January 2005

Qproteome Glycoprotein Fractionation Handbook

For the fractionation of glycoproteins in proteomic samples

Contents

Kit Contents

Qproteome Total Glycoprotein Kit	
Catalog no.	**37541**
Number of preps	**6**
Glycoprotein Binding Buffer SB	1 x 30 ml
Glycoprotein Elution Buffer ME	2 x 1.5 ml
Glycoprotein Elution Buffer SE	2 x 1.5 ml
Total Glycoprotein Spin Columns (green screw-caps)	6
Collection Tubes, 2 ml	6
Detergent Solution	1 x 2 ml
Protease Inhibitor Solution (100x)	1 x 300 µl

Qproteome Mannose Glycoprotein Kit	
Catalog no.	**37551**
Number of preps	**6**
Glycoprotein Binding Buffer MB	1 x 30 ml
Glycoprotein Elution Buffer ME	2 x 1.5 ml
ConA Spin Columns (green screw-caps)	2
GNA Spin Columns (blue screw-caps)	2
LCH Spin Columns (yellow screw-caps)	2
Collection Tubes, 2 ml	6
Detergent Solution	1 x 2 ml
Protease Inhibitor Solution (100x)	1 x 300 µl

Qproteome Sialic Glycoprotein Kit	
Catalog no.	**37561**
Number of preps	**6**
Glycoprotein Binding Buffer SB	1 x 30 ml
Glycoprotein Elution Buffer SE	1 x 1.5 ml
Glycoprotein Elution Buffer SLE	1 x 1.5 ml
WGA Spin Columns (green screw-caps)	2
SNA Spin Columns (blue screw-caps)	2
MAL Spin Columns (yellow screw-caps)	2
Collection Tubes, 2 ml	6
Detergent Solution	1 x 2 ml
Protease Inhibitor Solution (100x)	1 x 300 µl

Qproteome O-Glycan Glycoprotein Kit	
Catalog no.	**37571**
Number of preps	**6**
Glycoprotein Binding Buffer OB	1 x 30 ml
Glycoprotein Elution Buffer OE	1 x 1.5 ml
Glycoprotein Elution Buffer OGE	1 x 1.5 ml
AIL Spin Columns (green screw-caps)	3
PNA Spin Columns (yellow screw-caps)	3
Collection Tubes, 2 ml	6
Protease Inhibitor Solution (100x)	1 x 300 µl

Storage Conditions

Spin Columns, Glycoprotein Binding Buffers, Protease Inhibitor Solution (100x), and Detergent Solution should be stored at 2–8°C.

Glycoprotein Elution Buffers should be stored at –20°C.

Product Use Limitations

Qproteome Glycoprotein Kits are developed, designed, and sold for research purposes only. They are not to be used for human diagnostic or drug purposes or to be administered to humans unless expressly cleared for that purpose by the Food and Drug Administration in the USA or the appropriate regulatory authorities in the country of use. All due care and attention should be exercised in the handling of many of the materials described in this text.

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At QIAGEN we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of QIAGEN® products. If you have any questions or experience any difficulties regarding Qproteome Glycoprotein Kits or QIAGEN products in general, please do not hesitate to contact us.

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For technical assistance and more information please call one of the QIAGEN Technical Service Departments or local distributors (see inside back cover).

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). These are available online in convenient and compact PDF format at www.qiagen.com/ts/msds.asp where you can find, view, and print the MSDS for each QIAGEN kit and kit component.

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Poison Information Center Mainz, Germany Tel: +49-6131-19240

Introduction

The sheer number of proteins present in an organism's cells is not the only factor contributing to the complexity of its proteome. A second factor is the post-translational modifications of many of these proteins. Generally, such modifications regulate the in vivo activity or localization of a protein.

