A range of ready-to-use kits reduces complexity of protein samples for proteomics studies

Winfried Girg, Christian Feckler, Manuela Hosbach, Nicole Brinker-Krieger, Christina Schaaf, Nicole Seip, Peter Porschewski, Anja Weiß, Uritza von Groll, Heike Wolgast, Annette Zacharias, Thibault Géoui, and Kerstin Steinert

QIAGEN GmbH, Hilden, Germany

Introduction

A major problem facing protein researchers is the sheer complexity of the proteome. Purification and quantification of low-abundance proteins is especially challenging. To address these problems, QIAGEN has used its expertise in sample preparation to develop the Qproteome range of kits for standardized and reproducible protein fractionation.

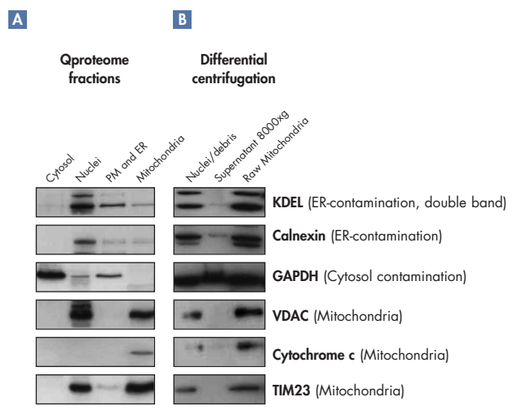
Qproteome kits offer:

- Reduced sample complexity according to highly specific separation criteria enabling efficient isolation of targeted subsets of proteins for easier analysis of low-abundance species
- Simple, easy-to-use kit formats with no specialized equipment required
- Highly reproducible, standardized separation for reliable results, time after time
- Intact, native-conformation proteins suitable for enzymatic assays and activity-based downstream applications

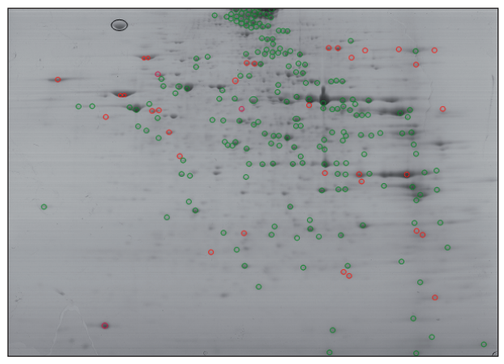
Qproteome kit	Protein categories	Fractions isolated
FFPE Tissue Kit	Whole proteome isolation	Full-length protein from FFPE Tissue
Soluble Protein Separation Kit	Whole proteome isolation	6 fractions according to protein solubility
Albumin/IgG Depletion Kit	Selective depletion	Albumin and IgG-free serum or plasma samples
Cell Compartment Kit	Organelle/Cell-compartment-specific	Cytosolic, membrane, nuclear and cytoskeletal proteins
Nuclear Protein Kit	Nuclear and nucleic acid binding proteins	Cytosolic proteins; Nucleic acid binding proteins; "insoluble" nuclear proteins (eg., Histones)
Nuclear Subfractionation Kit	Nuclear and nucleic acid binding proteins	As Nuclear Protein Kit with added nuclear protein subfractionation
Mitochondrial Isolation Kit	Organelle/Cell-compartment-specific	Mitochondrial proteins
Plasma Membrane Protein Kit	Organelle/Cell-compartment-specific	Plasma membrane proteins
PhosphoProtein Kit	Posttranslational modification	Phosphorylated and unphosphorylated proteins
Glycoprotein Kits	Posttranslational modification	Enriched fractions containing glycoproteins carrying lectin-specific moieties

Efficient isolation of high-purity mitochondria from cultured cells and tissue samples

One of the major problems in subcellular separation is obtaining high-purity fractions free from contaminants from other cell structures. Traditional methods are laborious, time-consuming, and require expensive equipment (e.g., ultracentrifuge).



Approximately 10^7 HeLa cells were lysed and fractionated either by using the Qproteome Mitochondria Isolation Kit A or by differential centrifugation B. The western blot data reveal that mitochondrial fractions isolated with the Qproteome Kit contain much lower levels of contaminating proteins than the differential centrifugation fractions.



