Protein expression analysis in Formalin-Fixed, Paraffin-Embedded (FFPE) tissue

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Introduction

- As the standard method for histological tissue preparation, millions of Formalin-Fixed Paraffin-Embedded (FFPE) samples are classified and archived each year.
- FFPE archives constitute a huge library of clinically appraised specimens, whose analysis on the molecular level can be directly related to progression of a disease or therapy.
- Previously, due to the crosslinks formed during the fixation process, their analysis was limited to histochemical techniques.
- Here we present a method that opens this vast, highly valuable resource up to proteomic and protein expression analysis by enabling efficient extraction of full-length proteins suitable for western blotting and protein arrays.

Methods

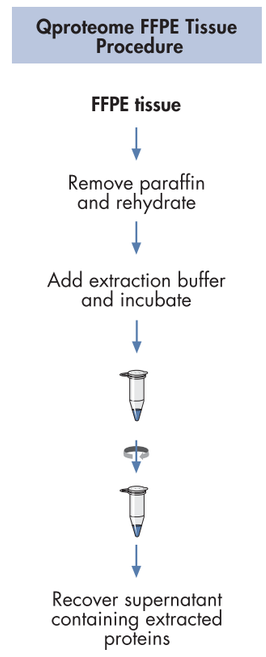
- Unstained FFPE samples are deparaffinized and rehydrated by sucessive washes in xylene, 100%, 96%, and 70% ethanol and water. Proteins are extracted by incubation in an extraction buffer at 100°C for 20 min followed by a 2 h incubation at 80°C. After incubation, proteins are recovered by centrifugation.

Table 1. Protein Yields from Different Tissues as Starting Material

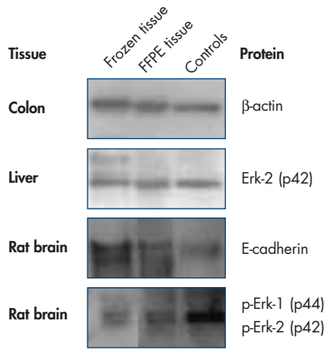
Tissue	Total size (mm²)	No. of sections	Protein yield
Heart (rat)*	~150	3	40 µg
Liver (rat) *	~150	3	80 µg
Colon (rat) *	~100–150	3	20 µg
Brain (rat) *	~150	3	60 µg
Breast cancer (human)†	~100	1	25–80 µg

* Proteins were extracted from FFPE tissue sections (10 µm) directly cut from a FFPE tissue sample block.

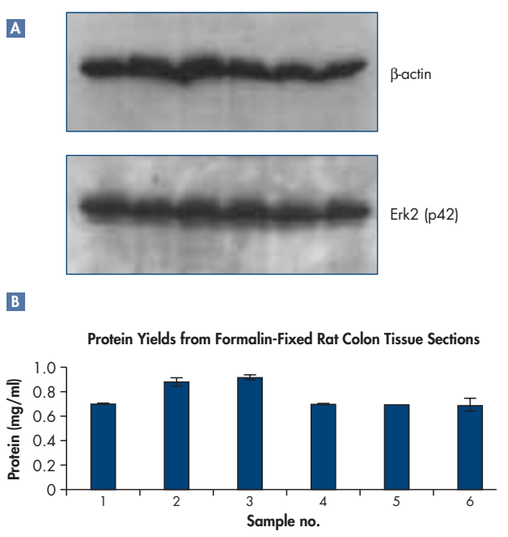
† Proteins were extracted from two areas with different morphological structures in the same FFPE tissue section (10 µm) mounted on a microscope slide.



Efficient and reproducible isolation of full-length proteins from FFPE tissues

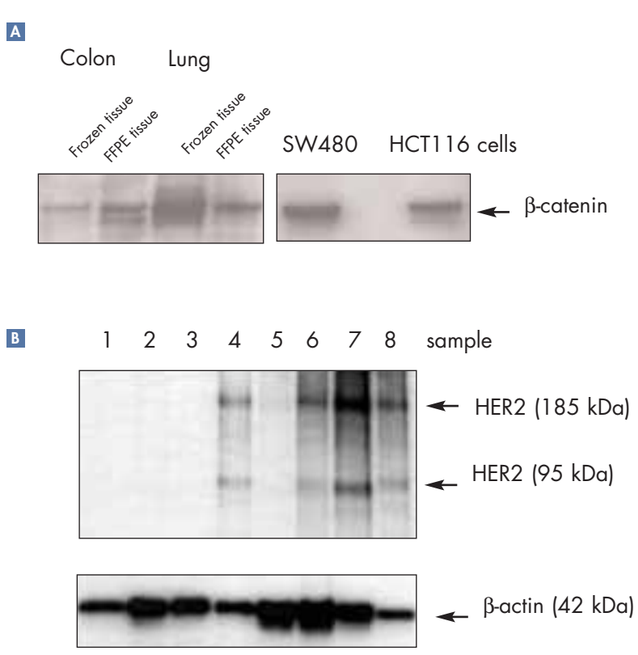


Protein yields from FFPE tissues using the Qproteome kit are comparable to those from frozen material. Protein yields from frozen and FFPE tissues were compared by processing material in parallel, separation by SDS-PAGE, and western blotting. Controls were lysates from SW480 colorectal adenocarcinoma cells (colon), Huh7 human hepatoma cells (liver), or SH-SY5Y neuroblastoma cells (rat brain).



