Standardized and reproducible protein fractionation and depletion with Qproteome® Kits

A major challenge facing protein researchers is the sheer complexity of the proteome. Purification, quantification, and identification of low-abundance proteins is especially problematic. To address these problems, QIAGEN's expertise in sample preparation has been used to develop Qproteome Kits for standardized and reproducible protein fractionation and depletion.

Benefits of Qproteome Kits:

- Consistently reproducible and reliable results
- Reduced sample complexity for analysis of less-abundant proteins
- Easy-to-use kits and no need for special equipment
- Intact, native-conformation proteins suitable for all assays
- Compatibility with various starting materials (body fluid, cells, or tissue)

Qproteome Kits enhance assay sensitivity and, combined with various downstream applications (e.g., western blot, mass spectrometry, and ELISA), help address the following biological questions:

- Is the protein expressed?
- Where is the protein localized?
- How is the protein modified?
- What is the function of the protein?

In the following sections, the challenges associated with the analysis of the proteome, critical factors for success, and information on dedicated products optimized for specific applications are presented.

Figure 1. Qproteome Kits ensure highly reproducible, standardized separation of proteins.

Kits to suit specific applications

Versatile Qproteome Kits can be used for a variety of applications and workflows (Figure 2). Dedicated kits for applications such as whole proteome isolation, protein localization, compartment enrichment, and enrichment of posttransationally modified proteins, as well as selective depletion to improve assay sensitivity are available. To choose the right product for your application, see Table 1.

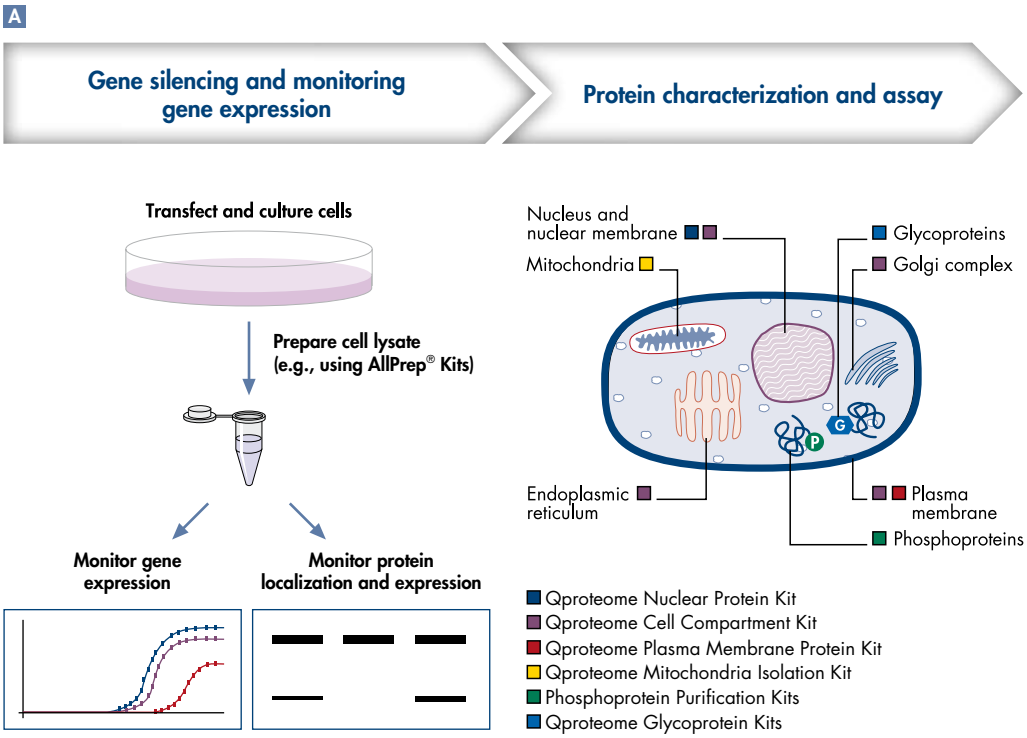
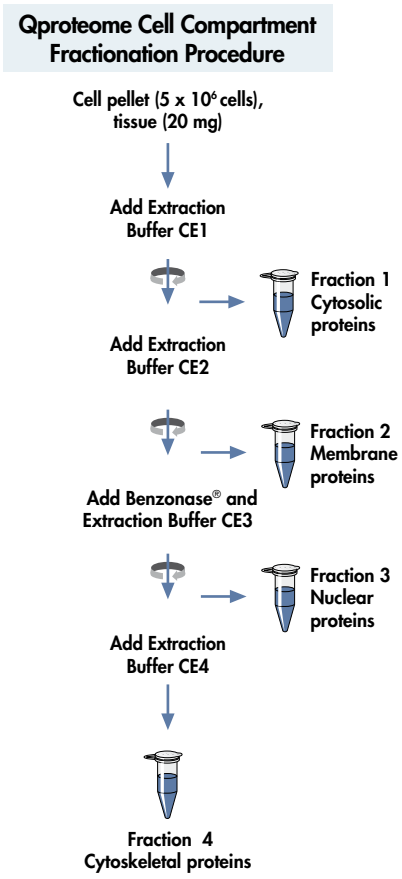


