PreAnalytiX Supplementary Protocol

Manual processing of tissue specimens treated with the PAXgene® Tissue System

This protocol is designed for manual processing of tissue specimens fixed and stabilized in either the PAXgene Tissue Container (cat. no. 765112) or the PAXgene Tissue FIX Container (50 ml) (cat. no. 765112).

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.

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.

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.

- Glass beaker with 2 liter volume
- Wire basket, round (e.g., 120 x 100 mm VWR, cat. no.216-2841)
- Large magnetic stirrer*, i.e., 50–80 mm
- Oven or incubator*, capable of heating to 60°C
- 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)†

Starting material

Starting material for manual processing should be a tissue sample, cut to a maximum size of 4 x 15 x 15 mm, placed into a standard tissue cassette and fixed and stabilized in either the PAXgene Tissue Container or the PAXgene Tissue FIX Container (50 ml).

* Ensure that instruments have been checked and calibrated according to the manufacturer's recommendations.

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

Important points before starting

- 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.
- 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 tissue samples in liquid paraffin for longer than 3 hours. Extensive incubation times above 60°C lead to degradation of RNA.

Things to do before starting

- In a hybridization or drying oven at 60°C, melt 1.6 liters of low-melting paraffin placed in a 2 liter glass beaker.
- Prepare 80% and 90% ethanol by mixing ethanol (100%) and water (deionized can be used).
- See Table 1 for manual processing parameters.

Procedure

1. **Fill a 2 liter glass beaker with 1.6 liters of 80% ethanol. Add a large magnetic stirring bar (e.g., 50 mm), and place beaker onto a magnetic stirrer.**
2. **Transfer tissue cassettes with PAXgene Tissue fixed and stabilized tissue specimens into a suitable round wire basket and place the basket into the glass beaker (see Figure 1).**

   Note: When using a 2 liter glass beaker filled with 1.6 liters of 80% ethanol, up to 40 tissue cassettes can be processed at the same time in a wire basket having a volume of 1.2 liters.
3. **Incubate for 1 h at room temperature (15–25°C) with slowly rotating stirring bar.**

   Note: Stirring should be slow with no more than 30–40 revolutions per min. Tissue cassettes must not be subjected to strong agitation.
4. **After 1 h, replace the 80% ethanol with 90% ethanol. Subsequently, replace the ethanol twice with 100% ethanol, incubating for 1 h after each change.**
5. **For clearing, replace the ethanol with xylene and incubate twice, incubating for 1 h after each change with slowly rotating stirring bar.**

   Note: Xylene substitutes may be used.

   Note: For optimal preservation of biomolecules, do not use clearing agents based on D-limonene.
6. **For infiltrating, place the wire basket holding the tissue cassettes into the 2 liter glass beaker filled with 1.6 liters of melted paraffin and incubate in a hybridization or drying oven at 60°C for an additional 2 h. Carefully stir the cassettes every 30 min with a glass stirrer or by moving the wire basket up and down.**
7. **Immediately, after the final paraffin incubation step, embed the tissue samples into a block of paraffin. For embedding, you may use the same low-melting paraffin used for infiltration.**

8. **For storage and sectioning, follow the recommendations in the *PAXgene Tissue Container Product Circular* or the *PAXgene Tissue FIX Container (50 ml) Product Circular*.**

Table 1. Manual processing protocol

Step	Media	Time	Temperature	Agitation
–	PAXgene Tissue STABILIZER	Up to 7 days	18–22°C	–
1	80% Ethanol	60 min	18–22°C	Slowly stirring
2	90% Ethanol	60 min	18–22°C	Slowly stirring
3	100% Ethanol	60 min	18–22°C	Slowly stirring
4	100% Ethanol	60 min	18–22°C	Slowly stirring
5	Xylene	60 min	18–22°C	Slowly stirring
6	Xylene	60 min	18–22°C	Slowly stirring
7	Paraplast X-tra	120 min	60°C	Manual stirring every 30 min

Figure 1. Beaker with tissue cassettes in a wire basket placed on magnetic stirrer for manual processing of PAXgene Tissue treated specimens.

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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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