Efficient processing of tissue samples using different protein fractionation technologies

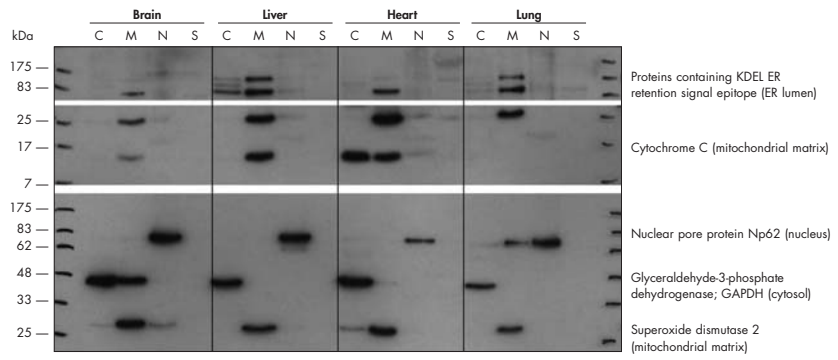
Christian Feckler, Heike Wolgast, Christina Schaaf, Nicole Seip, Peter Porschewski, and Kerstin Steinert
QIAGEN GmbH, Hilden, Germany

Introduction

- The multiplicity and poor reproducibility of sample isolation procedures and the scarcity and/or low quality of biosamples has meant that the number of biomarkers identified and validated in tissues has remained relatively low.
- Reproducible and universally applicable proteomics protocols offer the only way to overcome the challenges of obtaining valid biomarker information from tissues.
- Here we present a range of applications for which commercially developed protein sample preparation kits have provided excellent results.
- Newly developed protocols for tissues enable isolation and fractionation of proteins from the following classes:
- Complete proteome from fresh, frozen, and FFPE tissues
- Phosphorylated proteins
- Glycosylated proteins
- Mitochondria and mitochondrial proteins
- Proteins from different cell compartments (cytosol, membranes, nucleus, cytoskeleton)
- Nuclear proteins

Localizing proteins in different cell compartments

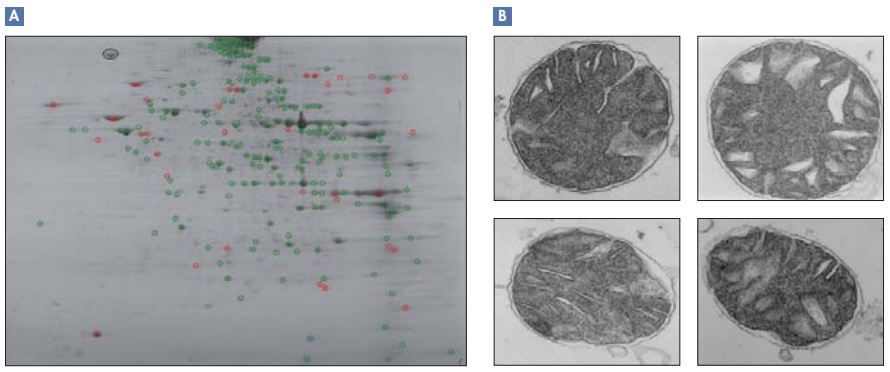
- Localizing proteins can give information about knockdown levels in RNAi experiments or can be used to follow translocation of proteins between cellular compartments in processes such as apoptosis.
- The robustness of the preparation method was tested by detecting cell compartment-specific marker proteins in fractions derived from four rat tissue samples.



The indicated rat tissue was processed using the Qproteome Cell Compartment Kit. The respective fractions (**C**: cytosol, **M**: membrane, **N**: nucleus, and **S**: cytoskeleton) were separated by SDS-PAGE and transferred by western blotting to nitrocellulose membrane. The blot was probed with antibodies against the indicated cell-compartment–specific marker proteins.

Isolation of mitochondria

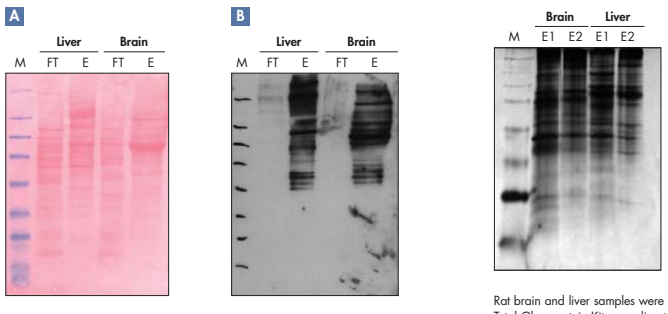
- Contamination of selected subproteomes with proteins from other cellular compartments is often a problem in proteomics procedures.
- After purification of a mitochondrial fraction using a kit-based method, 85% of extracted proteins were found to be of mitochondrial origin.
- Electron micrographs of the isolated mitochondria showed that both inner and outer membranes were intact.