Figure 2. A variety of workflows and a range of applications. A Qproteome Kits can be used in cell biology experiments to monitor the changes in the target protein. For instance, they enable detection of altered protein expression levels, changes in posttranslational modifications, or changes in subcellular localization that are caused by transfection with siRNAs. B Qproteome Kits can be used to prepare protein samples for assays (e.g., ELISA) or proteomics experiments by selective depletion of abundant proteins or enrichment of a protein subtype or cell compartment. This increases sensitivity and enables discovery of low-abundance biomarkers.

Table 1. Qproteome selection guide

	Product	Sample input per prep	Isolated fractions	Yield	Cat. no.
Whole proteome isolation	Qproteome Bacterial Protein Prep Kit	Bacterial cultures (scalable)	Soluble and insoluble proteins	Dependent on protein expression	37900 (for 4.5 liter culture)
	Qproteome Mammalian Protein Prep Kit	5–10 x 10^6 cells, 40 mg tissue	Soluble and insoluble proteins	Up to 98% protein solubilization (up to 1.7 mg protein)	37901 (for ~100 preps)
Protein localization/ compartmental enrichment	Qproteome Cell Compartment Kit	5 x 10^6 cells, 20 mg tissue	Cytosolic, membrane, nuclear, and cytoskeletal proteins	0.5–1 mg protein spread over the 4 fractions	37502 (10 preps)
	Qproteome Mitochondria Isolation Kit	5 x 10^6 – 2 x 10^7 cells, 20–60 mg tissue	Intact, highly active mitochondria, virtually free from cytosolic contaminants	20–80 µg (cells) or up to 400 µg (liver tissue) mitochondria	37612 (12 preps)
	Qproteome Nuclear Protein Kit	5 x 10^6 – 1 x 10^7 cells, 20 mg tissue	Cytosolic proteins, "insoluble" nuclear proteins (e.g., histones), and nucleic acid–binding proteins	1.5–2.5 mg protein (spread over the 3 fractions)	37582 (12 preps)
	Qproteome Plasma Membrane Kit	1 x 10^7 cells	Plasma membrane proteins	30–100 µg plasma membrane proteins	37601 (6 preps)
Enrichment of posttranslationally modified proteins	PhosphoProtein Purification Kit/ Cartridge	1 x 10^7 cells, 30 mg tissue	Phosphorylated and unphosphorylated proteins	100–300 µg phosphorylated proteins	37101 (kit for 6 preps); 37145 (FPLC cartridge)
	Qproteome Glycoprotein Kits*	1 x 10^7 cells, 50 µl serum, 10 mg tissue	Glycosylated and unglycosylated proteins	20–150 µg glycoproteins depending on lectin used	Varies
	Lectin Cartridges	100 µl serum, 10 ml cell culture supernatant	Glycosylated and unglycosylated proteins	250 µg – 1 mg glycoproteins depending on lectin used	Varies
Increase of assay sensitivity with selective depletion	Albumin and Albumin/ IgG Depletion Cartridges	100 µl serum, plasma, or CSF	Albumin- or albumin/ IgG-depleted fraction	3–5 mg protein	Varies
	Albumin and Albumin/ IgG Depletion Kits*	25 µl serum, plasma, or CSF	Albumin- or albumin/ IgG-depleted fraction	0.5–0.8 mg protein	Varies
	Albumin and Albumin/ IgG Depletion Plate	5 µl serum, plasma, or CSF	Albumin- or albumin/ IgG-depleted fraction	50–100 µg protein	37009

* Automatable on the QIAcube®.