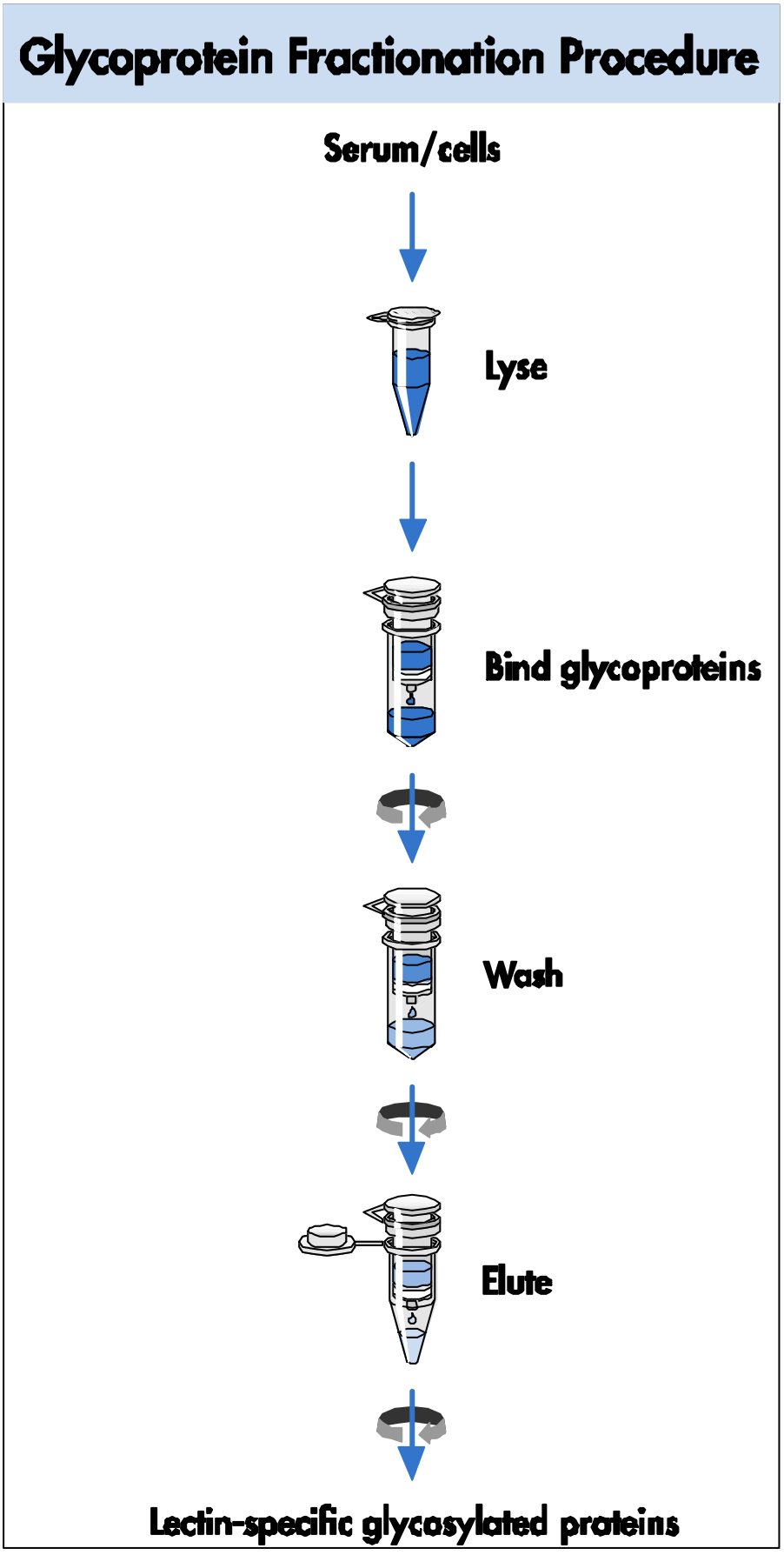
One of the most common post-translational protein modifications is the glycosylation of serine, threonine, and asparagine residues with mono- or oligosaccharides. Glycosylation of proteins plays a vital role in a wide range of cellular processes, such as cell adhesion and signaling, stabilization of protein structure and function, protein trafficking and sorting, and oncogenesis. Several diseases (e.g., rheumatoid arthritis) may be caused by a defect in protein glycosylation.

It has been shown that half of all eukaryotic gene products can be post-translationally glycosylated. Glycoproteins can be divided into two major classes. One class is characterized by the presence of glycans which are attached to the nitrogen atom of an asparagine residue (N-linked). The second class is characterized by the presence of glycans which are attached to the oxygen atom of serine or threonine residue (O-linked).

Glycosylation is catalyzed by glycosyltransferases, which transfer the appropriate sugar from a donor to the protein. The transfer of sugar residues occurs in the endoplasmic reticulum (ER). Following sugar transfer, the newly glycosylated protein is transported from the ER or Golgi apparatus to the plasma membrane, where it remains or is secreted to the extracellular matrix. Most cellular glycoproteins are located at the plasma membrane. Glycoproteins fulfill a wide range of functions in the cell and new functions are constantly being discovered. Therefore, their analysis and characterization is currently of great interest.

Principle and procedure

In proteomics studies, fractionation of samples can help to reduce their complexity and to enrich specific classes of proteins for subsequent downstream analyses, such as 2-D gel electrophoresis or mass spectroscopy. A method for fractionation and enrichment of glycoproteins is lectin affinity chromatography. Lectins are proteins that are able to specifically and reversibly bind carbohydrates. Subjecting cell lysates to lectin affinity chromatography enables enrichment of different classes of glycoproteins and allows initial characterization of a protein's glycan structure or the extent of glycosylation in the cells under investigation. The site of glycosylation in a protein can be identified by mass spectroscopy. Analysis of the glycosylation pattern allows not only the identification and quantification of glycoproteins; it also provides an insight into the molecular function of a glycoprotein.


Glycoprotein Fractionation Procedure
Serum/cells
Lyse
Bind glycoproteins
Wash
Elute
Lectin-specific glycosylated proteins

Qproteome Glycoprotein fractionation kits are designed for fast and easy fractionation of glycoproteins from proteomic samples.

Qproteome Total Glycoprotein Kit

The Total Glycoprotein Spin Columns in the Qproteome Total Glycoprotein Kit contain ConA and WGA lectins. They are used for a general enrichment of the total glycoprotein population from a cell or serum sample.

Qproteome Mannose Glycoprotein Kit

The ConA, GNA, and LCH lectin spin columns in the Qproteome Mannose Glycoprotein Kit are used for specific enrichment of glycoproteins with mannose-rich glycan moieties. The three lectins each bind different subclasses of these moieties.

Qproteome Sialic Glycoprotein Kit

The WGA, SNA, and MAL lectin spin columns in the Qproteome Sialic Glycoprotein Kit are used for specific enrichment of glycoproteins with sialic-acid-rich glycan moieties. The three lectins each bind different subclasses of these moieties.

Qproteome O-Glycan Glycoprotein Kit

The AIL, and PNA lectin spin columns in the Qproteome O-Glycan Glycoprotein Kit are used for specific enrichment of glycoproteins with a glycan structure of the type that are found on T-antigens. The two lectins each bind different subclasses of these glycoproteins.

Schematic representations of the glycan structures to which the individual lectins bind can be found in Appendix A, page 23.

The Total, Mannose, and Sialic Glycoprotein Kits are designed for the fractionation of glycoproteins from serum or cultured-cell samples. The O-Glycan Glycoprotein Kit is designed for the fractionation of glycoproteins from serum. The spin columns in each kit contain different lectin resins which are specific for individual glycoprotein modifications (see Figure 1 and Table 1, page 12 and Appendix, page 23). Proteins that carry a glycan moiety are bound specifically by the corresponding lectin and can be eluted using a buffer containing the appropriate sugar. Due to the dilute concentrations of protein and the presence of high concentrations of eluting sugars, serial processing of eluates using different spin columns is not recommended.

