PreAnalytiX Supplementary Protocol

Purification of full-length proteins from sections of PAXgene® Tissue fixed, paraffin-embedded (PFPE) tissue cut directly from a block of 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 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)
- Xylene
- Ethanol (100%, 96%, and 70% [v/v])*
- 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, cut from a block of PFPE tissue.

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).

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 18. Set the temperature of the shaker–incubator to 70°C.
- A cooling block is required in step 19. Equilibrate the cooling block to 4°C.
- Xylene washes (steps 3–6) should be performed in a fume hood.

Procedure

1. **Using a microtome, cut up to 3 sections with a thickness of up to 10 µm from a PFPE block of tissue.**

   Note: If the sample surface has been exposed to air, discard the first 2 or 3 sections.

2. **Place sections in a 1.5 ml collection tube (supplied with the Qproteome FFPE Tissue Kit).**
3. **Add 1 ml xylene to the sample. Vortex vigorously for 10 s, and incubate for 10 min at room temperature (15–25°C).**

4. **Centrifuge at maximum speed for 5 min (but do not exceed 20,000 x *g*).**

   To prevent damage to processing tubes, do not exceed 20,000 x *g*.

5. **Remove the supernatant by pipetting. Do not remove any of the pellet. Proceed immediately to the next step.**

   Note: In some cases the pellet may be loose. Remove the supernatant carefully.

6. **Repeat steps 3 to 5 twice, using 1 ml fresh xylene each time.**
7. **Add 1 ml 100% ethanol.* Mix by vortexing, and incubate for 5 min. Centrifuge at maximum speed for 2 min.**
8. **Remove the supernatant by pipetting. Do not remove any of the pellet.**

   Note: In some cases the pellet may be loose. Remove the supernatant carefully.

9. **Repeat steps 7 and 8, using 1 ml fresh 100% ethanol* each time.**
10. **Add 1 ml of 96% ethanol.* Mix by vortexing, and incubate for 5 min. Centrifuge at maximum speed for 2 min.**
11. **Remove the supernatant by pipetting. Do not remove any of the pellet.**

    Note: In some cases the pellet may be loose. Remove the supernatant carefully.

12. **Repeat steps 10 and 11, using 1 ml fresh 96% ethanol* each time.**
13. **Add 1 ml of 70% ethanol.* Mix by vortexing, and incubate for 5 min. Centrifuge at maximum speed for 2 min.**
14. **Remove the supernatant by pipetting. Do not remove any of the pellet.**

    Note: In some cases the pellet may be loose. Remove the supernatant carefully.

15. **Repeat steps 13 and 14, using 1 ml fresh 70% ethanol* each time.**
16. **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.

17. **Incubate on ice for 15 min, and mix by vortexing.**
18. **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.

19. **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.

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

20. **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.

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

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