Qproteome Cell Compartment Kit

Fractionation of proteins according to cellular localization can be achieved using the Qproteome Cell Compartment Kit (Figure 3). The kit enables:

- Efficient and reproducible separation of cellular proteins
- Monitoring of protein localization under different growth conditions
- Enrichment of a particular subset of proteins from a cell compartment

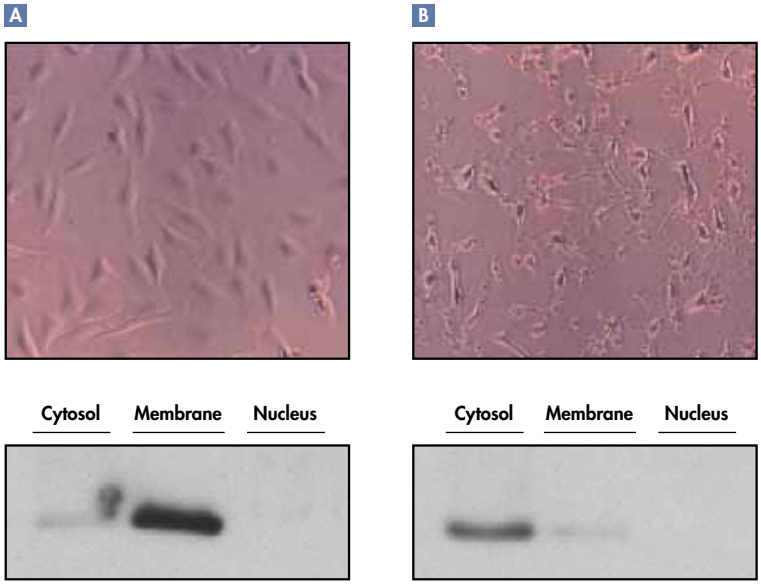


Figure 3. Reliable fractionation of proteins using the Qproteome Cell Compartment Kit. Micrographs (upper panels) of HeLa cells. A Viewed under normal growth conditions and B after a 4-hour incubation with staurosporine, an inducer of apoptosis. Lower panels show western blots prepared after cell lysates were processed using the Qproteome Cell Compartment Kit. The blots were probed with anti-cytochrome c antibody. During apoptosis, cytochrome c translocates from the intermembrane space of mitochondria to the cytosol, a process that is reflected in the detection of cytochrome c in the cytosolic fraction.

Qproteome Mitochondria Isolation Kit

The Qproteome Mitochondria Isolation Kit ensures efficient isolation of high-purity, active mitochondria (Figure 4). The benefits of the kit include:

- High-quality preparations virtually free of contaminating proteins
- Highly reproducible, fast, and standardized procedure
- Easy-to-use kit and no need for special equipment or extra reagents

