# Lectin Cartridge Handbook

For FPLC<sup>™</sup> purification of glycosylated proteins



# Sample & Assay Technologies

Trademarks: QIAGEN®, Qproteome™(QIAGEN Group); ÄKTAdesign™, FPLC™ (Amersham plc); Triton® (Rohm & Haas, Inc.);

Hoffmann-La Roche owns patents and patent applications pertaining to the application of Ni-NTA resin (Patent series: RAN 4100/63: USP 4.877.830, USP 5.047.513, EP 253 303 B1), and to 6xHis-coding vectors and His-labeled proteins (Patent series: USP 5.284.933, USP 5.130.663, EP 282 042 B1). All purification of recombinant proteins by Ni NTA chromatography for commercial purposes, and the commercial use of proteins so purified, requires a license from Hoffmann-La Roche.

Registered names, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law.

© 2007 QIAGEN, all rights reserved.

### Contents

Kit Contents	4
Storage	4
Technical Assistance	5
Product Use Limitations	5
Safety Information	5
Product Warranty and Satisfaction Guarantee	6
Quality Control	6
Introduction	7
Lectin Cartridge Connections	9
Protocols	
Serum/Cell Culture Supernatant Protocol	15
Troubleshooting Guide	17
Appendix A: Buffer Compositions	18
Appendix B: Cleaning Lectin Cartridges	20
Appendix C: Acetone Precipitation of Protein Fractions	20
Ordering Information	21

### **Kit Contents**

ConA Lectin Cartridges (3 x 1 ml)	Cat. no. 38003
ConA Lectin Cartridges (1 ml)	3
Handbook	1

WGA Lectin Cartridges (3 x 1 ml)	Cat. no. 38013
WGA Lectin Cartridges (1 ml)	3
Handbook	1

**ConA, WGA, GNA**, **LCH**, **SNA**, **MAL**, **AIL**, and **PNA** lectins can be supplied on demand as 1 ml or 5 ml Cartridges. Please inquire.

### Storage

After delivery, Lectin Cartridges should be stored at 2–8°C. Do not freeze! Cartridges can be stored under these conditions for one year without any reduction in performance.

When not in use, store Lectin Cartridges in the appropriate Binding Buffer (see Appendix A, page 20) supplemented with 0.05% (w/v) sodium azide.\*

<sup>\*</sup> When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

### **Technical Assistance**

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 Lectin Cartridges or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, please see our Technical Support center at <u>www.qiagen.com/goto/TechSupportCenter</u> or call one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit <u>www.qiagen.com</u>).

### **Product Use Limitations**

Qproteome Lectin Cartridges 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.

### **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.

#### 24-hour emergency information

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

### **Product Warranty and Satisfaction Guarantee**

QIAGEN guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, QIAGEN will replace it free of charge or refund the purchase price. We reserve the right to change, alter, or modify any product to enhance its performance and design. If a QIAGEN product does not meet your expectations, simply call your local Technical Service Department or distributor. We will credit your account or exchange the product — as you wish. Separate conditions apply to QIAGEN scientific instruments, service products, and to products shipped on dry ice. Please inquire for more information.

A copy of QIAGEN terms and conditions can be obtained on request, and is also provided on the back of our invoices. If you have questions about product specifications or performance, please call QIAGEN Technical Services or your local distributor (see back cover or visit <u>www.qiagen.com</u>).

### **Quality Control**

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

### Introduction

Qproteome Lectin Cartridges are pre-filled with 1 ml or 5 ml lectin resin and are ready to use for purification of glycoproteins from serum or cell culture supernatants using liquid chromatography systems, such as the ÄKTAdesign™ or FPLC<sup>™</sup> System.

Qproteome Lectin Cartridges are comprised of individual lectins coupled to a synthetic polymer resin that combines superior mechanical stability with outstanding flow characteristics and high dynamic binding capacity. These resins allow the efficient isolation and purification of glycoproteins up to production-scale using FPLC systems. Tables 1 and 2 give an overview of the cartridge specifications and the connectors required for processing cartridges on FPLC instruments.

