Second Edition July 2011

Qproteome® Glycoprotein Fractionation Handbook

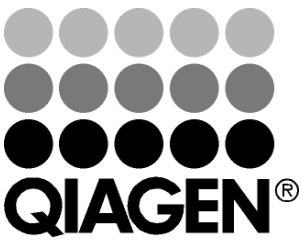
Qproteome Total Glycoprotein Kit

Qproteome Mannose Glycoprotein Kit

Qproteome Sialic Glycoprotein Kit

Qproteome O-Glycan Glycoprotein Kit

For the fractionation of glycoproteins in proteomic samples



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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 1.9 ml
Glycoprotein Elution Buffer SE	2 x 1.9 ml
Total Glycoprotein Spin Columns (green screw-caps)	6
Collection Tubes, 2 ml	6
Detergent Solution	1 x 2 ml
Protease Inhibitor Solution (100x)	2 x 300 µl
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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
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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
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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 1.9 ml
Glycoprotein Elution Buffer OGE	1 x 1.9 ml
AIL Spin Columns (green screw-caps)	3
PNA Spin Columns (yellow screw-caps)	3
Collection Tubes, 2 ml	6
Protease Inhibitor Solution (100x)	2 x 300 µl
Handbook	1

Storage

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.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of Qproteome Glycoprotein Kits is tested against predetermined specifications to ensure consistent product quality.

Product Use Limitations

Qproteome Glycoprotein Kits are intended for molecular biology applications. These products are not intended for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN® products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

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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 sample and assay technologies 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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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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Emergency medical information in English, French, and German can be obtained 24 hours a day from:

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.

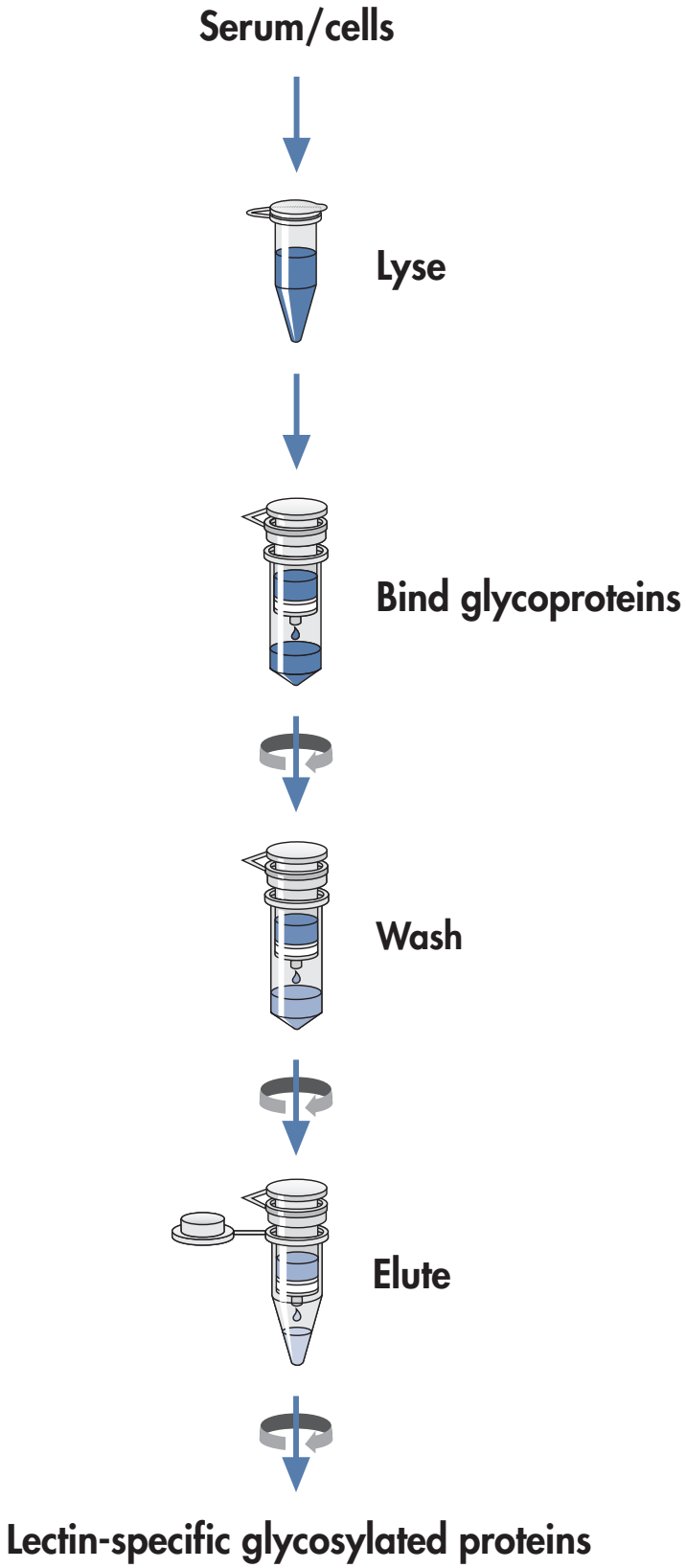
One of the most common post-translational protein modifications is the glycosylation of serine, threonine, and asparagine residues with mono- or oligosaccharides. Glycosylation of proteins plays a vital role in a wide range of cellular processes, such as cell adhesion and signaling, stabilization of 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.