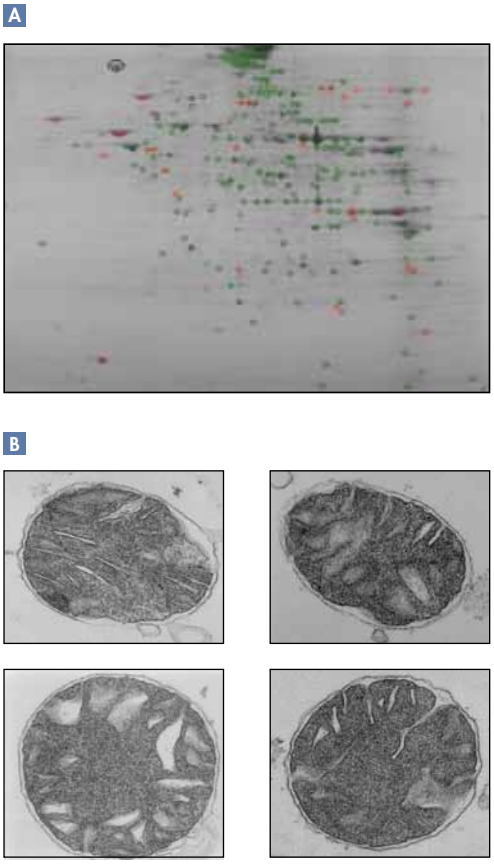
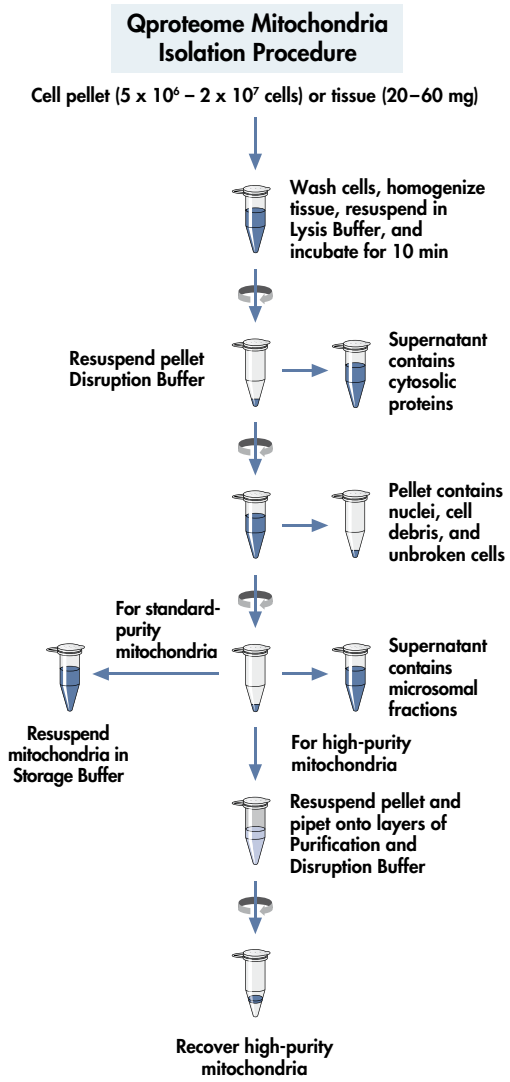
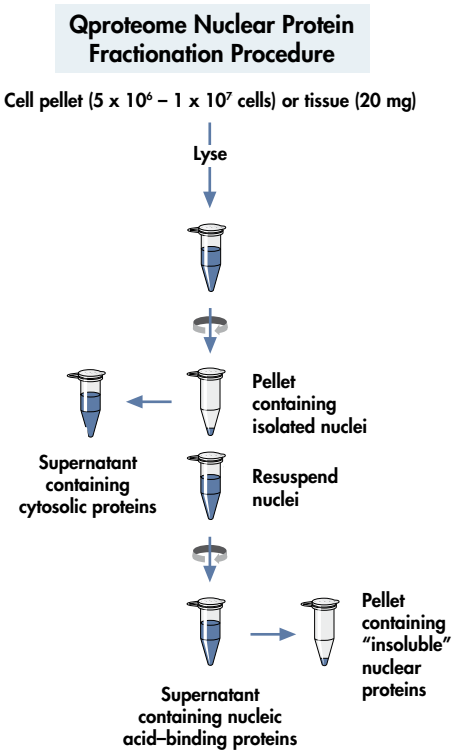


Figure 4. High-purity isolation of intact mitochondria with fully active proteins.
A Mitochondria were prepared from 60 mg of liver tissue using the Qproteome Mitochondria Isolation Kit and subjected to 2D-PAGE. Using MALDI-MS, 259 proteins were identified, 219 (85%) of which were predicted to have a mitochondrial localization. B Electron micrographs of mitochondria isolated using the Qproteome Mitochondria Isolation Kit showing intact inner and outer membranes.





Qproteome Nuclear Protein Kit

The Qproteome Nuclear Protein Kit ensures reliable separation of nuclear and nucleic acid–binding proteins (Figure 5). The dedicated kit is designed for specific enrichment of nuclear proteins from cultured mammalian cells and tissues and ensures:

- Efficient separation of nuclei from cytosolic proteins
- Reduction in sample complexity
- Easier analysis of low-abundance nuclear proteins
- Consistently reliable results

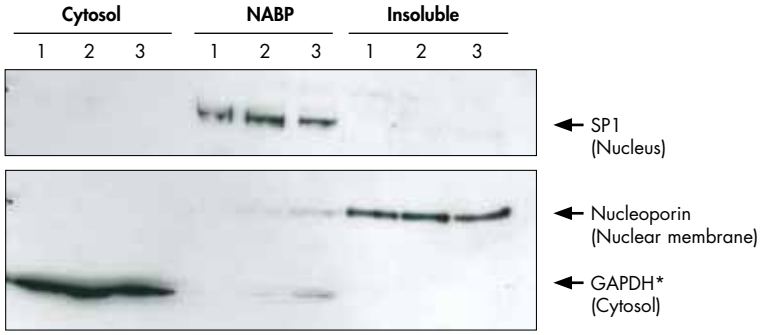


Figure 5. Nuclear preparations free of cytosolic proteins. Three cell lysate preparations were processed in parallel using the Qproteome Nuclear Protein Kit. Fractions were separated by SDS-PAGE. Fraction-specific markers were detected using protein-specific antibodies in a western blotting procedure. **NABP:** Nucleic acid–binding protein fraction.