Serum samples must be diluted in binding buffer to ensure an efficient binding of the glycoproteins to the lectin resin. To prevent proteolytic degradation of the proteins in the lysate, a protease inhibitor is added. Since the majority of cellular glycoproteins are located in the plasma membrane as integral membrane proteins, the addition of a detergent is necessary for the solubilization of glycosylated membrane proteins. Cell disruption is easily performed using a needle and syringe. A centrifugation step separates insoluble material to prevent clogging of the spin columns. After equilibration of the spin column the cleared lysate is applied to the column. The spin column is centrifuged and glycosylated proteins bind to lectins in the column matrix. The flow-through fraction can be collected for other applications. The bound glycoproteins are eluted with a buffer containing a sugar that competes for binding sites specific to the appropriate glycan structure.

Starting material for each spin column procedure is 50 µl of serum or 1 x 10^7 cells. Expected yields from different lectin spin column procedures using serum samples are shown in Table 2. The procedure has been used successfully with several different mammalian cell lines including HeLa, Huh7, HT29, HEK293, and Jurkat. The expected yield from one spin column fractionation procedure is 30–150 µg, depending on the cell line and lectin spin column used. For some downstream applications concentration of the elution fractions might be necessary. A protocol for concentration using acetone precipitation can be found on page 21.

Description of protocols

This handbook contains two protocols, one for serum samples (page 18) and one for cultured-cell samples (page 20). The buffers used in the cultured-cell protocol must be supplemented with a detergent solution before use.
The Total Glycoprotein Kit contains Total Glycoprotein Spin Columns, which bind most glycosylated proteins. Proteins are eluted over 6 fractions by sequential elution using two different buffers.

Glycoprotein Fractionation using Lectin Spin Columns

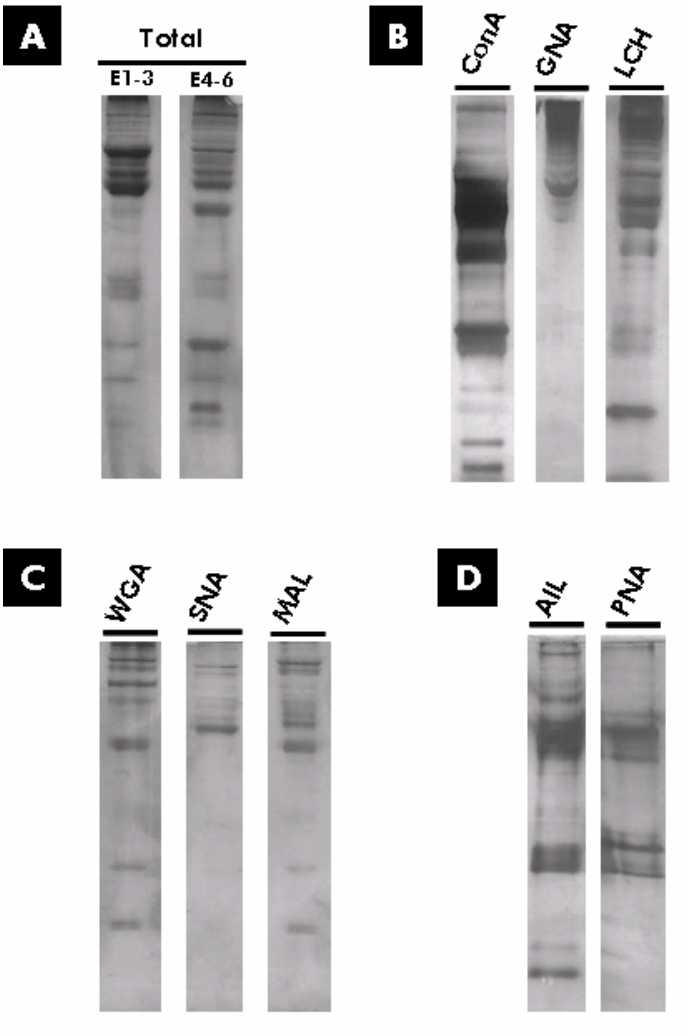


Figure 1 Glycoproteins were fractionated from serum using the different lectin spin columns in glycoprotein fractionation kits and analyzed by SDS-PAGE followed by silver staining. **A** Elution steps 1–3 and 4–6 from Total Lectin Spin Columns in the Total Glycoprotein Kit. **B** Eluted glycoproteins 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.

Table 1. Overview of Glycan-Binding Specificities of Lectins used in Glycoprotein Fractionation Kits*

Lectin specificity	Kit	Lectin	Organism	Glycan/Carbohydrate structure
Mannose binding lectins	Mannose Glycoprotein Kit	ConA (concanavalin A)	*Canavalia ensiformis*	Branched α-mannosidic structures High-mannose type, hybrid type, and biantennary complex type N-glycans
		LCH (lentil lectin)	*Lens culinaris*	Fucosylated core region of bi- and triantennary complex type N-glycans
		GNA (snowdrop lectin)	*Galanthus nivalis*	α1-3 and α1-6 linked high mannose structures
Sialic acid/ N-acetyl-glucosamine binding lectins	Sialic Glycoprotein Kit	WGA (Wheat germ agglutinin)	*Triticum vulgaris*	GlcNAcβ1-4GlcNAcβ1-4GlcNAc, Neu5Ac (sialic acid)
		SNA (Elderberry lectin)	*Sambucus nigra*	Neu5Acα2-6Gal(NAc)-R
		MAL (Maackia amurensis lectin)	*Maackia amurensis*	Neu5Ac/Gcα2-3Galβ1-4GlcNAcβ1-R
Galactose/ N-acetyl-galactosamine binding lectins	O-Glycan Glycoprotein Kit	AIL (Jacalin)	*Artocarpus integrifolia*	(Sia)Galβ1-3GalNAcα1-Ser/Thr (e.g., T-Antigen)
		PNA (Peanut agglutinin)	*Arachis hypogaea*	Galβ1-3GalNAcα1-Ser/Thr (e.g., T-Antigen)

* The Appendix on page 23 shows glycan structures to which lectins in Qproteome Glycoprotein Kits bind.

