PreAnalytiX Supplementary Protocol

Preparation of sections from PAXgene® Tissue fixed, paraffin-embedded (PFPE) and PAXgene Tissue fixed, cryo-embedded (PFCE) tissues for manual or laser microdissection (LMD)

This protocol describes the preparation and staining of sections from PAXgene Tissue fixed, paraffin-embedded (PFPE) and PAXgene Tissue fixed, cryo-embedded (PFCE) blocks of tissue for manual or laser microdissection (LMD). Tissue specimens must be fixed and stabilized with the PAXgene Tissue System, prior to processing and paraffin-embedding or freezing and cryo-embedding.

IMPORTANT: The tissue samples must be fixed and stabilized in PAXgene Tissue Containers. The *PAXgene Tissue Container Product Circular* includes information on tissue fixation and stabilization. For instructions on the preparation of PFCE tissue blocks, read the PreAnalytiX® Supplementary Protocol *Cryo-embedding tissue specimens fixed and stabilized with the PAXgene Tissue System*.

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Equipment and reagents

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.

- RNase-free water
- Acetic acid
- Ethanol (96–100%, purity grade p.a.)
- Xylene (96–100%, purity grade p.a.)
- Reagents for H&E staining: Hematoxylin (e.g., Roth, Cat. no. 3816); Aluminium potassium sulfate dodecahydrate (e.g., Merck®, Cat. no. 101042); Sodium iodate (e.g., Merck, Cat. no. 106525); Eosin (e.g. Eosin Y, Merck, Cat. no. 115935); Acetic Acid (e.g., Merck, Cat. no. 1000631000)*
- 14.3 M β-mercaptoethanol, β-ME (commercially available solutions are usually 14.3 M)
- Microtome or cryostat†
- Water bath†

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

† Make sure that instruments have been checked and calibrated according to the manufacturer's recommendations.

- Incubator*
- Adhesion slides for manual microdissection (e.g., SuperFrost® Plus Slides, VWR® Cat. no. 631-0108)† or frame slides for laser microdissection, (e.g., PPS-membrane 25mm x 76 mm, Leica® Cat. no. 11505273).† For general information regarding properties of LMD slides refer to product circulars of the supplier/manufacturer.

Starting material

Starting material for manual or LMD from slide-mounted sections is a block of PFPE tissue (see procedure A) or PFCE tissue (see procedure B).

The tissue samples must be fixed and stabilized in PAXgene Tissue Containers and processed according to the *PAXgene Tissue Container Product Circulars* and the Supplementary Protocol *Cryo-embedding tissue specimens fixed and stabilized with the PAXgene Tissue System*.

Things to do before starting

- Prepare RNase-free equipment and reagents (e.g., microtome, slides, water, water bath, incubator, staining devices).
- Pre-cool 96% ethanol on ice.
- Heat the water bath and incubator to 40°C.
- If performing laser microdissection, switch on the LMD system to warm up the laser and set up the collection devices (follow the manufacturer's instructions).
- Prepare a fresh RNase-free hematoxylin solution.

Step	Preparation
1	Dissolve 50 g aluminium potassium sulfate in 425 ml RNase-free water in a heated water bath.
2	Dissolve 2.5 g hematoxylin in 25 ml 96% ethanol in a heated water bath.
3	Carefully mix the hematoxylin solution and aluminium potassium sulfate solution.
4	Add 125 mg sodium iodate, chill to room temperature and add 2 ml 100% acetic acid.

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- Prepare fresh eosin solution

Step	Preparation
1	Add 2.5 g Eosin Y to 500 ml RNase-free water. Stir for 2 h at room temperature.
2	Add 2 ml 100% acetic acid.

Note: Commercially available, ready-to-use staining solutions can also be used for staining LMD slides. For subsequent RNA preparations, however, we recommend using the described freshly made RNase-free solutions.