* Control protein.

Qproteome Plasma Membrane Protein Kit

Traditional methods for preparation of high-purity plasma membrane protein fractions are tedious and time-consuming, and require access to an ultracentrifuge. The Qproteome Plasma Membrane Protein Kit provides plasma membrane fractions of unparalleled purity with a fast, reproducible, and standardized procedure using a bench-top centrifuge (Figure 6). The benefits of the kit include:

- Highly reproducible procedure ensuring consistently reliable results
- Easy-to-use kit format with a convenient and fast procedure
- No need for specialized equipment or extra reagents

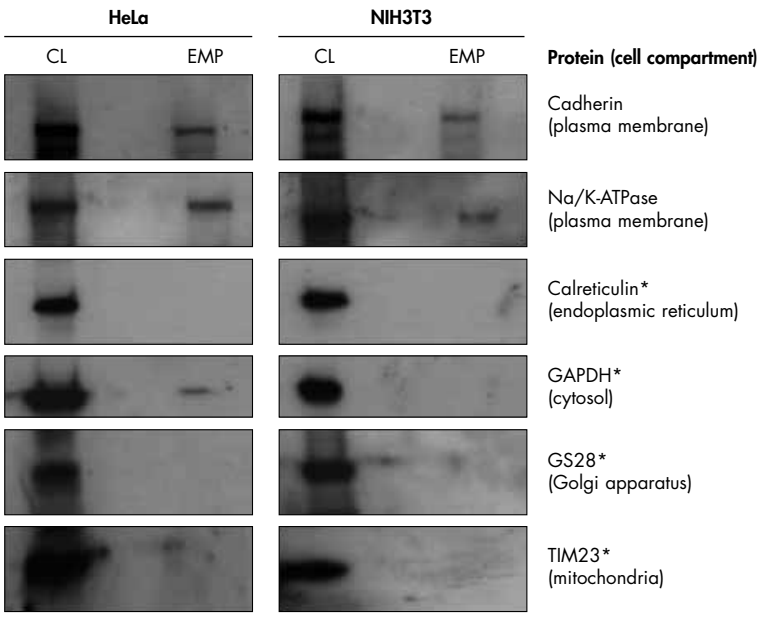
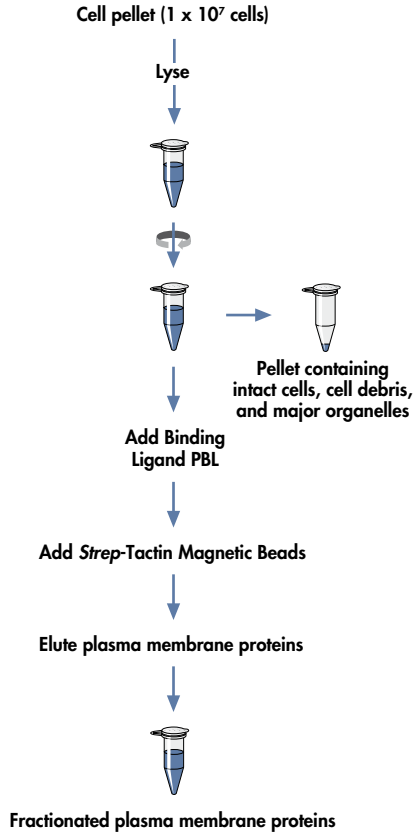
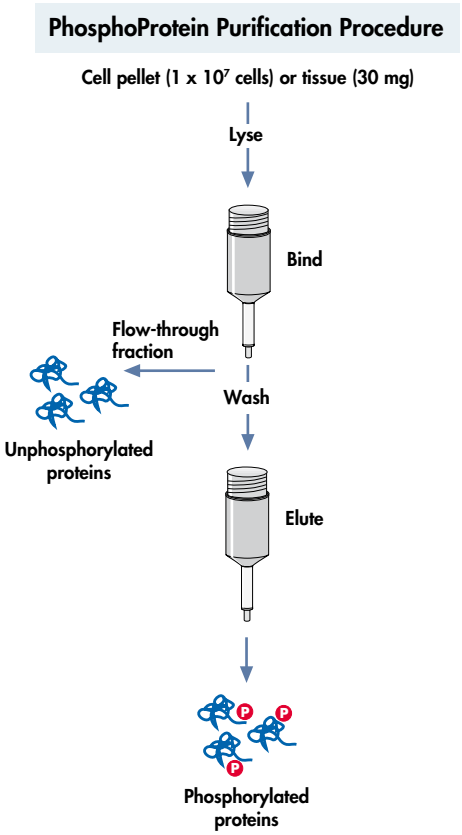


