PreAnalytiX Supplementary Protocol

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

This protocol describes use of the PAXgene Tissue miRNA Kit to purify total RNA, including miRNA, 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*.

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

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.

- Isopropanol (100%, purity grade p.a.)
- 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 15,000–20,000 x *g*, and equipped with a rotor for 2 ml microcentrifuge tubes

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

- Shaker–incubator* capable of incubating at 56°C and shaking at ≥400 rpm, not exceeding 1400 rpm (e.g., Eppendorf® Thermomixer Compact)†
- Vortex mixer*
- Forceps
- Safe-lock microcentrifuge tube (1.5 ml)
- Ice

Starting material

As starting material for RNA, including miRNA purification, use 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, see "Appendix A: General Remarks on Handling RNA" in the *PAXgene Tissue miRNA 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.
- Before using the kit for the first time, prepare 80% (v/v) ethanol by mixing ethanol (96–100%, purity grade p.a.) and RNase-free water.
- Buffer TM1 may form a precipitate upon storage. If necessary, incubate at 37°C to redissolve.
- Add β-Mercaptoethanol (β-ME) to Buffer TM1 before use. Add 10 µl β-ME per 1 ml Buffer TM1. Dispense in a fume hood and wear appropriate protective clothing. Buffer TM1 containing β-ME can be stored at room temperature (15–25°C) for up to 1 month.

* 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.

Procedure

1. **Label the lid and the side of a 1.5 ml safe-lock microcentrifuge tube (not provided). Prepare the lysis reagent by adding 150 µl Buffer TM1 and 290 µl RNase-free water to the tube. Mix by gently flicking the tube. Add 10 µl Proteinase K, mix again, and centrifuge briefly to collect residual liquid from the sides of the tube.**

   Note: Do not mix Buffer TM1 and Proteinase K before adding water.

2. **Pre-cool the lysis reagent on ice.**
3. **Using a cryostat, prepare a tissue section of 8–12 µm thickness from the PFCE tissue.**
4. **Using pre-cooled forceps, transfer the PFCE tissue section into the pre-cooled lysis reagent and mix by vortexing for 5 s.**
5. **If required, repeat steps 3 and 4 for a maximum of 3 sections.**
6. **Incubate for 15 min at 56°C using a shaker–incubator set to 1400 rpm.**
7. **Centrifuge for 3 min at 15,000–20,000 x *g*.**
8. **Carefully transfer the supernatant to a new 1.5 ml safe-lock microcentrifuge tube without disturbing the pellet.**
9. **Continue with step 12 of the protocol "Purification of Total RNA, Including miRNA, from Sections of PFPE Tissue", in the *PAXgene Tissue miRNA Kit handbook*.**

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**.