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



Principle

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 27.

Procedure

The Total, Mannose, and Sialic Glycoprotein Kits are designed for the fractionation of glycoproteins from serum, cultured-cell, or tissue 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 14 and Appendix A, page 27). 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, 1 x 10^7 cells, or 10 mg easy-to-lyse tissue. Expected yields from different lectin spin column procedures using serum samples are shown in Table 3. 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 25.

Description of protocols

This handbook contains three protocols, one for serum samples (page 19), one for cultured-cell samples (page 21), and one for tissues (page 23). The buffers used in the cultured-cell and tissue protocols 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.

Automated separation of glycoproteins

The QIAcube® can be used together with two Qproteome Total Glycoprotein Kits (cat. no. 37541; 12 x total glycoprotein preps) or four O-Glycan Glycoprotein Kits (cat. no. 37571; 12 x AIL lectin plus 12 x PNA lectin preps) for fully automated separation of glycoproteins from serum. Twelve samples are processed simultaneously, and protocols can be easily downloaded from the QIAcube web portal at www.qiagen.com/myQIAcube.

Separating glycoproteins using FPLC™-compatible Lectin Cartridges

All lectin resins available as Qproteome Glycoprotein spin columns are also available in prefilled 1 ml and 5 ml FPLC-compatible cartridges for use on fast liquid protein chromatography systems (e.g., ÄKTA™, BioLogic™, etc.). For a full list of available resins and formats see Table 1 and the Ordering Information.

Table 1. Available lectins and formats for isolation of glycoproteins.

Lectin	Spin Columns	Automatable on QIAcube	FPLC Cartridges
ConA	■	■	■
GNA	■		□
LCH	■		□
WGA	■	■	■
SNA	■		□
MAL	■		□
AIL	■	■	□
PNA	■	■	□

■ Available format, see Ordering Information.

□ Format available on request; please inquire.