Figure 6. High-purity plasma membrane fractions. Plasma membrane proteins were purified from either HeLa or NIH3T3 cell cultures using the Qproteome Plasma Membrane Protein Kit. Cell lysates (CL) and eluted membrane protein fractions (EMP) were separated by SDS-PAGE and transferred to a nitrocellulose membrane and analyzed 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. After fractionation using the kit, the sample is free of non-plasma membrane proteins as shown (see control proteins).*

Qproteome Plasma Membrane Fractionation Procedure



* Control proteins.



PhosphoProtein Purification Kit

The PhosphoProtein Purification Kit, which is based on affinity chromatography, ensures complete separation of phosphorylated and unphosphorylated proteins from a cell lysate, and facilitates investigation of the phosphorylation status of both entire cells and specific proteins (Figures 7 and 8). The benefits of the kit include:

- Complete separation without the need for tedious optimization
- Kit includes columns, buffers, reagents, and ultrafiltration columns
- Facilitation of cell-signaling studies without the need for radioactivity
- Suitability for automated liquid chromatography systems*

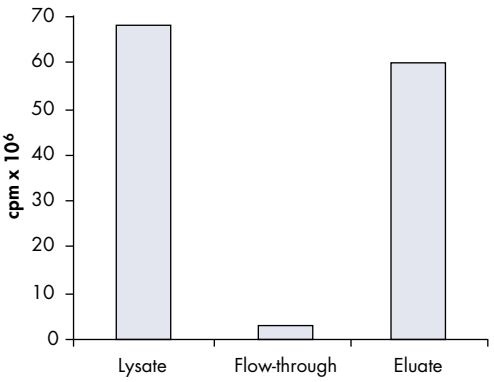


Figure 7. Efficient separation of phosphorylated proteins. Nonstimulated Jurkat cells were radioactively labeled in vivo using ^{32}P. The cell lysate was processed using the PhosphoProtein Purification Kit and the radioactivity in each fraction measured. (Data kindly provided by Gudrun Rehg and Sascha Dammeier, Byk Gulden, Konstanz, Germany.)

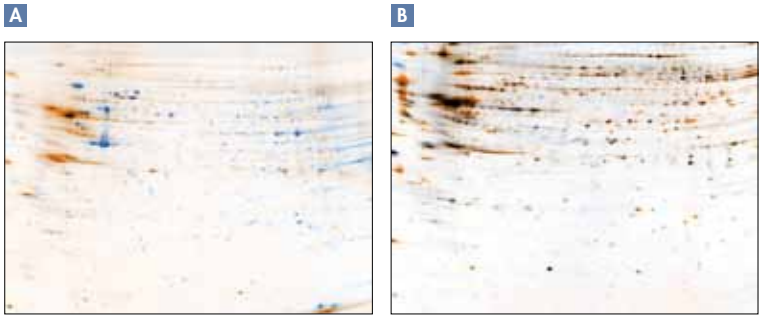


Figure 8. Efficient enrichment for 2D-PAGE phosphoproteome analysis. HeLa cells were treated with 2 phosphatase inhibitors, calyculin (100 nM) and orthovanadate (1 mM), for 20 minutes at 37°C. A After harvest and cell lysis, an aliquot of the lysate was separated by 2D-PAGE and proteins visualized using a nonspecific total protein stain (blue) and a phosphoprotein-specific stain (orange, images overlaid). B A second aliquot was processed using the PhosphoProtein Purification Kit and the eluate (containing phosphoproteins) treated as before. Overlapping protein spots are colored black. Image analysis indicated that 87% of eluted proteins are phosphorylated.

* Cartridges are suitable for ÄKTA™, ÄKTAdesign™, or BioLogic workstation.