Table 2. Expected Yields from Lectin Spin Columns Using 50 µl Serum Sample.

Spin column	Protein in pooled eluate (µg)
Total Glycoprotein Kit	
Total Spin Column (Elution steps 1–3)	150
Total Spin Column (Elution steps 4–6)	60
Mannose Glycoprotein Kit	
ConA Spin Column	60
GNA Spin Column	20
LCH Spin Column	30
Sialic Glycoprotein Kit	
WGA Spin Column	80
SNA Spin Column	30
MAL Spin Column	40
O-Glycan Glycoprotein Kit	
AIL Spin Column	80
PNA Spin Column	20

Glycoprotein Fractionation Spin Protocols

Important notes before starting

- All steps are performed at room temperature (15–25°C). Use pre-cooled buffers and spin columns. Separated protein fractions should be stored at 4°C, or for longer term storage, at –80°C.
- For downstream applications such as SDS-PAGE or 2-D gel analysis the elution fractions should be pooled and concentrated, for example using acetone (see page 21).
- Starting material for one fractionation procedure using the Glycoprotein Fractionation Kit Serum Protocol is 50 µl serum. Starting material for one fractionation procedure using the Glycoprotein Fractionation Kit Cell Protocol is 1 x 10^7 cells.
- For quantification of glycoprotein yield from the Glycoprotein Fractionation Kit Serum Protocol use the Bradford method (e.g., Bio-Rad Protein Assay Kit, cat. no. 500-0001). For quantification of glycoprotein yield from the Glycoprotein Fractionation Kit Cell Protocol use the Lowry (e.g., Bio-Rad DC protein Assay Kit, cat. no. 500-0111) or BCA method (e.g., Pierce Micro BCA Protein Assay Kit, cat. no. 23235).
- Before use, add Protease Inhibitor Solution (100x) and Detergent Solution to the Binding and Elution Buffers according to the tables below.
- All centrifuge steps are carried out using a bench-top microcentrifuge (e.g., Eppendorf® Micro Centrifuge 5417C or Heraeus Biofuge® 15).*
- Ensure that the correct Elution Buffer is used (see Table 4).
- When processing whole cells, do not use phosphate buffer to wash the cells as this will interfere with binding of glycosylated proteins to lectin resins. Use a HEPES-based buffer (e.g., 10 mM HEPES; 150 mM NaCl, pH 7.4) or TBS to wash cells.
- Certain chemicals can adversely affect binding of glycoproteins to lectin columns and therefore their use in buffers should be avoided. These chemicals include reducing agents (e.g., DTT, β-mercaptoethanol), chelating reagents (e.g., EDTA, EGTA), detergents (other than those provided in the kit), denaturants (e.g., urea, GuHCl), and proteases.

* This is not a complete list of suppliers and does not include many important vendors of biological supplies.

Table 3. Preparing Binding and Elution Buffers for Glycoprotein Purification Procedures

Serum protocol

Buffer	Buffer required per column used	Protease Inhibitor Solution (100x) to be added
Binding Buffer	2.5 ml	25 µl
Each Elution Buffer	300 µl	3 µl

Cell protocol

Buffer	Buffer required per column used	Protease Inhibitor Solution (100x) to be added	Detergent Solution to be added
Binding Buffer	3 ml	30 µl	300 µl
Each Elution Buffer	300 µl	3 µl	30 µl

Table 4. Spin Columns and Relevant Elution Buffers

Spin Column	Elution Buffer
Total Glycoprotein Kit	
Total Spin Column (green screw-caps) (Elution steps 1–3)	Glycoprotein Elution Buffer ME
Total Spin Column (Elution steps 4–6)	Glycoprotein Elution Buffer SE
Mannose Glycoprotein Kit	
ConA Spin column (green screw-caps)	Glycoprotein Elution Buffer ME
LCH Spin column (yellow screw-caps)	Glycoprotein Elution Buffer ME
GNA Spin column (blue screw-caps)	Glycoprotein Elution Buffer ME
Sialic Glycoprotein Kit	
WGA Spin column (green screw-caps)	Glycoprotein Elution Buffer SE
SNA Spin column (blue screw-caps)	Glycoprotein Elution Buffer SLE
MAL Spin column (yellow screw-caps)	Glycoprotein Elution Buffer SLE
O-Glycan Glycoprotein Kit	
AIL Spin column (green screw-caps)	Glycoprotein Elution Buffer OE
PNA Spin column (yellow screw-caps)	Glycoprotein Elution Buffer OGE

Protocol: Glycoprotein Fractionation Serum Protocol

This protocol can be used to fractionate glycoproteins from serum using the Total, Mannose, Sialic, and O-Glycan Glycoprotein Kits.