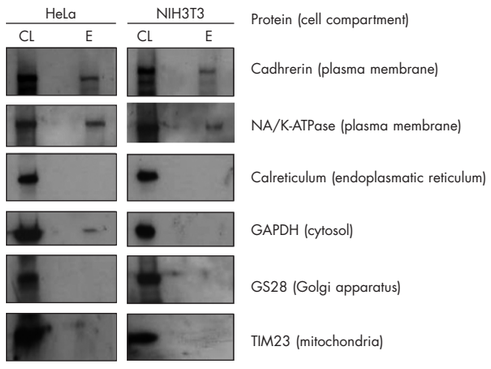
Mitochondria were prepared from 60 mg of liver tissue and subjected to 2D-PAGE. 384 spots were picked, 259 were identified by MALDI-MS and 219 (85%) were predicted to have a mitochondrial localization (85%). A total of 88 different proteins were identified, 66 with a predicted mitochondrial localization (75%).

Green circles indicate mitochondrial proteins, red circles indicate impurities, the black circle indicates a protein which is probably located in mitochondria and ER.

Efficient recovery of high-purity active plasma membrane proteins

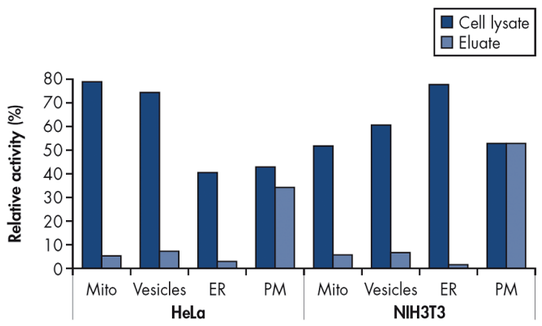
- Using the Qproteome kit we were able to isolate fully active proteins, virtually free of contaminating proteins from other cell compartments.
- The kit format provides a procedure that is highly reproducible and there is no need for expensive specialized equipment, protein derivatization, or extra reagents.

Efficient Removal of Non-PM Proteins



Plasma membrane proteins were purified from either HeLa or NIH3T3 cell cultures using the Qproteome Plasma Membrane Protein Kit. Cell lysates (CL) and elution fractions (E) were seperated by SDS-PAGE and transferred to a nitrocellulose membrane by western blotting. Proteins regarded as markers for different cell compartments were detected using protein-specific antibodies and an HRP-conjugated secondary antibody with chemiluminescent detection.

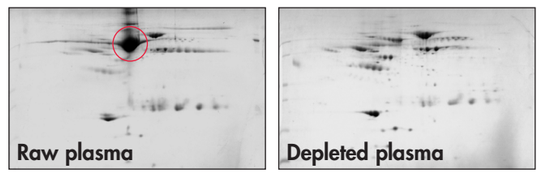
Efficient Recovery of Active PM Proteins



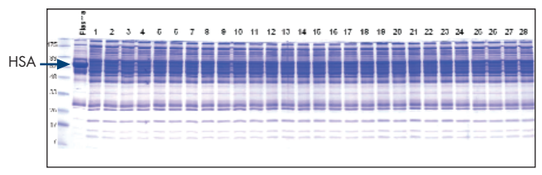
The relative activitiy of the cell compartment-specific marker proteins fumarase (mitochondria), acid phosphatase (vesicles), NADH-cytochrome-c reductase (ER, endoplasmic reticulum), and Na/K ATPase (PM, plasma membrane) was compared in cell lysate and elution fractions.

Albumin/IgG depletion facilitates analysis of low-abundance proteins

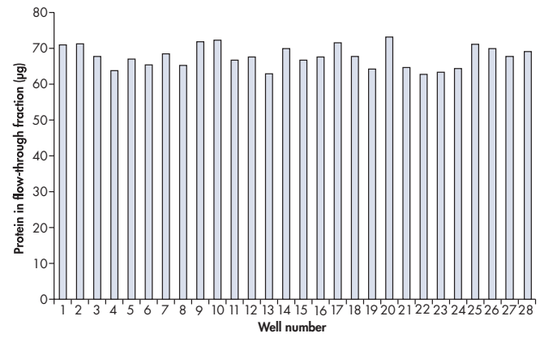
- Body fluids, such as serum and plasma, are widely used in clinical research and diagnostic procedures. A major problem in analyzing the makeup of these samples is the huge dynamic range of concentrations of their constituent proteins.



Coomassie® stained 2-D PAGE gels showing non-depleted and depleted plasma samples.