Qproteome Glycoprotein Kits

Glycosylated proteins can be easily isolated using Qproteome Glycoprotein Kits and cartridges (Figures 9 and 10). An assortment of lectin cartridges are also available separately and can be ordered individually (Table 1). For greater convenience, kits can also be automated on the QIAcube. The advantages of the kits include:

- Specific separation of glycoproteins according to their glycan moieties
- Profiling of glycoproteins in cells grown under different conditions
- Vast selection of lectin columns and cartridges*
- Precise glycoprotein characterization

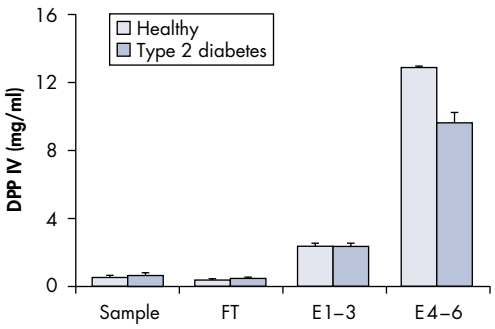
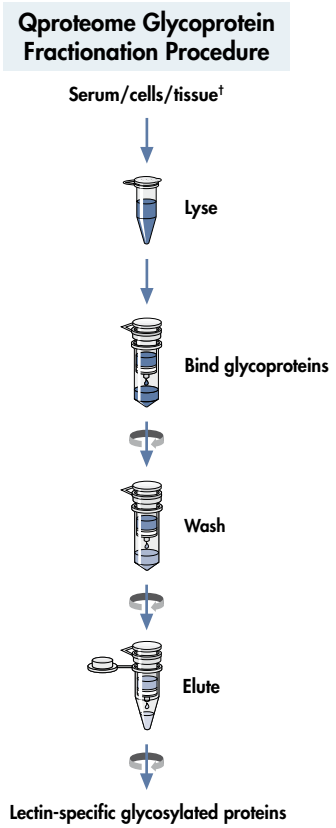


Figure 9. ELISA analysis of DPP IV in glycoprotein elution fractions. ELISA of DPP IV was performed after fractionation of glycoproteins according to their glycan structure. The amount of DPP IV in E4–6 from type 2 diabetes samples is decreased compared to healthy specimens. This result could be due to changes in the degree of sialylation, which might indicate reduced sialylation of the glycan structures on DPP IV. **E**: Elution fractions.



* Cartridges are suitable for ÄKTA, ÄKTAdesign, or BioLogic workstation.

† Quantity varies depending on whether a spin column or a cartridge is used.

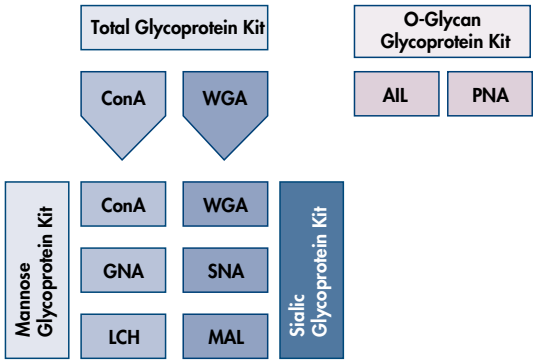


Figure 10. Comprehensive and precise analysis of glycan moieties. Initial analysis of glycoproteins can be carried out using the Qproteome Total Glycoprotein Kit and the Qproteome O-Glycan Glycoprotein Kit. Depending on which lectin column binds a protein of interest, further studies on its precise nature can be performed using either the Qproteome Mannose Glycoprotein Kit or the Qproteome Sialic Glycoprotein Kit.

Table 2. Lectins used in Qproteome Glycoprotein Kits

	Lectin	Name	Affinity
Mannose-binding lectins	Con A	Concanavalin A	Branched α -mannosidic structures; high-mannose type, hybrid type, and biantennary complex type N-glycans
	LCH	Lentil lectin	Fucosylated core region of bi- and tri-antennary complex type N-glycans
	GNA	Snowdrop lectin	α 1-3 and α 1-6 linked high mannose structures
Galactose/ N-acetylgalactosamine-binding lectins	PNA	Peanut agglutinin	Galβ1-3GalNAcα1-Ser/Thr (T-Antigen)
	AIL	Jacalin	(Sia)Galβ1-3GalNAcα1-Ser/Thr (T-Antigen)
Sialic acid/ N-acetylglucosamine-binding lectins	WGA	Wheat germ agglutinin	GlcNAcβ1-4GlcNAcβ1-4GlcNAc, Neu5Ac (sialic acid)
	SNA	Elderberry lectin	Neu5Acα2-6Gal(NAc)-R
	MAL	Maackia amurensis lectin	Neu5Ac/Gcα2-3Galβ1-4GlcNAcβ1-R