Procedure

1. **For each spin column you plan to use, supplement a 2.5 ml aliquot of Binding Buffer with 20 µl of Protease Inhibitor Solution (100x) as described in Table 3 on page 16.**
2. **Add 500 µl of the Binding Buffer prepared in step 1 to 50 µl of serum. Mix by vortexing gently.**
3. **Prepare spin columns by loosening the screw cap of the column a quarter turn, snap off the bottom closure, and place the spin column in a 2 ml collection tube (provided).**
4. **Centrifuge the spin column for 2 min at 500 rpm in a microcentrifuge.**
5. **Discard the flow-through and pipet 500 µl Binding Buffer supplemented with Protease Inhibitor Solution (100x) onto the spin column. Centrifuge for 2 min at 500 rpm in a microcentrifuge.**
6. **Discard the flow-through and apply the sample from step 2 to the spin column.**
7. **Incubate for 1 min and centrifuge for 2 min at 500 rpm.**
   Collect the flow-through if analysis of other serum proteins is desired.
8. **Apply 750 µl of Binding Buffer supplemented with Protease Inhibitor Solution (100x) to wash the spin column, centrifuge for 2 min at 500 rpm and discard the flow-through. Repeat this step.**
9. **During centrifugation add 3 µl of Protease Inhibitor to a 300 µl aliquot of Elution Buffer as described in Table 3 on page 15.**
   Refer to Table 4 on page 16 to ensure that you use the relevant elution buffer. When using the Total Glycoprotein Kit, prepare 300 µl aliquots of Elution Buffer ME and Elution Buffer SE.
10. **Transfer the spin column to a clean microcentrifuge tube.**

11. **Apply 100 µl of the Elution Buffer prepared in step 9 to the spin column, incubate for 1 min and centrifuge for 2 min at 500 rpm.**

    When using the Total Glycoprotein Kit, use Glycoprotein Elution Buffer ME for this elution step.

12. **Repeat step 11 two times with two further 100 µl aliquots of Elution Buffer. Pool the eluate fractions and determine protein concentration (e.g., using the Bradford method).**

    When using the Total Glycoprotein Kit, use Glycoprotein Elution Buffer ME for these elution steps.

13. **When using the Total Glycoprotein Kit, carry out 3 further elution steps using 100 µl aliquots of Glycoprotein Elution Buffer SE. Pool the eluate fractions and determine protein concentration (e.g., using the Bradford method).**

Protocol: Glycoprotein Fractionation Kit Cell Protocol

This protocol can be used to fractionate glycoproteins from cells using the Total, Mannose, and Sialic Glycoprotein Kits. This protocol is **NOT** suitable for use with the O-Glycan Glycoprotein Kit.

Equipment and reagents to be supplied by the user

- Buffer for washing cells (see Appendix B, page 25)
- Blunt-ended needle and syringe for cell disruption and homogenization

Procedure

1. **Collect cells by using a cell scraper. Centrifuge for 5 min at 450 x *g* and wash the cell pellet with HEPES-based buffer or TBS (see Appendix B, page 25). Repeat this step once. Store cell pellet on ice.**
   Do not use phosphate buffer to wash the cells as this will interfere with binding of glycosylated proteins to lectin resins.
2. **During centrifugation, for each spin column you plan to use, supplement a 3 ml aliquot of Binding Buffer with 30 µl of Protease Inhibitor Solution (100x) and 300 µl Detergent Solution as described in Table 3 on page 16.**
3. **By gently pipetting, resuspend a cell pellet corresponding to 1 x 10^7 cells in 1 ml of Binding Buffer containing the Protease Inhibitor and Detergent Solution prepared in step 2.**
4. **Incubate for 15 min at 4°C. Mix briefly every 5 min.**
5. **Complete cell disruption by using a blunt-ended needle and a syringe (not provided). Draw the lysate slowly into the syringe and eject with one stroke. Repeat this 10 times.**
6. **Centrifuge the cell lysate at 10,000 x *g* and 4°C for 20 min.**
7. **During centrifugation of the cell lysate, prepare spin columns by loosening the screw cap of the column a quarter turn, snap off the bottom closure, and place the spin column in a 2 ml collection tube (provided).**
8. **Centrifuge the spin column for 2 min at 500 rpm.**
9. **Discard the flow-through and pipet 500 µl Binding Buffer onto the spin column. Centrifuge for 2 min at 500 rpm in a microcentrifuge.**
10. **Discard the flow through and apply 500 µl of the sample from step 6 to the spin column.**
11. **Incubate for 1 min and centrifuge for 2 min at 500 rpm.**
    Collect the flow-through fraction if analysis of other proteins is desired.

12. **Repeat steps 10 and 11 with the second 500 µl sample aliquot from step 6.**
13. **Apply 750 µl of Binding Buffer containing Protease Inhibitor and Detergent solution to wash the spin column, centrifuge for 2 min at 500 rpm and discard the flow-through. Repeat this step.**
14. **During centrifugation add 3 µl of Protease Inhibitor and 30 µl Detergent Solution to a 300 µl aliquot of Elution Buffer.**

    Refer to Table 4 on page 16 to ensure that you use the relevant elution buffer. When using the Total Glycoprotein Kit, prepare 300 µl aliquots of Elution Buffer ME and Elution Buffer SE.
15. **Transfer the spin column to a clean microcentrifuge tube.**
16. **Apply 100 µl of the Elution Buffer prepared in step 14 to the spin column, incubate for 1 min and centrifuge for 2 min at 500 rpm.**

    When using the Total Glycoprotein Kit, use Glycoprotein Elution Buffer ME for this elution step.
17. **Repeat step 16 two times with two further 100 µl aliquots of Elution Buffer. Pool the eluate fractions and determine protein concentration using the Lowry method.**

    When using the Total Glycoprotein Kit, use Glycoprotein Elution Buffer ME for these elution steps.
18. **When using the Total Glycoprotein Kit, carry out 3 further elution steps using 100 µl aliquots of Glycoprotein Elution Buffer SE. Pool the eluate fractions and determine protein concentration using the Lowry method.**

Protocol: Acetone Precipitation of Protein Fractions

This protocol is suitable for concentrating and desalting protein samples for downstream applications such as SDS-PAGE.