Glycoprotein Fractionation Using Lectin Spin Columns

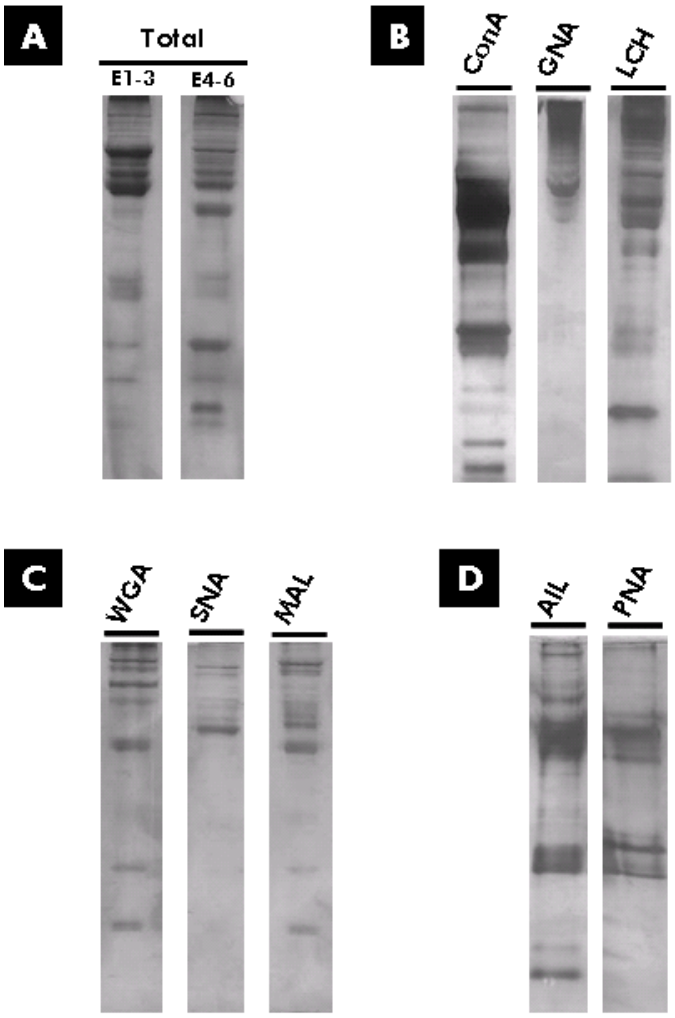


Figure 1 Glycoproteins were fractionated from serum using the different lectin spin columns in glycoprotein fractionation kits and analyzed by SDS-PAGE followed by silver staining. **A** Elution steps 1–3 and 4–6 from Total Lectin Spin Columns in the Total Glycoprotein Kit. **B** Eluted glycoproteins 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 2. 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 27 shows glycan structures to which lectins in Qproteome Glycoprotein Kits bind.

Table 3. 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 25).
- 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. Starting material for one fractionation procedure using the Glycoprotein Fractionation Kit Tissue Protocol is 10 mg easy-to-lyse tissue (e.g., liver or brain).
- 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 or Tissue Protocols 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 Table 4 on page 17.
- 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 5, page 18).
- 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 (see Appendix B, page 29).
- 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 4. Preparing binding and elution buffers for glycoprotein purification procedures

Serum protocol (page 19)

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 (page 21) and tissue protocol (page 23)

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 5. Spin columns and relevant elution buffers

Spin Column	Elution Buffer
Total Glycoprotein Kit	
Total Glycoprotein Spin Column (green screw-caps) (Elution steps 1–3)	Glycoprotein Elution Buffer ME
Total Glycoprotein 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 25 µl of Protease Inhibitor Solution (100x) as described in Table 4 on page 17.**
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 4 on page 15.**
   Refer to Table 5 on page 18 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 29)
- 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 29). 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 4 on page 17.**
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 5 on page 18 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: Glycoprotein Fractionation Kit Tissue Protocol

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

Starting material for one fractionation procedure using the Glycoprotein Fractionation Kit Tissue Protocol is 10 mg easy-to-lyse tissue (e.g., brain or liver).

Equipment and reagents to be supplied by the user

- TissueRuptor (QIAGEN cat. no. 9001271 [USA and Canada]; 9001272 [Europe (excluding UK and Ireland)]; 9001273 [UK and Ireland])

Important notes before starting

- Ensure that you are familiar with operating the TissueRuptor. Refer to the TissueRuptor User Manual for operating instructions.

Procedure

1. **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 4, page 17.**
2. **Using the TissueRuptor, homogenize the tissue sample in 1 ml of Binding Buffer containing the Protease Inhibitor and Detergent Solution prepared in step 1 for 30 s at the lowest speed.**
3. **Incubate the lysate for 15 min at 4°C. Mix briefly every 5 min.**
4. **Centrifuge the lysate at 10,000 x *g* and 4°C for 20 min.**
5. **During centrifugation of the 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).**
6. **Centrifuge the spin column for 2 min at 500 rpm.**
7. **Discard the flow-through and pipet 500 µl Binding Buffer onto the spin column. Centrifuge for 2 min at 500 rpm in a microcentrifuge.**
8. **Discard the flow through and apply 500 µl of the sample from step 4 to the spin column.**
9. **Incubate for 1 min and centrifuge for 2 min at 500 rpm.**

   Collect the flow-through fraction if analysis of other proteins is desired.

10. **Repeat steps 8 and 9 with the second 500 µl sample aliquot from step 4.**
11. **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.**
12. **During centrifugation add 3 µl of Protease Inhibitor and 30 µl Detergent Solution to a 300 µl aliquot of Elution Buffer.**

    Refer to Table 5 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.
13. **Transfer the spin column to a clean microcentrifuge tube.**
14. **Apply 100 µl of the Elution Buffer prepared in step 12 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.
15. **Repeat step 14 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.
16. **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

This troubleshooting guide may be helpful in solving any problems that may arise. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or sample and assay technologies (for contact information, see back cover or visit www.qiagen.com).

	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 25) 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 25.

