

Protein Extraction from PFPE

- Extraction of full length proteins from PAXgene® Tissue fixed, paraffin-embedded (PFPE) without crosslinks or chemical modifications
- Detection of membrane, cytoplasmic, nuclear, and phosphorylated proteins possible
- Comparable results to cryopreserved samples
- Applicable for all types of standard protein assays



Protein extraction principle and procedure

Sections of PFPE samples with a thickness of up to 10 µm, mounted on slides or cut directly from a block of PFPE tissue, are deparaffinized with xylene. After rehydration in a series of graded ethanol solutions, samples are transferred into extraction buffer EXB Plus (Qproteome® FFPE Tissue Kit) and incubated for 15 minutes on ice, followed by heating at 70°C for 2 hours with agitation. For a detailed description, see supplementary protocols on www.preanalytix.com or www.qiagen.com.

Protein quality

Proteins extracted from PFPE tissue using the Qproteome FFPE Tissue Kit are nondegraded, immunoreactive, and can be used for standard protein applications such as Western blot analysis and reverse-phase protein arrays.

This protein extraction procedure is suitable for use with:

- Purification of full-length proteins from sections of PFPE tissue cut directly from a block.
- Purification of full-length proteins from slide-mounted sections of PFPE tissue.
- ☑ **Effective extraction of full-length proteins from PFPE tissue**

Extraction of full-length proteins from PFPE tissue using the Qproteome FFPE Tissue Kit

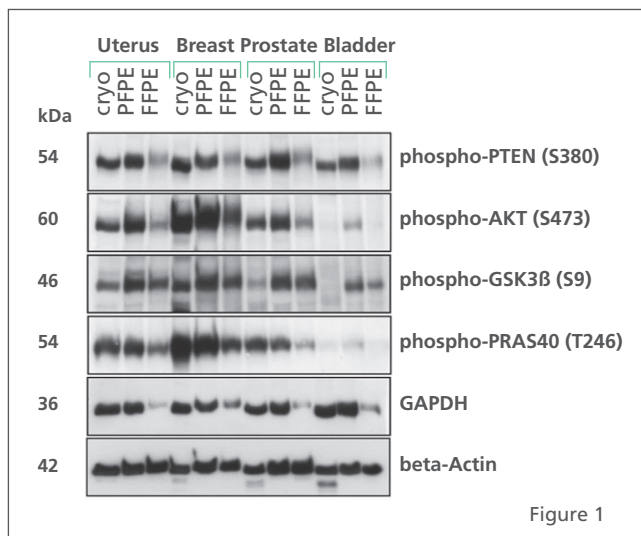


Figure 1. Preservation of phosphoproteins in PFPE samples from human clinical tissue specimens.

Non-malignant uterus, breast, prostate and bladder specimens were divided into three samples and either flash-frozen in liquid nitrogen (cryo), PAXgene Tissue fixed, paraffin-embedded (PFPE), or formalin-fixed, paraffin-embedded (FFPE). Proteins from cryo, PFPE, and FFPE tissues were extracted using the extraction buffer EXB Plus (Qproteome FFPE Tissue Kit; described in Ergin et al. 2010 and Gündisch et al. 2013, and PAXgene Tissue supplementary protocols). SDS-PAGE and Western blot analysis were performed with 15 µg protein and the indicated antibodies.

Detection of nondegraded, immunoreactive phosphoproteins from human clinical PFPE tissue

Data kindly provided by Karl-Friedrich Becker, Technical University of Munich, Germany.

Extraction of full-length proteins from PFPE tissue for special applications, such as 2-D PAGE and ELISA

Protein extraction principle and procedure

Sections of PFPE samples with a thickness of up to 10 µm, mounted on slides or cut directly from a block of PFPE tissue, are deparaffinized with xylene. After rehydration in a series of graded ethanol, samples are transferred into an appropriate extraction buffer and, if required, supplemented with protease, phosphatase, and kinase inhibitors. For enzyme-linked immunosorbent (ELISA) and two dimensional gel electrophoresis (2-D PAGE), we recommend using buffers from the manufacturer of the ELISA or 2-D PAGE kit for protein extraction. Proteins can then be detected using antibodies against directed denatured proteins.

Figure 2. Analysis of protein extracts from PFPE tissue in 2D-PAGE.

Non-malignant human duodenum tissue was divided into three samples and either flash-frozen in liquid nitrogen (cryo), PFPE, or FFPE. Proteins from cryo and PFPE tissue were extracted in 2D buffer (30 mM Tris-HCl pH8.8, 7 M urea, 2 M thiourea, 4% CHAPS, 75 mM DTT) supplemented with protease inhibitor. Proteins from FFPE tissue were extracted in EXB Plus buffer supplemented with protease inhibitor, precipitated with acetone, and resuspended in 2D buffer (as described in Gündisch et al. 2013) and 150 µg protein from each sample was separated by 2-D PAGE.

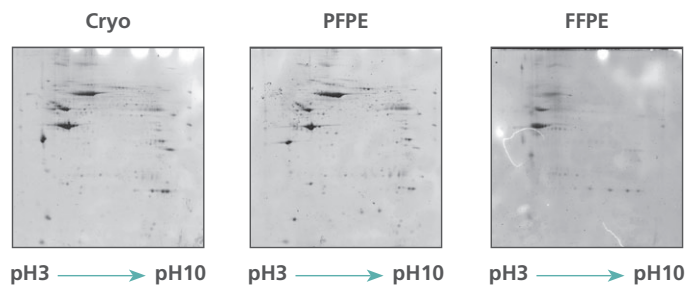


Figure 2

Protein extracted from PFPE is suitable for two-dimensional gel electrophoresis

Data kindly provided by Karl-Friedrich Becker, Technical University of Munich, Germany.

Order Information: Qproteome FFPE Tissue Kit (20)

cat. no. 767134

To find the distributor closest to you: www.qiagen.com and www.preanalytix.com

For research use only. Not for use in diagnostic procedures.

For up-to-date licensing information and product-specific disclaimers about QIAGEN products, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

Trademarks: PAXgene®, PreAnalytiX® (PreAnalytiX GmbH); Qproteome® (QIAGEN Group).