1. **Add four volumes of ice-cold acetone to the protein fraction and incubate for 15 min on ice.**
2. **Centrifuge for 10 min at 12,000 x *g* in a pre-cooled microcentrifuge at 4°C. Discard the supernatant and air dry the pellet.**

   Do not overdry the pellet as this may make it difficult to resuspend.
3. **Depending on the application, resuspend the pellet in the required sample buffer.**

Troubleshooting Guide

	Comments and Suggestions
Protein assays give inaccurate or inconsistent results	The binding and elution buffers contain components that might interfere with protein quantification assays. Protein fractions must be precipitated (e.g., using acetone, see page 21) and dissolved in a reagent suitable for your protein assay of choice.
When storing the Elution Buffers at –20°C, a precipitate may occur after thawing	This does normally not affect the result of your experiment. In case of a precipitation of buffer components from the Elution buffer, gently warm the buffer at 37°C, mix well and cool on ice before use.
Cell culture-medium components interfere with binding	Wash cells before harvesting in HEPES-based buffer or TBS to prevent possible interference with column binding. Do not use phosphate buffer for washing the cells as this will interfere with binding of glycosylated proteins to the columns.
Eluate fractions too dilute to use in 2-D gel	For 2-D gel analysis, protein fractions must be concentrated and desalted. Use the protocol on page 21.

Appendix A: Glycan Structures Bound by Lectins in Qproteome Glycoprotein Kits

The diagrams below show schematic representations of the glycans to which individual lectins in Qproteome Glycoprotein Kits bind. Lectins can bind multiple complex oligosaccharides/glycans and therefore the depicted structures should be regarded as typical examples and not a comprehensive list. Sugar structures surrounded by a broken line may be present in the glycan structure but are not required for binding.

Key:

NeuNAc — *N*-Acetylneuraminic acid (Sialic Acid)

Gal — Galactose

GlcNAc — *N*-Acetylglucosamine

Man — Mannose

Fuc — Fucose

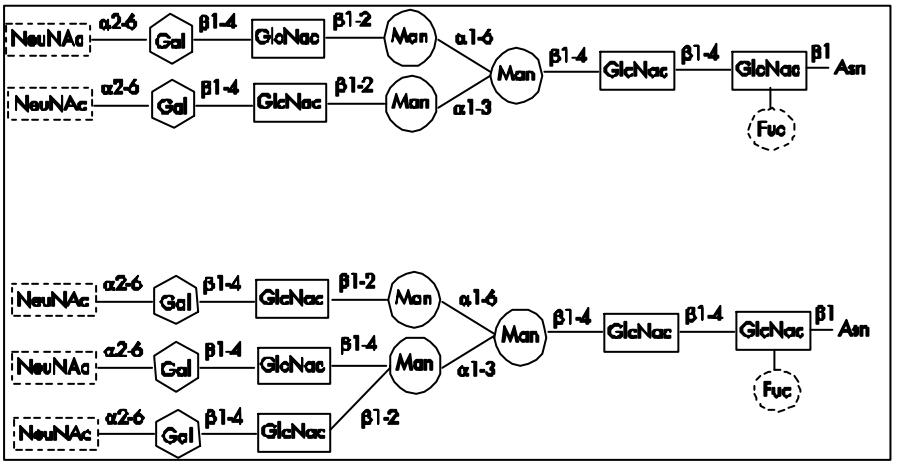
GalNAc — *N*-Acetylgalactosamine

Asn — Asparagine

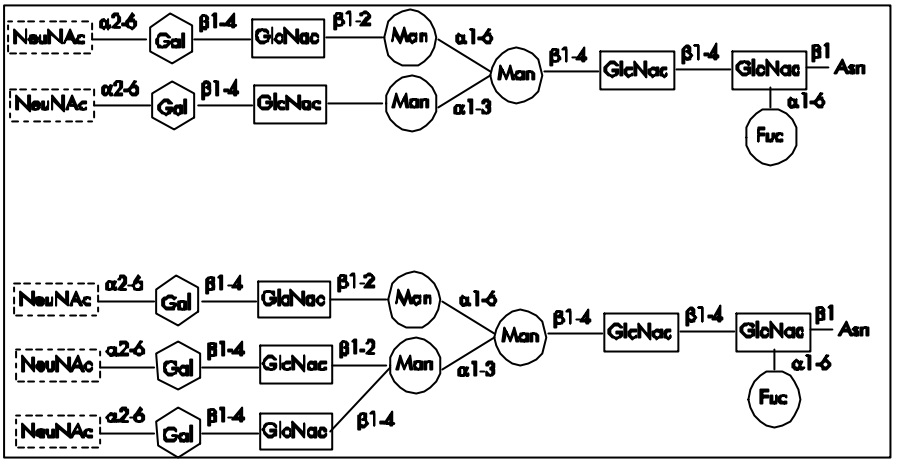
Ser/Thr — Serine/Threonine

Qproteome Mannose Glycoprotein Kit

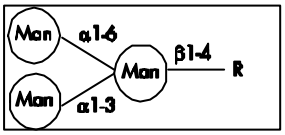
ConA — binds biantennary and triantennary complex type N-glycans



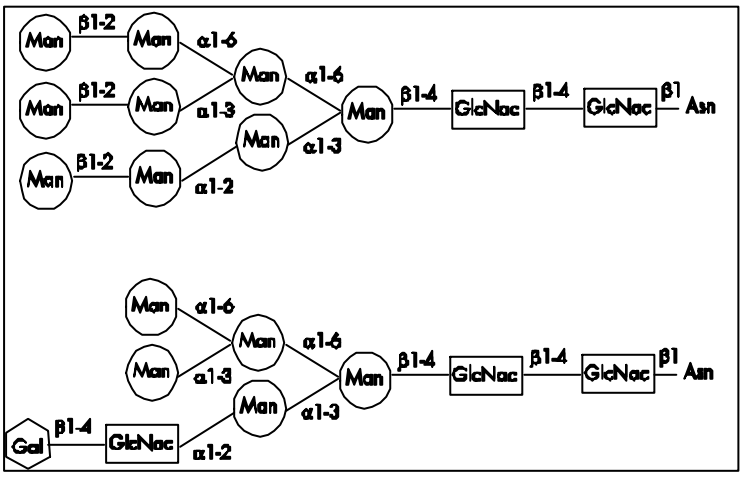
LCH — binds biantennary and triantennary complex type N-glycans with core fucose