Affinity chromatography purification is commonly used in the downstream processing of proteins that are to be used for clinical research or therapeutic purposes. In general, proteins used as therapeutics (e.g., therapeutic antibodies) are glycosylated. The glycan makeup of a glycoprotein has a great impact on its stability (e.g., resistance to proteases) and its therapeutic half-life after injection into the human body. Using specific lectin affinity chromatography, glycosylated proteins can be separated for further analysis from non or differently-glycosylated proteins, and initial evidence about the level of glycosylation, type of glycosylation, and type(s) of glycan moiety present (e.g., sialic acid content) can be obtained. Table 3 on page 10 gives an overview of the lectins used in the cartridges and their glycan-binding specificities.

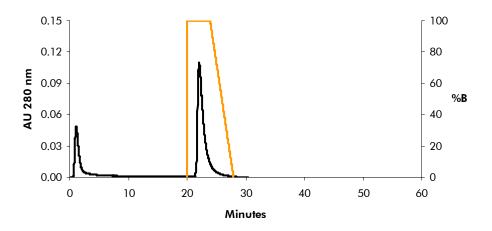


Figure 1. Typical elution profile using a Lectin Cartridge. Ovalbumin (500  $\mu$ l of a 25 mg/ml solution) was loaded onto a 5 ml ConA Lectin Cartridge and eluted using a methylmannose step gradient.

	1 ml Cartridge	5 ml Cartridge
Support	Synthetic po	olymer resin
Mean particle size	75	μm
Cartridge dimensions	6.7 mm x 28.0 mm	14.7 mm x 29.8 mm
Maximum pressure*	5 bar, 0.5 MPa	5 bar, 0.5 MPa
Typical back pressure	2–3 bar	2–4 bar
Recommended flow rate	0.6–1 ml/min	3–4 ml/min
Maximum flow rate	1.2 ml/min	5 ml/min
Cartridge connections	See Table 2, page 9	See Table 2, page 9
pH stability (short-term, <20 min)	0.1 M acetic acid, pH 3	0.1 M acetic acid, pH 3
pH stability (long-term, > 2h)	Use recommended storage buffers ( see Appendix B)	Use recommended storage buffers ( see Appendix B)
System compatibility	ÄKTA, FPLC, BioLo	ography systems (e.g., gic, BioCAD, Vision tation)
Cartridge body material	Polypro	opylene
Connectors	1/16″ (inlet)	); M6 (outlet)

Table 1. Qproteome Lectin Cartridge specifications

\* The maximum pressure usable with the lectin matrix is 7 bar. However, stability of the cartridges is only guaranteed up to 5 bar.

### Lectin Cartridge Connections

Qproteome Lectin Cartridges can be used for fractionation of glycoproteins in an automated procedure using a chromatography system, such as the ÄKTAdesign or FPLC<sup>™</sup> systems. The cartridge inlet and outlet dimensions and required connectors and adapters for manual and automated procedures are detailed in the table below.

	Inlet	Outlet
Qproteome Lectin Cartridge	1/16″ female (ÄKTAdesign)	M6 male (FPLC)
Adapters for manual procedure using a syringe	1/16" male/luer female (e.g., Amersham Product Code 18-1112-51)	
Connector for automated procedure (ÄKTAdesign 1/16″ connectors)	No adapter required	Union M6 female / 1/16″ female (e.g., Amersham Product Code 18-1123-94)
Connector for automated procedure (M6 fittings, [FPLC])	Union M6 female / 1/16" male (e.g., Amersham Product Code 18-3858- 01)	SRTC-2, M6 female (0.5 mm i.d.) (e.g., Amersham Product Code 18-3856-01)

