PreAnalytiX Supplementary Protocol

Purification of full-length proteins from PAXgene® Tissue fixed and stabilized (PF) tissue samples

This protocol describes using the Qproteome® FFPE Tissue kit for purification of full-length proteins from PAXgene Tissue fixed and stabilized (PF) tissue samples.

IMPORTANT: The tissue samples must be fixed and stabilized in PAXgene Tissue Containers. The *PAXgene Tissue Container Product Circular* includes information on tissue fixation and stabilization.

Also 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 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
- Pipets and pipet tips
- β-mercaptoethanol (β-ME), (commercially available solutions are usually 14.3 M)
- Phosphate-buffered saline (PBS)
- Variable-speed microcentrifuge* capable of attaining 14,000 x *g*, cooling to 2–8°C, and equipped with a rotor for 1.5 ml and 2 ml microcentrifuge tubes
- Shaker-incubator* capable of incubating at 95°C (e.g. Eppendorf® Thermomixer Compact, or equivalent)†
- Equipment for tissue disruption and homogenization (e.g. TissueLyser LT* system (QIAGEN cat.no. 85600), or TissueLyser II* system (QIAGEN cat.no. 85300))
- Vortex mixer*

* 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.

- Forceps
- Ice

Starting material

Starting material for protein purification is up to 30 mg of a tissue sample fixed with *PAXgene Tissue FIX* and stabilized with *PAXgene Tissue STABILIZER* (see the *PAXgene Tissue Container Product Circular* for information about tissue fixation and stabilization). Weighing the tissue is important for adequate determination of protein concentration in a sample.

Things to do before starting

- Unless otherwise indicated, all steps of this protocol, including centrifugation steps, should be carried out at 2–8°C.
- 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 PBS and Extraction Buffers on ice.
- Pre-cool a microcentrifuge tube rack on ice.

Procedure

1. **Label the lid and the side of a 2 ml round-bottom safe-lock microcentrifuge tube.**
2. **Retrieve the tissue sample from the PAXgene Tissue STABILIZER solution using forceps. Transfer 30 mg tissue into the labeled 2 ml microcentrifuge tube.**

   Note: Do not use more than 30 mg tissue sample. If required, cut the tissue into smaller pieces using a scalpel.
3. **If using the TissueLyser LT or TissueLyser II, add one stainless steel bead (5 or 7 mm mean diameter, QIAGEN cat. no. 69989 or 69990) to each tube.**

   If using a different instrument for tissue disruption, follow the manufacturer's instructions.
4. **Add 650 µl PBS, and mix by vortexing for 5 s. Using a pipet, remove the supernatant.**
5. **Add 100 µl Extraction Buffer EXB Plus.**

   Note: For samples larger than 30 mg, increase the volume of Buffer EXB Plus by 3.4 µl per additional mg of tissue.

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

6. **Disrupt and homogenize the sample using the TissueLyser LT or TissueLyser II or an equivalent homogenizer. Place the tubes in the TissueLyser LT Adapter (TissueLyser LT) or the TissueLyser Adapter Set 2 x 24 (TissueLyser II) and homogenize the sample for 2 min at 25 Hz.**

   The time of tissue disruption using the TissueLyser depends on the processed tissue size and type and can be extended for up to 5 min until the tissue is completely homogenized.

   Optional: Complete disruption and homogenization of some tissue types (e.g. fibrous or lipid tissue) may require an additional sonication step, e.g., 5 cycles of 10 s sonication each in a water bath.

   For detailed protocols, refer to the instrument handbook or manufacturer's instructions.

7. **Briefly centrifuge the tube to remove any foam that may have formed in step 6. Remove the stainless steel bead from the processing tube using forceps.**
8. **Incubate the tube on a heating block at 95°C for 10 min.**
9. **After incubation, place the tube on ice for 5 min.**
10. **Centrifuge for 5 min at 14,000 x *g* and 2–8°C. Transfer the supernatant containing the extracted proteins to a new 1.5 ml collection tube. Keep the tube 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). Dilute an aliquot of the extracted protein fraction with distilled water and perform the assay protocol according to the 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 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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