PreAnalytiX Supplementary Protocol

Purification of full-length proteins from sections of PAXgene® Tissue fixed, paraffin-embedded (PFPE) tissue

This protocol describes using the Qproteome® FFPE Tissue kit to purify full-length proteins from sections of PAXgene Tissue fixed, paraffin-embedded (PFPE) tissue mounted on a slide (procedure A) or placed directly into a microcentrifuge tube (procedure B).

IMPORTANT: The tissue sample must be fixed and stabilized in PAXgene Tissue Containers, dehydrated, and embedded in paraffin (see the *PAXgene Tissue Container Product Circulars* for information on tissue fixation, stabilization and paraffin-embedding).

Also read the *Qproteome FFPE Tissue Handbook*, paying careful attention to the "Safety Information" section, 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.

- Extraction Buffer EXB Plus (provided in the Qproteome FFPE Kit (QIAGEN cat. no. 37623))
- 1.5 ml Collection Tubes (provided in the Qproteome FFPE Kit)
- 2 ml round-bottom safe-lock microcentrifuge tubes
- Adhesion slides, e.g. SuperFrost® Plus Slides (e.g., VWR® cat. no. 631-0108)*
- Ethanol (96–100%, purity grade p.a.)†
- Xylene
- β-mercaptoethanol, β-ME (commercially available solutions are usually 14.3 M)
- Ice
- Container for removal of paraffin, e.g. staining dishes or Coplin jars
- Pipets and pipet tips

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

† Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.

- Forceps
- Variable-speed microcentrifuge* capable of attaining 14,000 x *g*, cooling to 2–8°C, and equipped with a rotor for 1.5 ml microcentrifuge tubes
- Shaker-incubator* capable of incubating at 95°C (e.g., Eppendorf® Thermomixer Compact)†
- Vortex mixer*
- Microtome*
- Optional: Equipment for tissue disruption and homogenization. We recommend the TissueLyser LT* system (QIAGEN cat.no. 85600), or the TissueLyser II* system (QIAGEN cat.no. 85300)

Starting material

Starting material for protein purification is a section of a PFPE tissue sample mounted on a slide (procedure A) or 1–3 sections placed directly into a microcentrifuge tube (procedure B). The sections should have a thickness of 8–12 µm and a tissue surface ≤225 mm^2.

Things to do before starting

- Unless otherwise indicated, all steps of this protocol, including centrifugation steps, should be carried out at 2–8°C.
- Tissue specimens must be fixed and stabilized, dehydrated and embedded in paraffin according to the *PAXgene Tissue Container Product Circular*.
- β-ME‡ must be added to Extraction Buffer EXB Plus before use. For each extraction of 30 mg tissue sample, add 6 µl β-ME to 94 µl of Extraction Buffer EXB Plus to obtain a working solution.

  Optional: Protease-, phosphatase- and kinase-inhibitors may be added to Extraction Buffer EXB Plus if required.
- Set the temperature of the shaker–incubator to 95°C.
- Pre-cool Extraction Buffer EXB Plus on ice.
- Pre-cool a microcentrifuge tube rack on ice.

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

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‡ Perform procedures using β-ME in a fume hood with appropriate protective clothing.

Procedure A: Purification of full-length proteins from slide-mounted sections of PFPE tissue

1. **Using a microtome, prepare a tissue section of 8–12 µm thickness from a PFPE tissue block.**
2. **Place the section onto the surface of a water bath at 40°C. Transfer the floating and stretched out section without folds onto an adhesion slide.**
3. **Remove embedding medium and stain (optional) according to the Supplementary Protocol *Preparation of sections from PAXgene Tissue fixed, paraffin-embedded (PFPE) and PAXgene Tissue fixed, cryo-embedded (PFCE) tissue for manual or Laser microdissection (LMD)*.**

   Note: Repeat deparaffinization procedure if the paraffin is not completely removed.
4. **Remove the slide from the ethanol solution using forceps. Using an absorbent sheet, wipe away the liquid on the slide that surrounds the tissue section.**

   Note: Tissue areas of interest can be isolated by scratching away other tissue parts with a scalpel blade or cell scraper. Avoid completely drying the section because this makes it harder to dissolve the tissue from the slide.
5. **Place the slide on a horizontal working plate and overlay it with 100 µl ice-cold Extraction Buffer EXB Plus.**

   Note: Work quickly. We recommend using a dark underlay as a work surface to make it easier to see the tissue.