#### Table 2. Connectors required for Qproteome Lectin Cartridges

Table 3. Overviev	Table 3. Overview of glycan-binding specificities of Lectin Cartridges	ities of Lectin Cartridge	Se
Lectin specificity	Lectin	Organism	Glycan/Carbohydrate structure
Mannose binding lectins	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	lpha1-3 and $lpha$ 1-6 linked high mannose structures
Sialic acid/ N-acetyl- glucosamine binding lectins	WGA (Wheat germ agglutinin) SNA (Elderberry lectin) MAL (Maackia amurensis lectin)	Triticum vulgaris Sambucus nigra Maackia amurensis	GlcNAcβ1-4GlcNAcβ1-4GlcNAc, Neu5Ac (sialic acid) Neu5Acα2-6Gal(NAc)-R Neu5Ac/Gcα2-3Galβ1-4GlcNAcβ1-R
Galactose/ N-acetyl- galactosamine binding lectins	AlL (Jacalin) PNA (Peanut agglutinin)	Artocarpus integrifolia Arachis hypogaea	Artocarpus integrifolia (Sia)Galβ1-3GalNAcα1-Ser/Thr (e.g., T-Antigen) Arachis hypogaea Galβ1-3GalNAcα1-Ser/Thr (e.g., T-Antigen)

#### Sample preparation

Qproteome Lectin Cartridges are designed for the fractionation of glycoproteins from serum or cell culture supernatants. Starting material for each purification procedure is  $100 \,\mu$ l of serum or 10 ml cell culture supernatant.

Serum samples must be diluted in binding buffer to ensure an efficient binding of glycoproteins to the lectin resin. To prevent proteolytic degradation of the proteins in the sample, a protease inhibitor is added.

After equilibration of the cartridge, the diluted sample is applied to the column, where glycosylated proteins bind to lectins on the 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.

Table 4 shows typical recoveries obtained using model glycoproteins. Yields from ConA Lectin Cartridge procedures using serum samples are shown in Table 5. For some downstream applications, concentration of the elution fractions may be necessary. A protocol for concentration using acetone precipitation can be found in Appendix C on page 22.

Lectin	Tested crude glycoprotein	Protein loaded (µg)	Protein recovered (µg)	Yield
WGA	Bovine fetuin	500	172	34%
ConA	Ovalbumin	500	264	53%
GNA	Glucose oxidase	500	387	78%
LCH	Glucose oxidase	500	402	81%
SNA	Human transferrin	500	211	42%
MAL	Bovine fetuin	2000	325	16%
AIL	Asialofetuin	500	259	52%
PNA	Asialofetuin	500	241	48%

Table 4: Recoveries from lectin cartridges using model glycoproteins

### Table 5. Glycoprotein yields from serum samples.

Sample	Sample size*	Yield of glycosylated proteins <sup>†</sup> ( $\mu$ g)
Fetal calf serum	50 <i>µ</i> l	233
Human serum	50 <i>µ</i> l	1062

\* Serum was diluted 1 in 10 with binding buffer before being loaded onto cartridge.

<sup>†</sup> Determined by the Bradford method.

#### Compatibility with buffer components

In general, only the recommended buffers should be used. Table 6 lists reagents that should be avoided when purifying glycoproteins using Lectin Cartridges.

Cleaning of the resins is described in Appendix B (page 22).

Reagent	Effect	Comments
Chelating reagents		
EDTA, EGTA	Strips cations from resin and reduces lectin binding activity	Use of chelating reagents is not recommended.
Sulfhydryl reagents		
β-mercaptoethanol, DTT, DTE	Can cause breakage of disulfide cross-linkages leading to reduced binding capacity	Use of sulfhydryl reagents is not recommended.
Detergents		
Nonionic and ionic detergents (Triton <sup>®</sup> , CHAPS, SDS, etc.)	Reduced binding capacity	Use the recommended buffers for the appropriate lectin resin
Denaturants		
GuHCI, Urea	Interfere with binding of glycoproteins to the resin	Use of denaturants is not recommended
Other additives		
Salts and organic molecules	Can interfere with binding of glycoproteins to the resin	Use of additives is not recommended

#### Selecting the appropriate Lectin Cartridge

The selection of the appropriate lectin glycoprotein purification cartridge for the purification of a glycoprotein depends on the nature of the protein, its glycosylation status, and on the accessibility of the glycan moiety. Since the procedure is limited to purifying glycoproteins under native conditions, the assumed glycan structure must be present and accessible for the lectin. Purification cannot be performed under denaturing conditions.