GNA — binds α1–3 and α1–6 linked high mannose structures

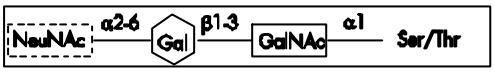


ConA/GNA — binds high mannose type N-glycan/α1–3 and α1–6 linked high mannose structures



Qproteome O-Glycan Glycoprotein Kit

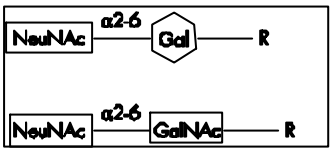
AIL



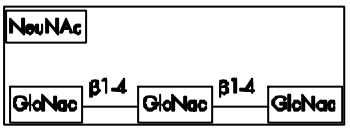
PNA

Qproteome Sialic Glycoprotein Kit

SNA



WGA



MAL

Appendix B: Buffers Used to Wash Cells

HEPES buffer (1 liter):

10 mM HEPES	2.38 g HEPES (MW 238.31 g/mol)
150 mM NaCl	8.77 g NaCl (MW 58.44 g/mol)

Dissolve HEPES and NaCl in 900 ml distilled water and adjust pH to 7.4 using NaOH. Adjust volume to 1 liter.

1xTBS buffer (1 liter):

10 mM Tris·Cl	1.21 g Tris base (MW 121.14 g/mol)
150 mM NaCl	8.77 g NaCl (MW 58.44 g/mol)

Dissolve Tris base and NaCl in 900 ml distilled water and adjust pH to 7.4 using HCl. Adjust volume to 1 liter.

Ordering Information

Product	Contents	Cat. no.
Qproteome Total Glycoprotein Kit	For 6 total glycoprotein preps: Buffers, Lectin Spin Columns (6), Detergent Solution, Protease Inhibitor Solution, Collection Tubes (6 x 2 ml)	37541
Qproteome Mannose Glycoprotein Kit	For 6 mannose glycoprotein preps: ConA, GNA, and LCH Lectin Spin Columns (2 each); Buffers; Detergent Solution; Protease Inhibitor Solution; Collection Tubes (6 x 2 ml)	37551
Qproteome Sialic Glycoprotein Kit	For 6 sialic acid glycoprotein preps: WGA, SNA, and MAL Lectin Spin Columns (2 each); Buffers; Detergent Solution; Protease Inhibitor Solution; Collection Tubes (6 x 2 ml)	37561
Qproteome O-Glycan Glycoprotein Kit	For 6 O-glycan glycoprotein preps: AIL and PNA Lectin Spin Columns (3 each); Buffers; Protease Inhibitor Solution; Collection Tubes (6 x 2 ml)	37571
Related products		
Qproteome Albumin/IgG Depletion Kit	For albumin/IgG depletion of 6 serum or plasma samples: Albumin/IgG Depletion Spin Columns (6)	37521
Qproteome Nuclear Subfractionation Kit	For 6 nuclear protein preparations: Buffers, Reagents, Nuclear protein Fractionation Columns (6), Nuclear Protein Fractionation Resin, Protease Inhibitor Solution, Benzonase®	37531
Qproteome Soluble Protein Separation Kit	For 10 soluble protein fractionations: Fractionation Buffer, Precipitation Reagents, Protease Inhibitor Solution, Benzonase	37512
Qproteome Cell Compartment Kit	For 10 subcellular fractionations: Extraction buffers, Protease Inhibitor Solution, Benzonase	37502

QIAGEN Companies

Please see the back cover for contact information for your local QIAGEN office.

QIAGEN Distributors

Argentina
Tecnolab S.A.
Tel: (011) 4555 0010
Fax: (011) 4553 3331
E-mail: info@tecnolab.com.ar
Web site: www.tecnolab.com.ar

Brazil
Uniscience do Brasil
Tel: 011 3622 2320
Fax: 011 3622 2323
E-mail: info@uniscience.com
Web site: www.uniscience.com

Chile
Biosonda SA
Tel: 562 209 6770
Fax: 562 274 5462
E-mail: ventas@biosonda.cl
Web site: www.biosonda.cl

China
Gene Company Limited
Tel: (852)2896-6283
Fax: (852)2515-9371
E-mail:
Hong Kong:
info@genehk.com
Beijing:
info_bj@genecompany.com
Shanghai:
info_sh@genecompany.com
Chengdu:
gene@public.cd.sc.cn
Guangzhou:
info_gz@genecompany.com

Croatia
INEL Medicinska Tehnika d.o.o.
Tel: (01) 2984-898
Fax: (01) 6520-966
E-mail:
inel-medicinska-tehnika@zg.htnet.hr

Cyprus
Scientronics Ltd
Tel: 02-357 22 765416
Fax: 02-357 22 764614
E-mail:
a.sarpetsas@biotronics.com.cy

Czech Republic
BIO-CONSULT spol. s.r.o.
Tel/Fax: (420) 2 417 29 792
E-mail: info@bioconsult.cz
Web site: www.bioconsult.cz

Egypt
Clinilab
Tel: 52 57 212
Fax: 52 57 210
E-mail: Clinilab@link.net

Estonia
PEAI-Est OÜ
Tel: (051) 65 830
Fax: (07) 383 360
E-mail: langel@ut.ee

Greece
BioAnalytica S.A.
Tel: (210)-640 03 18
Fax: (210)-646 27 48
E-mail: bioanalyt@hol.gr
Web site: www.bioanalytica.gr

Hungary
Kasztel-Med Co. Ltd.
Tel: (01) 385 3887
Fax: (01) 381 0695
E-mail: info@kasztel.hu
Web site: www.kasztel.hu

India
Genetix
Tel: (011)-2542 1714
or (011)-2515 9346
Fax: (011)-2546 7637
E-mail: genetix@nda.vsnl.net.in