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

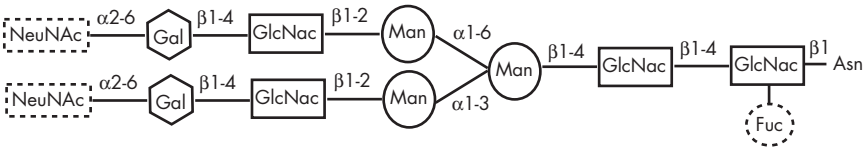
GalNAc — *N*-Acetylgalactosamine

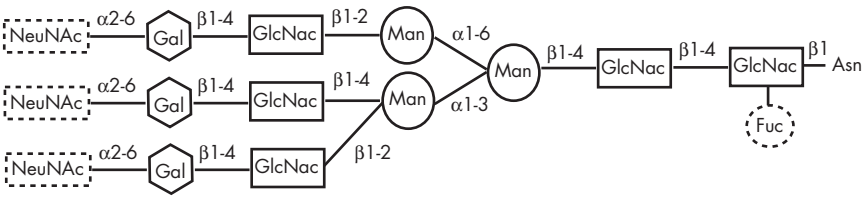
Asn — Asparagine

Ser/Thr — Serine/Threonine

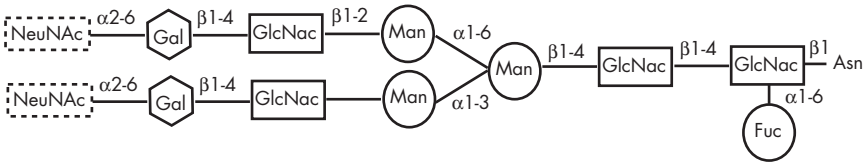
Qproteome Mannose Glycoprotein Kit

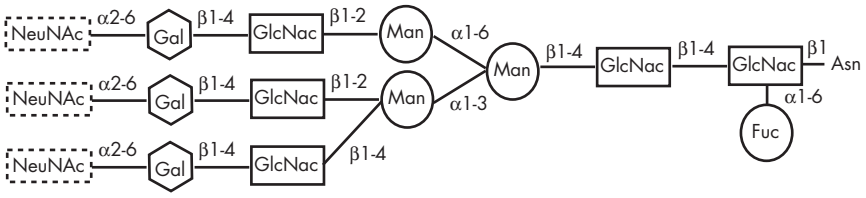
ConA — binds biantennary and triantennary complex type N-glycans



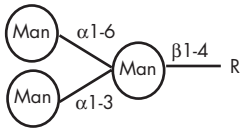


LCH — binds biantennary and triantennary complex type N-glycans with core fucose

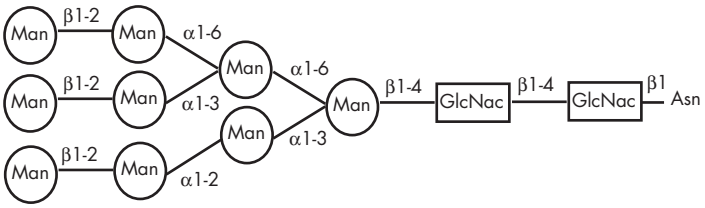


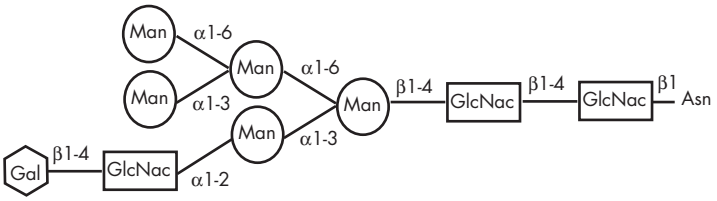


GNA — binds α1–3 and α1–6 linked high mannose structures



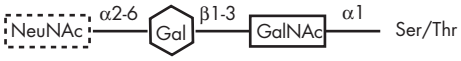
ConA/GNA — binds high mannose type N-glycan/α1–3 and α1–6 linked high mannose structures





Qproteome O-Glycan Glycoprotein Kit

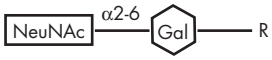
AIL



PNA

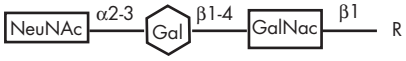
Qproteome Sialic Glycoprotein Kit

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
ConA Cartridges (3 x 1 ml)	3 FPLC-compatible cartridges prefilled with 1 ml ConA lectin resin	38003
WGA Cartridges (3 x 1 ml)	3 FPLC-compatible cartridges prefilled with 1 ml WGA lectin resin	38013
Related products		
QIAcube (110 V) QIAcube (230 V)	Robotic workstation for automated purification of DNA, RNA, or proteins using QIAGEN spin-column kits, 1-year warranty on parts and labor	9001292 9001293
TissueRuptor	Handheld rotor–stator homogenizer, 5 TissueRuptor Disposable Probes	9001271* 9001272† 9001273‡ 9001274§

* 120 V, 60 Hz (for North America and Japan); † 235 V, 50/60 Hz (for Europe, excluding UK and Ireland); ‡ 235 V, 50/60 Hz (for UK and Ireland); § 235 V, 50/60 Hz (for Australia)

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