#### 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 2D gel analysis the elution fractions should be pooled and concentrated, for example using acetone (see page 22) or an ultrafiltration device.
- Starting material for one fractionation procedure using a 1 ml Lectin Cartridge is 100 μl serum or 10 ml cell culture supernatant.
- For quantification of glycoproteins, use the Bradford method (e.g., Bio-Rad Protein Assay Kit, cat. no. 500-0001)\*.
- Ensure that the correct Binding and Elution Buffers are used for the respective lectin cartridge (see Appendix A, page 18).
- 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 recommended), denaturants (e.g., urea, GuHCl), and proteases.

<sup>\*</sup> This is not a complete list of suppliers and does not include many important vendors of biological supplies.

### Protocol: Serum/Cell Culture Supernatant Protocol

#### Materials and reagents to be supplied by user

- Serum or cell culture supernatant
- 0.2 μm or 0.45 μm sterile filters
- Cell culture supernatant samples: Ultrafiltration devices (e.g., Amicon)\*
- Optional: Protease inhibitors (e.g., Complete, Mini, EDTA-free; Roche Cat. no. 11 836 170 001)\*
- Binding and Elution buffers (see Appendix A, page 20). All buffers should be sterile filtered (0.2 or  $0.45 \,\mu$ m) before use. Degassing buffers before use is also recommended.

#### Procedure

#### **Preparation of buffers**

1. Depending on the Lectin Cartridge being used, prepare 1 liter of the appropriate Binding buffer and 100 ml of the appropriate Elution Buffer according to Appendix A.

This amount of buffer should be sufficient for 5 runs. **Optional:** Supplement buffers with protease inhibitors according to manufacturer's instructions.

#### **Preparation of samples**

- 2. For preparation of serum samples, proceed using step 2a. For preparation of cell culture supernatant samples, proceed using step 2b.
- 2a. Add 900  $\mu$ l of the Binding Buffer prepared in step 1 to 100  $\mu$ l serum. Mix by vortexing gently and pass the sample through a sterile filter.
- 2b. Concentrate cell culture supernatant sample down to 100  $\mu$ l using an appropriate ultrafiltration device and add 900  $\mu$ l of the Binding Buffer prepared in step 1. Mix by vortexing gently and pass the sample through a sterile filter.

<sup>\*</sup> This is not a complete list of suppliers and does not include many important vendors of biological supplies.

#### **Purification procedure**

- 3. Fill system pumps with Binding Buffer and Elution Buffer and attach cartridge to the pump outlet, taking care not to introduce air into the system.
- 4. Remove cartridge outlet stopper and attach to the system tubing.
- 5. Equilibrate the cartridge with 10 column volumes of Binding Buffer. Use a flow rate of 1 ml/min (1 ml cartridges) or 4 ml/min (5 ml cartridges).
- 6. Use a sample loop, syringe, or Superloop<sup>®</sup> to load the sample onto the cartridge.

To ensure that the target protein has bound to the lectin resin, retain the flow-through fraction for analysis by SDS-PAGE.

- 7. Wash the cartridge until the  $A_{280}$  returns to the baseline value. Retain the wash fraction for analysis by SDS-PAGE.
- Use Elution Buffer to elute protein from cartridge.
  Protein usually elutes within 5–10 column volumes. An elution gradient over 10 column volumes may help separate proteins.
- 9. For regeneration, flush cartridge with 60 column volumes of Binding Buffer at the recommended flow rate.

### **Troubleshooting Guide**

	Comments and suggestions
Back pressure exceeds 5 bo	ar (0.5 MPa)
Column is clogged	Perform the cleaning-in-place procedure (see Appendix B).
	The sample loaded onto the cartridge may have contained particles. Sterile filter (0.2 or 0.45 $\mu$ m) or centrifuge prior to loading onto column.
Protein does not bind to the lectin resin	
Glycan moiety is not present	Perform a lectin blot using the appropriate lectin to check whether the glycan epitope is present.