Israel
Westburg (Israel) Ltd.
Tel: 08-6900655
or 1-800 20 22 20 (toll free)
Fax: 08-6900650
E-mail: info@westburg.co.il
Web site: www.westburg.co.il

Korea
LRS Laboratories, Inc.
Tel: (02) 924-86 97
Fax: (02) 924-86 96
E-mail: webmaster@lrslab.co.kr
Web site: www.lrslab.co.kr

Malaysia
RESEARCH BIOLABS SDN. BHD.
Tel: (603)-8070 3101
Fax: (603)-8070 5101
E-mail: biolabs@tm.net.my
Web site: www.researchbiolabs.com

Mexico
Quimica Valaner S.A. de C.V.
Tel: (55) 55 25 57 25
Fax: (55) 55 25 56 25
E-mail: ventas@valaner.com
Web site: www.valaner.com

Poland
Syngen Biotech Sp.z.o.o.
Tel: (071) 798 58 50 - 52
Fax: (071) 798 58 53
E-mail: info@syngen.pl
Web site: www.syngen.pl

Portugal
IZASA PORTUGAL, LDA
Tel: (21) 424 7312
Fax: (21) 417 2674
E-mail: consultasbiotec@izasa.es

Saudi Arabia
Abdulla Fouad Co. Ltd.
Tel: (03) 8324400
Fax: (03) 8346174
E-mail:
sadiq.omar@abdulla-fouad.com

Singapore
Research Biolabs Pte Ltd
Tel: 6777 5366
Fax: 6778 5177
E-mail: sales@researchbiolabs.com
Web site: www.researchbiolabs.com

Slovak Republic
BIO-CONSULT Slovakia spol. s.r.o.
Tel/Fax: (02) 5022 1336
E-mail: bio-cons@cdicon.sk
Web site: www.bioconsult.cz

Slovenia
MEDILINE d.o.o.
Tel: (01) 830-80-40
Fax: (01) 830-80-70
or (01) 830-80-63
E-mail: mediline@siol.net

South Africa
Southern Cross Biotechnology
(Pty) Ltd
Tel: (021) 671 5166
Fax: (021) 671 7734
E-mail: info@scb.co.za

Spain
IZASA, S.A.
Tel: (93) 902.20.30.90
Fax: (93) 902.22.33.66
E-mail: consultasbiotec@izasa.es

Taiwan
TAIGEN Bioscience Corporation
Tel: (02) 2880 2913
Fax: (02) 2880 2916
E-mail: taigen@ms10.hinet.net

Thailand
Theera Trading Co. Ltd.
Tel: (02) 412-5672
Fax: (02) 412-3244
E-mail: theetrad@samart.co.th

Turkey
Medek Medikal Ürünler
ve Saglik Hizmetleri A. S.
Tel: (216) 302 15 80
Fax: (216) 302 15 88
E-mail: makialp@med-ek.com

All other countries
QIAGEN GmbH, Germany

Australia ■ **QIAGEN Pty Ltd** ■ PO Box 641 ■ DONCASTER VIC 3108
Orders 03-9840-9800 ■ Fax 03-9840-9888 ■ Technical 1-800-243-066

Belgium ■ **QIAGEN Benelux B.V.** ■ Spoorstraat 50 ■ 5911 KJ Venlo ■ The Netherlands
Orders 0800-79612 ■ Fax 0800-79611 ■ Technical 0800-79556

Canada ■ **QIAGEN Inc.** ■ 2800 Argentia Road ■ Unit 7 ■ Mississauga ■ Ontario ■ L5N 8L2
Orders 800-572-9613 ■ Fax 800-713-5951 ■ Technical 800-DNA-PREP (800-362-7737)

France ■ **QIAGEN S.A.** ■ 3 avenue du Canada ■ LP 809 ■ 91974 COURTABOEUF CEDEX
Orders 01-60-920-920 ■ Fax 01-60-920-925 ■ Technical 01-60-920-930

Germany ■ **QIAGEN GmbH** ■ QIAGEN Strasse 1 ■ 40724 Hilden
Orders 02103-29-12000 ■ Fax 02103-29-22000 ■ Technical 02103-29-12400

Italy ■ **QIAGEN S.p.A.** ■ Via Grosio, 10/10 ■ 20151 Milano
Orders 02-33430411 ■ Fax 02-33430426 ■ Technical 02-33430414

Japan ■ **QIAGEN K.K.** ■ Forefront Tower II ■ 13-1, Kachidoki 3 Chome ■ Chuo-ku, Tokyo 104-0054
Telephone 03-5547-0811 ■ Fax 03-5547-0818 ■ Technical 03-5547-0811

Luxembourg ■ **QIAGEN Benelux B.V.** ■ Spoorstraat 50 ■ 5911 KJ Venlo ■ The Netherlands
Orders 8002-2076 ■ Fax 8002-2073 ■ Technical 8002-2067

The Netherlands ■ **QIAGEN Benelux B.V.** ■ Spoorstraat 50 ■ 5911 KJ Venlo
Orders 0800-0229592 ■ Fax 0800-0229593 ■ Technical 0800-0229602

Switzerland ■ **QIAGEN AG** ■ Garstligweg 8 ■ 8634 Hombrechtikon
Orders 055-254-22-11 ■ Fax 055-254-22-13 ■ Technical 055-254-22-12

UK and Ireland ■ **QIAGEN Ltd.** ■ QIAGEN House ■ Fleming Way ■ Crawley ■ West Sussex, RH10 9NQ
Orders 01293-422-911 ■ Fax 01293-422-922 ■ Technical 01293-422-999

USA ■ **QIAGEN Inc.** ■ 27220 Turnberry Lane ■ Valencia ■ CA 91355
Orders 800-426-8157 ■ Fax 800-718-2056 ■ Technical 800-DNA-PREP (800-362-7737)

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