PreAnalytiX Supplementary Protocol

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

This protocol is designed for use with the Qproteome® FFPE Tissue Kit (QIAGEN, cat. no. 37623) for purification of full-length proteins from slide-mounted sections of PFPE tissue.

IMPORTANT: Please read the *Qproteome FFPE Tissue Handbook*, paying careful attention to the Safety Information, before beginning 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 material safety data sheets (MSDSs), available from the product supplier.

- Qproteome FFPE Tissue Kit (QIAGEN, cat. no. 37623)
- Container for deparaffinization of PFPE sections, e.g., staining dishes or Coplin jars
- Xylene
- Ethanol (100%, 96%, and 70% [v/v])*
- Isopropanol
- Needles
- β-mercaptoethanol
- Crushed ice
- Pipets† (10 µl – 1 ml)
- Variable-speed microcentrifuge† capable of attaining 14,000 x *g*, cooling to 4°C, and equipped with a rotor for 1.5 ml microcentrifuge tubes
- Shaker-incubator† capable of incubating at 70°C and shaking at 750 rpm (e.g., Eppendorf® Thermomixer Compact, or equivalent)
- Cooling block capable of holding tubes at 4°C
- Vortex mixer†

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

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

Starting material

Starting material for protein purification should be up to 3 sections, each with a thickness of up to 10 µm and an area of up to 100 mm², of PFPE tissue mounted on microscope slides.

Important points before starting

- Ensure that the kit boxes are intact and undamaged, and that buffers have not leaked. Do not use a kit that has been damaged.
- When using a pipet, ensure that it is set to the correct volume and that liquid is carefully and completely aspirated and dispensed.
- To avoid transferring samples to the wrong tube or spin column, ensure that all tubes and spin columns are properly labeled. Label the lid and the body of each tube. For spin columns, label the body of its processing tube.
- Close each tube or spin column after liquid is transferred to it.
- Spillages of samples and buffers during the procedure may reduce the yield and purity of proteins.
- Unless otherwise indicated, all steps of this protocol, including centrifugation steps, should be carried out at room temperature (15–25°C).
- Xylene washes (steps 1 and 2) should be performed in a fume hood.

Things to do before starting

- β-mercaptoethanol (β-ME) must be added to Extraction Buffer EXB Plus before use. Add 6 µl β-mercaptoethanol to 94 µl of Extraction Buffer EXB Plus to obtain a working solution. Dispense in a fume hood and wear appropriate protective clothing.
- A shaker–incubator is required in step 10. Set the temperature of the shaker–incubator to 70°C.
- A cooling block is required in step 11. Equilibrate the cooling block to 4°C.

Procedure

1. **Transfer the PFPE tissue section mounted on a slide to a suitable reservoir containing fresh xylene. Incubate for 10 min at room temperature (15–25°C).**

   The slide should be completely covered.

2. **Repeat step 1 twice, using fresh xylene each time.**
3. **Transfer the slide to a reservoir containing fresh 100% isopropanol. Incubate for 10 min at room temperature. Repeat this step using fresh 100% isopropanol.**

   Note: 100% ethanol may be used instead of isopropanol.

4. **Transfer the slide to a reservoir containing 96% ethanol.* Incubate for 10 min at room temperature. Repeat this step using fresh 96% ethanol.***

5. **Transfer the slide to a reservoir containing 70% ethanol.* Incubate for 10 min at room temperature. Repeat this step using fresh 70% ethanol.***

6. **Immerse the slide in double-distilled water for 30 s and remove water by tapping the slide carefully on a paper towel.**

   Note: Ensure that the sections do not dry out. Slides may stay in water for up to 3 h.

7. **Excise areas of interest with a needle and transfer to a 1.5 ml collection tube (supplied with the Qproteome FFPE Tissue Kit).**

8. **Add 100 µl Extraction Buffer EXB Plus working solution (including β-mercaptoethanol; see page 2), and mix by vortexing. Seal the collection tube with a Collection Tube Sealing Clip (Qproteome FFPE Tissue Kit).**

   Note: Optionally protease inhibitors, phosphatase inhibitors, and kinase inhibitors may be added at this step.

9. **Incubate on ice for 15 min, and mix by vortexing.**

10. **Incubate the tube on a heating block at 70°C for 2 h with agitation at 750 rpm.**

    Note: Be sure that collection tubes are properly sealed with a Collection Tube Sealing Clip.

11. **After incubation, place the tube in a cooling block at 4°C for 1 min and remove the Collection Tube Sealing Clip.**

    Note: Be sure that Collection Tube Sealing Clip has been removed before starting the centrifugation step.

12. **Centrifuge for 15 min at 14,000 x *g* at 4°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). Dilute an aliquot of extracted protein fraction in a ratio of 1:3 with distilled water and perform the tube assay protocol according to manufacturer's instructions.

13. **If not used immediately, store protein extracts at –20°C or –70°C.**

    Note: For long-term storage freezing at –70°C to –80°C is recommended. Aliquot the protein extract to avoid multiple freeze-thaw cycles.

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

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Material safety data sheets (MSDS) for any QIAGEN or PreAnalytiX product can be downloaded from www.qiagen.com/Support/MSDS.aspx.

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