgene Tissue System	16
Appendix B: Other Processing Protocols Successfully Tested for Use with Specimens Treated with the PAXgene Tissue System	17
Appendix C: Optimizing Immunohistochemistry (IHC) Assays with Sections of PFPE (PAXgene Tissue-Fixed, Paraffin-Embedded) Tissue	19
Ordering Information	21
PreAnalytiX Worldwide	23

Kit Contents

PAXgene Tissue FIX Container (50 ml)	(10)
Catalog no.	765312
PAXgene Tissue FIX Container (50 ml)*	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 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 has not been determined.

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.

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.

PAXgene Tissue FIX Container (50 ml)



Contain methanol and acetic acid: Highly Flammable, Toxic. Risk and safety phrases:* R11-23/24/25-36/38-39/23/24/25, S7-16-36/37/39-45.

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 PAXgene Tissue FIX 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. In order 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), pre-filled 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 min.* The reagent preserves morphology and biomolecules without the destructive cross-linking and degradation found with formalin-fixed tissues. The 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 total RNA, RNA including miRNA, or DNA from PAXgene Tissue-fixed and stabilized tissue samples require 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 pre-filled 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 analysis.

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

Description of protocols

Sample collection and stabilization with the PAXgene Tissue FIX Container

PAXgene Tissue FIX Containers are single-chamber containers prefilled with 50 ml of 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 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 within the same container. 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.*

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.**

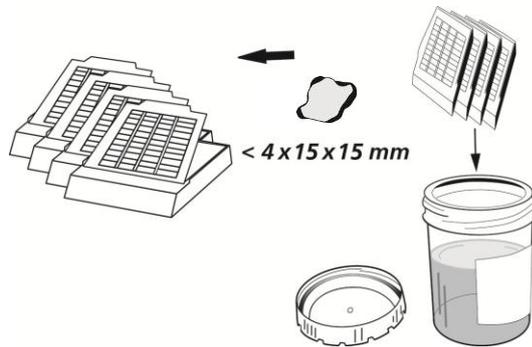
Stabilized samples can be embedded in paraffin for histological studies. Nucleic acids and proteins can be isolated from the stabilized samples before or after embedding in paraffin. See the *PAXgene Tissue Container Product Circulars* for information about DNA, RNA, or miRNA isolation, and the PAXgene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

See Figure 1 on page 8 for an illustration of the steps in the fixation process. See Figure 2 on page 9 for an illustration of the steps in the stabilization process.

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

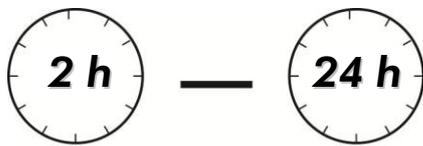
** For the latest results on long-term storage see Technical Notes at www.preanalytix.com.

A Multiple smaller tissue samples



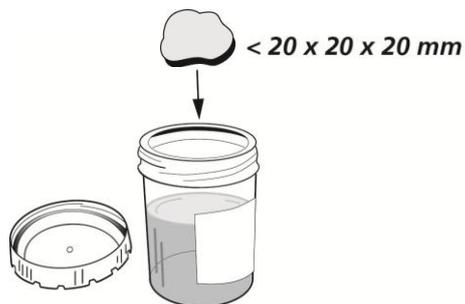
Resect and cut the tissue into max. 4 x 15 x 15 mm sections. Place in up to four (4) tissue cassettes.

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



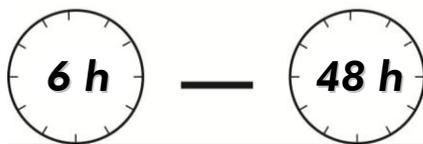
Fixation for 2 to 24 hours, depending on tissue sample and size.

B Single, larger tissue sample



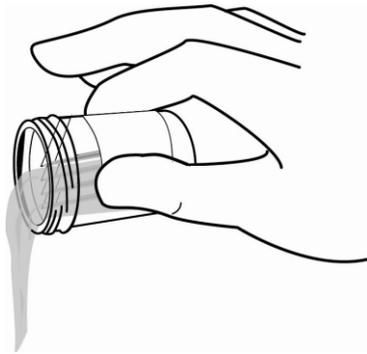
Tissue sample can have max. dimensions 20 x 20 x 20 mm.

