

PreAnalytiX Supplementary Protocol

Preparation of PFPE tissue sections for use with in situ hybridization (ISH) staining assays

This protocol describes the preparation of PAXgene Tissue fixed, paraffin-embedded (PFPE) tissue sections for use with in situ hybridization (ISH) assays, including fluorescence (FISH), chromogenic (CISH) and silver (SISH) in situ hybridization.

IMPORTANT: Please read the *PAXgene Tissue Container Product Circular* or the *PAXgene Tissue FIX Container (50 ml) Product Circular*, paying careful attention to the "Safety Information" and "Important Notes" sections, before starting this procedure.

Note: The following is a generic protocol for ISH staining, including FISH, CISH and SISH. It is the user's responsibility to ensure that the protocol is compatible with a specific assay and produces results that are comparable to FFPE tissue sections.

For Research Use Only. Not for use in diagnostic procedures. The performance characteristics of this product have not been fully established.

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.

- Adhesion slides, e.g. SuperFrost® Plus Slides (e.g., VWR® cat. no. 631-0108)*
- Xylene (or xylene substitute)
- 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)
- Histology staining jars
- Standard buffered formalin solution (commercially available formalin solutions are 37% formaldehyde dissolved in water and diluted 1:10 in phosphate buffer for a 3.7% fixation solution, pH 7; e.g., Sigma-Aldrich cat. no. HT501128, VWR cat. no. 95042-908)*
- Phosphate-buffered saline (PBS)
- Standard heating plate

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

Starting material

Starting material for ISH staining is a PFPE tissue section mounted on a slide. The tissue sample must be 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).

Things to do before starting

- Prepare RNase-free equipment and reagents (e.g., microtome, slides, water, water bath, incubator, staining devices).
- Tissue specimens must be fixed, stabilized, processed and embedded in paraffin according to the *PAXgene Tissue Container Product Circular*.
- Prepare 90%, 70% and 50% (v/v) ethanol by mixing ethanol (96–100%) and water (deionized water may be used).

Procedure

1. **Cut PFPE tissue sections according to the ISH assay manufacturer's instructions (typically 3–5 μm). Mount the sections on adhesion slides.**
2. **Heat the sections on a heating plate for 10 min at 70°C.**
3. **Deparaffinize the PFPE tissue sections by incubating them according to Table 1.**

Table 1. Incubation steps to deparaffinize PFPE tissue sections

Step	Media	Incubation time
1	Xylene	10 min
2	Xylene	10 min
3	96%–100% Ethanol	15 min
4	96%–100% Ethanol	15 min
5	90% Ethanol	2 min
6	70% Ethanol	2 min
7	50% Ethanol	2 min

4. **Wash the deparaffinized PFPE tissue sections twice for 10 min each in distilled water.**
5. **Wash the PFPE tissue sections once for 10 min in PBS.**
6. **Transfer the PFPE tissue sections to a staining dish containing standard buffered formalin solution.**

Makes sure that the slides are completely covered by the solution.

Note: Commercially available formalin solutions are 37% formaldehyde dissolved in water and diluted 1:10 in phosphate buffer for a 3.7% fixation solution.

7. **Incubate the sections for 24 hours at room temperature (15–25°C).**
8. **Remove excess formalin from tissue sections by washing 3 times for 10 min each in PBS followed by washing twice for 10 min each in distilled water.**
9. **Proceed with the heat pretreatment step of the planned in situ hybridization protocol.**

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

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