   Make sure that the entire section is covered. The volume of Extraction Buffer needed depends on the tissue surface area.
6. **Detach the tissue from the slide by pipetting the lysis mixture up and down. Transfer the tissue and the entire liquid to a labeled 1.5 ml collection tube and mix by vortexing for 5 s.**

   Note: If the tissue does not dissolve easily, use the pipet tip to scrape it from the slide. Additional mechanical or physical disruption of the sample does not increase total protein yield.
7. **Incubate the tube on ice for 15 min.**
8. **Incubate the tube on a heating block at 95°C for 10 min.**
9. **Incubate the tube on ice again for 5 min.**
10. **Centrifuge for 5 min at 14,000 x *g* at 2–8°C. Transfer the supernatant containing the extracted proteins to a new 1.5 ml collection tube.**

    Note: For quantification of protein yield, use the Lowry method (e.g., Bio-Rad® RC DC Protein Assay Kit, cat. no. 500-0122). Perform the tube assay protocol according to manufacturer's instructions. The assay used for quantification must be compatible with detergents and reducing agents such as β-ME.
11. **If not used immediately, store protein extracts at –15°C to –30°C.**

    Note: For long-term storage, we recommend freezing at –80°C. Aliquot the extracted protein to avoid multiple freeze-thaw cycles.

Procedure B: Purification of full-length proteins from sections of PFPE tissue placed directly into a microcentrifuge tube.

1. **Using a microtome, prepare a tissue section of 8–12 µm thickness from a PFPE tissue block.**
2. **Using pre-cooled forceps, transfer the PFPE tissue section into a pre-cooled, labeled 2 ml safe-lock microcentrifuge tube.**
3. **If required, repeat steps 1 and 2 for a maximum of 3 sections.**
4. **Follow the protocol for "Deparaffinization of FFPE Tissue Sections Cut Directly from an FFPE Sample Block" on page 15 of the *Qproteome FFPE Tissue Handbook*.**

   Note: It may be possible to reduce deparaffinization steps and times. Make sure to test any modifications to this protocol.
5. **Add 100 µl ice-cold Extraction Buffer EXB Plus to the tissue and mix by vortexing for 5 s.**

   Optional: Some fibrous and lipid tissues types may require additional mechanical disruption of the sample to improve total protein yield. When using the Qiagen TissueLyser LT or TissueLyser II, process the sample according to the protocol "Purification of RNA or Multiple Analytes from Animal and Human Tissues" in the *TissueLyser Handbook* (page 16). For sections from PFPE tissue, we recommend operating the TissueLyser LT or TissueLyser II for 2 min at 25 Hz.
6. **Incubate on ice for 15 min.**
7. **Incubate the tube on a heating block at 95°C for 10 min.**
8. **Incubate the tube on ice again for 5 min.**
9. **Centrifuge for 5 min at 14,000 x *g* at 2–8°C. Transfer the supernatant containing the extracted proteins to a new 1.5 ml collection tube. Keep supernatant on ice.**

   Note: For quantification of protein yield, use the Lowry method (e.g., Bio-Rad RC DC Protein Assay Kit, cat. no. 500-0122). Perform the tube assay protocol according to manufacturer's instructions. The assay used for quantification must be compatible with detergents and reducing agents such as β-ME.
10. **If not used immediately, store protein extracts at –15°C to –30°C.**

    Note: For long-term storage, we recommend freezing at –80°C. Aliquot the extracted protein to avoid multiple freeze-thaw cycles.

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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