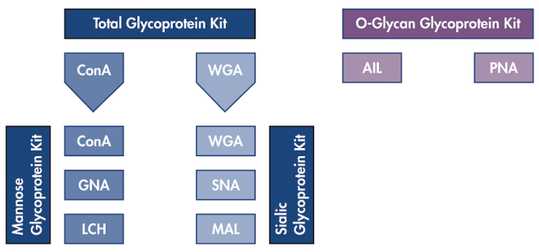
SDS-PAGE analysis of the 28 depleted serum samples and the input plasma. HSA; Human serum albumin.



5 µl aliquots of plasma were diluted with 15 µl buffer and loaded into wells of a 96-well plate containing 130 µl albumin/IgG depletion resin. The amount of protein in the flow-through of 28 samples was determined using Bradford method. Mean value: 68 µg; Standard deviation: 3 µg, CV: 4.6 %, Flow through volume: 68 µl.

Highly specific isolation of glycoproteins

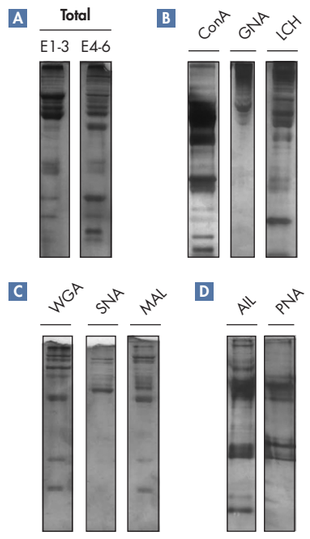
Comprehensive and Precise Analysis of Glycan Moieties



Initial analysis of glycoproteins can be carried out using the Total and O-Glycan Glycoprotein Kits. Depending on which lectin column binds a protein of interest, further studies on its precise nature can be performed using either the Mannose or Sialic Glycoprotein Kit.

One of the most common posttranslational protein modifications is glycosylation, which plays a vital role in a wide range of cellular processes such as cell adhesion and signaling, stabilization of protein structure and function, protein trafficking and sorting, and oncogenesis.

Highly Specific Glycoprotein Fractionation Using Lectin Spin Columns



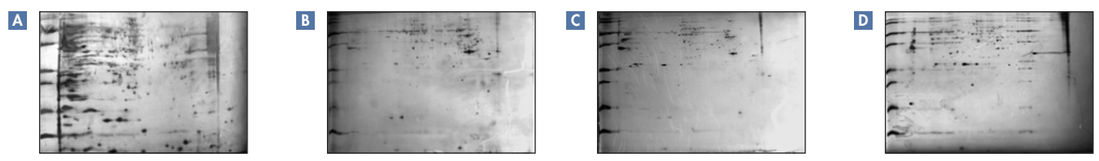
Glycoproteins were fractionated from serum using the different lectin spin columns in glycoprotein fractionation kits and analyzed by SDS-PAGE followed by silver staining. A Elution steps 1–3 and 4–6 from Total Lectin Spin Columns in the Total Glycoprotein Kit. B Eluted glycoproteins from ConA, GNA, and LCH Spin Columns in the Mannose Glycoprotein Kit. C Eluted glycoproteins from WGA, SNA, and MAL Spin Columns in the Sialic Glycoprotein Kit. D Eluted glycoproteins from AIL and PNA Spin Columns in the O-Glycan Glycoprotein Kit.

Summary

The Qproteome range of protein sample preparation kits offers:

- Facilitated analysis of low-abundance species — especially important when identifying or validating diagnostic biomarkers or drug targets
- Reproducible, standardized procedures — unlike home brew methods, the quality-controlled reagents in Qproteome kits offer the reassurance that each preparation is the same as the last, time after time
- Separation according to multiple, highly specific criteria — the Qproteome portfolio of kits currently offers the widest range of commercially available technologies, enabling researchers to focus on their specific separation requirements
- Gentle procedures that deliver fully active, native-conformation proteins — protein fractions can be used directly in a range of downstream applications, such as enzymatic activity tests, mass spectroscopy, further purification, or 2D-PAGE

Reduction in Complexity of Nucleic Acid Binding Protein Fraction Through Subfractionation



A Nucleic acid binding protein fraction isolated in the first stage of the Qproteome Nuclear Subfractionation Kit procedure. B – D Subfractionated nucleic acid binding proteins eluted from Nuclear Subfractionation Resin in the second stage of the Qproteome Nuclear Subfractionation Kit procedure.

06/2007

Sample & Assay Technologies