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



Fixation for 6 to 48 hours, depending on tissue type and size

Figure 1. Fixation. Resection and fixation of tissue samples using the PAXgene Tissue FIX Container is a straightforward two-step process. It is suitable for multiple smaller tissue samples **(A)** or a single, larger tissue sample **(B)**.



Pour off PAXgene Tissue FIX



Fill with PAXgene Tissue STABILIZER



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

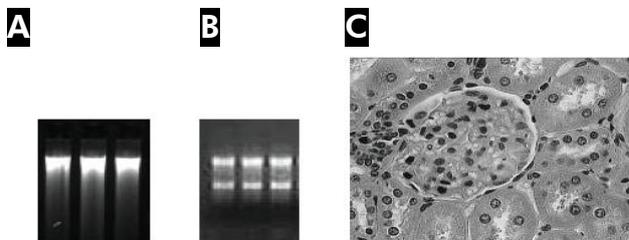


Figure 3. Preservation of nucleic acids and morphology. Later analysis of fixed and stabilized material shows stable preservation of DNA (A) and RNA (B). Microscope examination shows stable preservation of morphology (C).

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.

- 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): 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 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 FIX Container

In order to allow calculation 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). After replacing PAXgene Tissue FIX 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.

The image shows a label for a PAXgene Tissue FIX Container (50 ml). The label is white with a teal header and contains the following text and icons:

- PAXgene® Tissue FIX Container (50 ml)** (Teal header)
- For Research Use Only**
Not for Use in Diagnostic Procedures
- MAT** (Material icon) next to a dotted line for *Identification Number*
- LOT** (Lot icon) next to a dotted line for *Patient* and *DOB*
- Hourglass icon** next to a dotted line for *Person Collecting / Physician*
- Skull and crossbones icon**, **Flame icon**, and **2°C–25°C** (Temperature range icon) next to a dotted line for *Fixation Date/Time*
- Exchange with Tissue STABILIZER Date/Time** next to a dotted line and a **checkbox** with a checkmark.
- PreAnalytiX GmbH, 8634 Hombrechtikon, CH** (Company name and address)

Figure 4. Label of PAXgene Tissue FIX Container.

Protocol: Sample Fixation and Stabilization

Starting material

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

Important points before starting

- Do not use tissue samples larger than 20 x 20 x 20 mm as this will 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 transferring samples to the wrong container, 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 within 30 min.

2. Cut tissue into desired 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 overflows the edge of the container sample submersion, this is an indication that the tissue specimen(s) were too large. Multiple containers should be used instead.

- 4. Incubate tissue specimen(s) at room temperature (15–25°C) for a minimum of 2 hours, but preferably 3–24 hours (for samples up to 4 x 15 x 15 mm), or 6–48 hours, but preferably 8–24 hours (for samples up to 20 x 20 x 20 mm).**

Note: Longer fixation periods than indicated for the sample sizes 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.

- 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 position one. See Table 2, Appendix B on page 16 for an example of a processing protocol using PAXgene Tissue STABILIZER.

- 6. Process the samples further or store or transport tissue sample in PAXgene Tissue STABILIZER.**

Note: 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 (see footnote on page 7). Samples can be frozen at –20°C (–15°C to –30°C) or –80°C (–65°C to –90°C). 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.

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 13) and then incubated in PAXgene Tissue STABILIZER for a minimum of 2 h.
- Refer to Appendices A and B for detailed processing protocols (pages 16–18).
- Do not begin tissue processing either with 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.

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 station of a tissue processor filled with 80–99% ethanol to start dehydration.**
- 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 two 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 ($15\text{--}25^{\circ}\text{C}$).

Appendix A: Generic Processing Protocol 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 STABILZER
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. Contaminations in the alcohol used for sample processing can lead to significant reductions in DNA and RNA yield.

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

Appendix B: Other 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 see the tissue atlas at <http://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 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 — 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 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 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

Product	Contents	Cat. no.
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
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.

PreAnalytiX Worldwide

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Brazil • Orders 0800-557779 • Fax 55-11-5079-4001 • Technical 0800-557779
Canada • Orders 800-572-9613 • Fax 800-713-5951 • Technical 800-DNA-PREP (800-362-7737)
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Luxembourg • Orders 8002-2076 • Fax 8002-2073 • Technical 8002-2067
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