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 ≤225 mm².

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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