A Standardized and reproducible extraction from FFPE sections. Six FFPE rat colon tissue samples (2 sections each) were processed in parallel. Equal volumes of extracted protein were separated by SDS–PAGE. Proteins were detected using β-actin and Erk2 antibodies after western blotting. B Protein concentration in each sample was measured in duplicate using the BCA method.

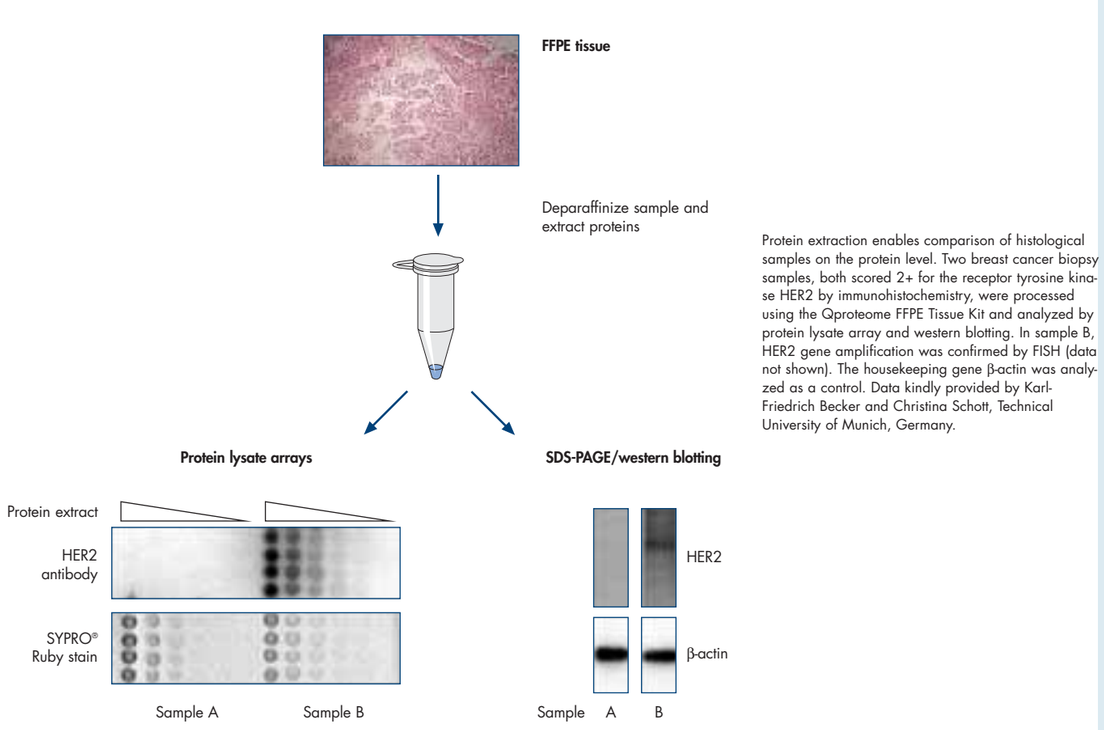
Detection and identification of biomarkers



- Over 90% of all colorectal carcinoma tumors harbor activating mutations within proteins of the canonical Wnt/β-catenin signaling cascade, with most mutations affecting either the β-catenin or APC proteins.
- Proteins such as β-catenin and HER2 are important biomarkers for monitoring tumor development and progression.

A The colorectal carcinoma biomarker β-catenin was detected in both frozen and FFPE tissue samples from colon and lung and in lysates from the colon carcinoma cell lines SW480 and HCT116. B FFPE breast tissue samples from 8 breast cancer patients was processed using the Qproteome FFPE Tissue Kit and proteins separated by SDS-PAGE. After western blotting, blots were probed with HER2 (upper panel) or β-actin (lower panel, control) antibodies. Both p185 and p95 forms of HER could be detected in the protein samples extracted from FFPE tissues. Data kindly provided by Karl-Friedrich Becker and Christina Schott, Technical University of Munich, Germany.

Proteins suitable for lysate arrays or western blot analysis



Protein extraction enables comparison of histological samples on the protein level. Two breast cancer biopsy samples, both scored 2+ for the receptor tyrosine kinase HER2 by immunohistochemistry, were processed using the Qproteome FFPE Tissue Kit and analyzed by protein lysate array and western blotting. In sample B, HER2 gene amplification was confirmed by FISH (data not shown). The housekeeping gene β-actin was analyzed as a control. Data kindly provided by Karl-Friedrich Becker and Christina Schott, Technical University of Munich, Germany.

Summary

- Proteins obtained using the FFPE kit are full-length and suitable for array or western blot analysis
- Investigation of FFPE samples enables detection and identification of biomarkers on the protein level
- Extraction of proteins from FFPE tissue is efficient and reproducible, and yields are comparable with those obtained from frozen tissue
- Any kind of FFPE material can be processed, including unstained slide-mounted sections, freshly-cut sections, and tissue punches from FFPE blocks.