Qproteome Depletion products

Proteomic analysis of serum, plasma, and other body fluids such as cerebrospinal fluid (CSF) is complicated by the large differences in relative abundances of their constituent proteins, which can span several orders of magnitude. Removal of abundant proteins greatly facilitates the analysis of potential biomarkers that are generally present in far lower concentrations. Qproteome Depletion products enable efficient depletion of albumin and IgG from human and murine serum, plasma, and CSF samples (Figures 11 and 12). Qproteome Depletion products provide:

- High specificity through monoclonal antibodies
- Easy-to-use spin-column and 96-well plate formats
- Suitability for automated liquid chromatography systems*
- Automated depletion on the QIAcube

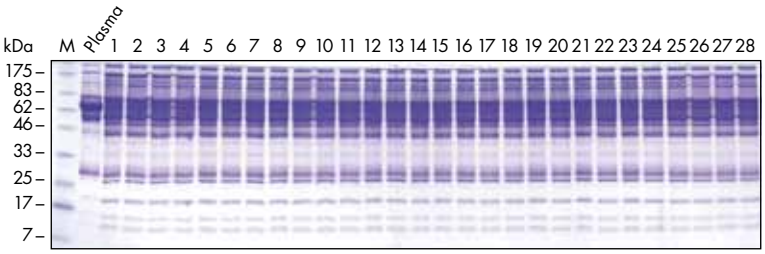


Figure 11. Evaluation of depletion efficiency. The data shows 28 flow-through samples that were analyzed following depletion using SDS-PAGE. Five microliters of human plasma was diluted with 15 µl PBS and applied to the Qproteome depletion plate. The plate was placed on a horizontal shaker for 5 minutes. The flow-through was harvested by centrifugation of the plate at 500 x *g* for 10 seconds. The resin was washed 6 times with 20 µl PBS and centrifuged at 500 x *g* for 10 seconds. The flow-through was then diluted. The mean protein amount was found to be 68 µg in 68 µl, which gives a concentration of 1 mg/ml. With a standard deviation of 3 µg, this leads to a relatively low coefficient of variation of 4.6%. Reproducible and efficient removal of the most abundant protein species albumin and IgG is achieved.

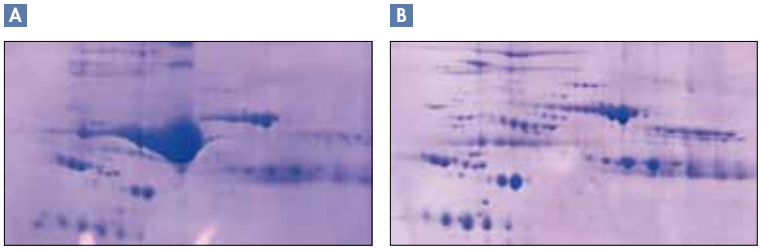
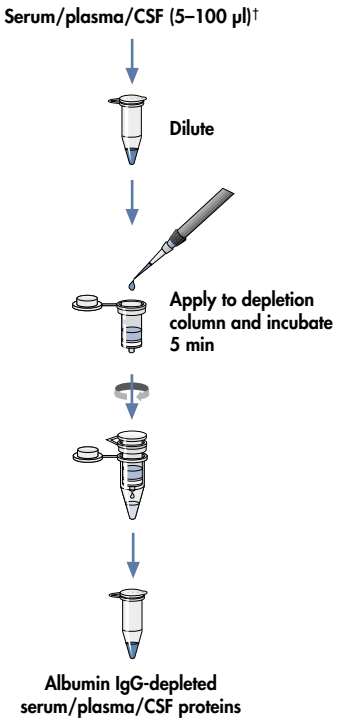


Figure 12. Albumin/IgG depletion facilitates analysis of low-abundance proteins. Coomassie® stained 2D-PAGE gels showing A nondepleted and B depleted plasma samples.

Qproteome Depletion Procedure



* ÄKTA, ÄKTAdesign, or BioLogic workstation.

† Quantity varies depending on product used.

Unravel the complexity of the proteome

QIAGEN's diverse selection of Qproteome Kits enable efficient isolation of targeted subsets of proteins for easier analysis of low-abundance species. Easy-to-use kits and straightforward protocols ensure reliable results every time and eliminate the need for specialized equipment or extra reagents. Automatable kits offer greater flexibility and convenience, while delivering the same high-quality results research demands. Trust QIAGEN's superior technologies and ensure standardization and reproducibility in proteomics research!

For up-to-date licensing information and product-specific disclaimers, 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.

Visit www.qiagen.com/goto/Qproteome for more information!

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