

PreAnalytiX Supplementary Protocol

Purification of total RNA from sections of PAXgene[®] Tissue fixed, cryo-embedded (PFCE) tissue placed directly into a microcentrifuge tube

This protocol is for using the PAXgene Tissue RNA Kit to purify total RNA from sections of PAXgene Tissue fixed, cryo-embedded (PFCE) tissue placed directly into a microcentrifuge tube.

IMPORTANT: The tissue sample must be fixed and stabilized in PAXgene Tissue Containers (see the *PAXgene Tissue Container Product Circular* for information on tissue fixation and stabilization), washed in a sucrose solution, snap-frozen and cryo-embedded. For details, see the PreAnalytiX[®] Supplementary Protocol *Cryo-embedding tissue specimens fixed and stabilized with the PAXgene Tissue System (PX14)*.

Also read the *PAXgene Tissue RNA Kit Handbook*, paying careful attention to the “Safety Information” and “Important Notes” sections, before beginning this procedure.

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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.

- Ethanol (96–100%, purity grade p.a.)
- 14.3 M β -mercaptoethanol, β -ME (commercially available solutions are usually 14.3 M)
- Pipets and pipet tips
- Cryostat*
- Variable-speed microcentrifuge* capable of attaining 1000–8000 x g, and equipped with a rotor for 2 ml microcentrifuge tubes
- Shaker-incubator* capable of incubating at 45–80°C and shaking at ≥ 400 rpm, not exceeding 1400 rpm (e.g., Eppendorf[®] Thermomixer Compact)[†]
- Vortex mixer*
- Forceps
- 1.5 ml safelock microcentrifuge tubes

* Make sure 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.

Starting material

Starting material for RNA purification is 1–3 PFCE tissue sections with a thickness of 8–12 μm and a tissue surface area $\leq 225 \text{ mm}^2$.

Things to do before starting

- If working with RNA for the first time, read “Appendix A: General Remarks on Handling RNA” in the *PAXgene Tissue RNA Kit Handbook*.
- Tissue specimens must be fixed and stabilized according to the *PAXgene Tissue Container Product Circular*. Fixed and stabilized tissue must be cryo-embedded according to the Supplementary Protocol *Cryo-embedding of tissue specimens fixed and stabilized with the PAXgene Tissue System*.
- Set the temperature of the shaker–incubator to 56°C.
- Buffer TR1 may form a precipitate during storage. If necessary, warm to 37°C to dissolve.
- Add β -Mercaptoethanol (β -ME) to Buffer TR1 before use. Add 10 μl β -ME per 1 ml Buffer TR1. Dispense in a fume hood and wear appropriate protective clothing. Buffer TR1 containing β -ME can be stored at room temperature (15–25°C) for up to 1 month.

Procedure

1. **Label the lid and the body of a 1.5 ml safelock microcentrifuge tube (not provided). Prepare a lysis mixture in the tube by mixing 150 μl Buffer TR1 with 290 μl RNase-free water. Mix by gently flicking the tube. Add 10 μl Proteinase K, mix again and centrifuge briefly (1–2 s at 500–1000 x g) to collect residual liquid from the sides of the tube.**
Note: Do not mix Buffer TR1 and Proteinase K before adding water.
2. **Pre-cool the lysis reagent mixture on ice.**
3. **Using a cryostat, make a tissue section of 8–12 μm thickness from the PFCE tissue.**
4. **Using pre-cooled forceps, transfer the PFCE tissue section into the lysis reagent cooled on ice and mix by vortexing for 5 s.**
5. **If required, repeat steps 2 and 3 for a maximum of 3 sections.**
6. **Incubate the tissue on a shaker–incubator for 15 min at 56°C and 1400 rpm.**
7. **Centrifuge for 3 min at maximum speed (but do not exceed 20,000 x g).**
8. **Carefully transfer the supernatant to a new 1.5 ml microcentrifuge tube without disturbing the pellet.**
9. **Continue with the addition of ethanol in step 12 of the protocol “Purification of Total RNA from Sections of PFPE Tissue” in the *PAXgene Tissue RNA Kit Handbook* (page 17).**

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.

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