A Mitochondria were prepared from 60 mg of liver tissue using the Qproteome Mitochondria Isolation Kit and subjected to 2D-PAGE. Out of 384 spots picked, 268 proteins were identified by MALDI-MS, 228 (85%) of which were predicted to have a mitochondrial localization. Green circles indicate mitochondrial proteins, red circles indicate impurities, the black circle indicates a protein which is probably located in mitochondria and ER. B Electron micrographs of mitochondria isolated using the Qproteome Mitochondria Isolation Kit showing intact inner and outer membranes.

Phospho- and glycoprotein extraction from tissues

- Protein phosphorylation is involved in cell signaling, cancer, apoptosis and immune disorders. A complete separation of non-phosphorylated from phosphorylated proteins facilitates studies of proteins involved in these processes.
- Using glycan-specific lectins enables separation of glycoproteins according to their glycan moieties, and can give a clue as to the structure and makeup of such moieties.

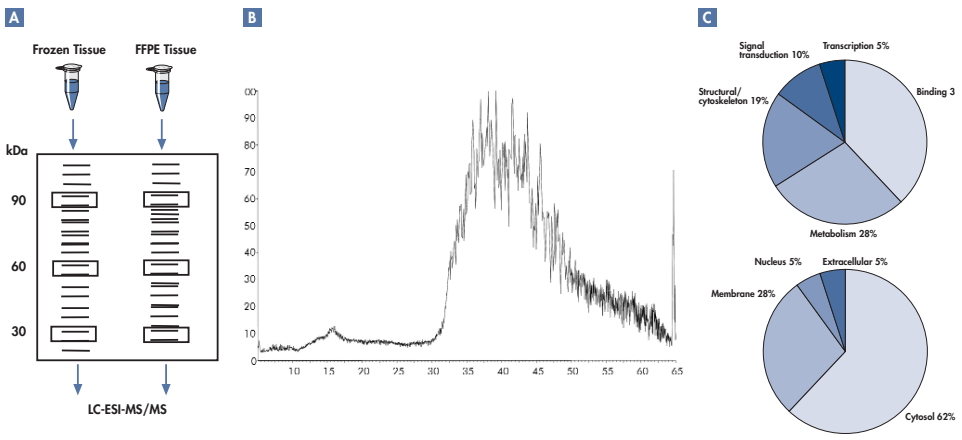


Frozen rat liver and brain (approximately 30 mg each) were disrupted at medium speed for 30–60 seconds in 350 µl PhosphoProtein Lysis Buffer. The homogenized samples were then diluted with 1500 µl PhosphoProtein Lysis Buffer and incubated at 4°C for 30 minutes, with brief vortexing every 10 minutes. Phosphoproteins were purified using the PhosphoProtein Purification Kit. A Transfer membrane stained with Ponceau S, B Western blot probed with antibodies specific for phosphorylated proteins. **M**: markers; **FT**: flow-through; **E**: elution fraction.

Rat brain and liver samples were processed using the TissueRuptor and the Qproteome Total Glycoprotein Kit according to the tissue protocol. The two-step elution from the ConA (E1) and WGA (E2) lectins in Total Glycoprotein Spin Columns enables separation of proteins carrying high antennarity or high-mannose glycans and those carrying highly sialylated glycans. Each eluate was pooled and aliquots separated by SDS-PAGE. A Silver stained gel of the eluates from each tissue. A Western blots probed with antibodies directed against beta-integrin and E-cadherin. In both tissues beta-integrin was found mainly in eluate 2, suggesting the presence of terminal sialic acid residues. E-cadherin was detected in both eluates, suggesting that it carries glycans with high-mannose or high antennarity and a high degree of sialylation.

MS identification of proteins in FFPE tissues

- The vast number of FFPE tissue sections prepared worldwide each year constitutes a huge resource for proteomics and molecular pathology research.
- Proteins in these tissues are now open to proteomic analysis by a range of methods, including mass spectrometry.



FFPE rat brain sample was separated by 1D-PAGE. A The protein bands around 90, 60, and 30 kDa were excised, subjected to tryptic digest and the resulting tryptic digests were analyzed B by LC-ESI-MS/MS (Ultimate nanoHPLC/QTOF2). Proteins were identified by MS/MS ion search using the Mascot search engine. A total of 567 unique peptides originating from 48 proteins were isolated and identified by MS/MS from the FFPE samples. C The distribution of function (upper panel) and cellular location (lower panel) of proteins isolated from the FFPE tissue 30 kDa band reflects normal cellular conditions.

Summary

- Meaningful comparisons between proteomics samples require reliable and reproducible sample preparation procedures. Using standardized kit-based methods eliminates the variations inherent to "homebrew" methods and enables intra- and inter-laboratory comparison and sharing of data.
- The development of standardized, reproducible methods for protein isolation from tissues offers great potential for the discovery and validation of biomarkers
- Biomarkers discovered in tissue samples probably have a greater relevance to the “real-life” disease situation than those found in cell culture models
- Above all, the opening of the vast worldwide resource of FFPE tissue libraries to proteomic analysis represents a major opportunity for advances in the fields of diagnostic and clinical molecular pathology

HB_PO_2007_Tissue