Procedure A: Sectioning, deparaffinization and staining of PFPE samples for manual or laser microdissection (LMD) from slide-mounted sections

1. **Using a microtome, make a tissue section of 6–12 µm thickness from a PFPE tissue block.**

   Note: If the sample surface has been exposed to air, discard the first 2 or 3 sections.

2. **Place the section onto the surface of a water bath at 40°C. Transfer the floating and stretched out section without folds onto an adhesion slide for manual microdissection or onto the membrane of a frame slide for LMD.**
3. **Dry the slide on a heating plate or in an incubator for 10 min at 40°C.**
4. **Deparaffinize the PFPE tissue sections, following the incubation steps in Table 1.**

Table 1. Deparaffinization of PFPE Slides for LMD

Step	Media	Incubation time
1	Xylene	1 min
2	Fresh Xylene	30 s
3	96% Ethanol	1 min
4	Fresh 96% Ethanol	1 min
5	70% Ethanol	1 min

5. **Optional: Perform H&E staining. Follow the incubation steps in Table 3 (page 4).**

   Note: If H&E staining is not performed immediately, transfer the slide to ice-cold 96% ethanol for transport or short-term storage.

Procedure B: Sectioning and staining of PFCE samples for manual or laser microdissection (LMD) from slide-mounted sections.

1. **Using a cryostat, make a tissue section of 6–12 µm thickness from a PFCE tissue block.**

   Note: If the sample surface has been exposed to air, discard the first 2 or 3 sections.

2. **Carefully transfer the section without folds onto an adhesion slide for manual microdissection or onto the membrane of a frame slide for LMD.**

   Note: Several sections can be placed on a single slide, if necessary.

3. **Air-dry the slide for 1 min at room temperature (15–25°C).**

4. **Perform the steps in Table 2 to remove the cryo-medium from the PFCE tissue sections.**

Table 2. Removal of cryo-medium from PFCE Slides for LMD

Step	Media	Incubation time
1	70% Ethanol	1 min
2	50% Fresh Ethanol	45 s
3	30% Fresh Ethanol	30 s

5. **Optional: Perform H&E staining. Follow the incubation steps in Table 3.**

   Note: If H&E staining is not performed immediately, transfer the slide to ice-cold 96% ethanol for transport or short-term storage.

Table 3. Hematoxylin and eosin quick-staining protocol

Step	Media	Incubation time
1	RNase-free water	30 sec
2	Hematoxylin solution	1 min
3	RNase-free water	30 sec
4	RNase-free water	30 sec
5	Eosin solution	10 sec
6	70% Ethanol	30 sec
7	96% Ethanol	30 sec
8	100% Ethanol	Until use

Procedure C: Manual or laser microdissection (LMD) of PFPE and PFCE slides

1. **Keep freshly prepared slides in ice-cold ethanol until microdissection.**

   Note: For long-time storage, freeze PFPE and PFCE slides at –80°C. Thaw frozen slides at 4°C for 30 min.

2. **Perform microdissection manually or using a LMD-microscope according to the manufacturer's instructions (e.g., Leica LMD6500 & LMD7000 system, Leica Microsystems).**

   For further instructions on the microdissection procedure, see one of the PreAnalytiX Supplementary Protocols available to purify total RNA, genomic DNA or total RNA including miRNA from microdissected PAXgene Tissue fixed, paraffin-embedded (PFPE) and PAXgene Tissue fixed, cryo-embedded (PFCE) tissues.

   Note: Avoid storing slides in ethanol after LMD because of diffusion between frame and membrane. Avoid multiple freeze-thaw cycles for RNA applications.

For up-to-date licensing information and product-specific disclaimers, see the respective PreAnalytiX or QIAGEN® kit handbook or user manual. Handbooks and user manuals are available at **www.qiagen.com** and **www.preanalytix.com** or can be requested from QIAGEN Technical Services or your local distributor.

Safety data sheets (SDS) for any QIAGEN or PreAnalytiX product can be downloaded from **www.qiagen.com/safety**.