#### Protein elutes in the wash buffer

Expected glycan moiety is	Check glycan moiety e.g., in a lectin blot using
underrepresented	different glycan-specific lectins as probe

### Protein precipitates during purification

Temperature is too low	Perform purification at	room temperature.
		· · · · ·

### **Appendix A: Buffer Compositions**

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

To prevent microbial growth during storage, all buffers should be supplemented with 0.05% (w/v) sodium azide.

#### Buffers for use with ConA, GNA, and LCH cartridges

#### Binding Buffer (1 Liter):

10 mM BIS-TRIS	6.90 g BIS-TRIS (MW 209.24 g/mol)	
150 mM NaCl	8.77 g NaCl (MW 58.44 g/mol)	
1 mM CaCl <sub>2</sub>	0.147 g CaCl <sub>2</sub> ·2 H <sub>2</sub> O (MW 147.01 g/mol)	
1 mM MnCl <sub>2</sub>	0.198 g MnCl <sub>2</sub> ·4 H <sub>2</sub> O (MW 197.91 g/mol)	
Adjust pH to 6.0 using HCl and sterile filter (0.2 or 0.45 $\mu$ m).		

#### Elution Buffer (100 ml):

10 mM BIS-TRIS	0.69 g BIS-TRIS (MW 209.24 g/mol)	
150 mM NaCl	0.88 g NaCl (MW 58.44 g/mol)	
1 mM CaCl <sub>2</sub>	0.01 g CaCl <sub>2</sub> ·2 H <sub>2</sub> O (MW 147.01 g/mol)	
1 mM MnCl <sub>2</sub>	0.02 g MnCl <sub>2</sub> ·4 H <sub>2</sub> O (MW 197.91 g/mol)	
200 mM Methylmannose	3.88 g Methylmannose (MW 194.18 g/mol)	
Adjust pH to 6.0 using HCl and sterile filter (0.2 or 0.45 $\mu$ m).		

#### Buffers to use with WGA, SNA, MAL cartridges

#### Binding Buffer (1 Liter):

10 mM BIS-TRIS	6.90 g BIS-TRIS (MW 209.24 g/mol)	
150 mM NaCl	8.77 g NaCl (MW 58.44 g/mol)	
1 mM CaCl <sub>2</sub>	0.147 g CaCl <sub>2</sub> ·2 H <sub>2</sub> O (MW 147.01 g/mol)	
1 mM MnCl <sub>2</sub>	0.198 g MnCl <sub>2</sub> ·4 H <sub>2</sub> O (MW 197.91 g/mol)	
Adjust pH to 6.0 using HCl and sterile filter (0.2 or 0.45 $\mu$ m).		

#### Elution buffer for WGA (100 ml):

10 mM BIS-TRIS	0.69 g BIS-TRIS (MW 209.24 g/mol)
150 mM NaCl	0.88 g NaCl (MW 58.44 g/mol)
1 mM CaCl <sub>2</sub>	0.01 g CaCl <sub>2</sub> ·2 H <sub>2</sub> O (MW 147.01 g/mol)
1 mM MnCl <sub>2</sub>	0.02 g MnCl <sub>2</sub> ·4 H <sub>2</sub> O (MW 197.91 g/mol)
200 mM N-Acetylglucosamine	4.42 g N-Acetylglucosamine (MW 221.21 g/mol)

Adjust pH to 6.0 using HCl and sterile filter (0.2 or 0.45  $\mu$ m).

#### Elution Buffer for SNA and MAL (100 ml):

10 mM BIS-TRIS	0.69 g BIS-TRIS (MW 209.24 g/mol)	
150 mM NaCl	0.88 g NaCl (MW 58.44 g/mol)	
1 mM CaCl <sub>2</sub>	0.01 g CaCl <sub>2</sub> ·2 H <sub>2</sub> O (MW 147.01 g/mol)	
1 mM MnCl <sub>2</sub>	0.02 g MnCl <sub>2</sub> ·4 H <sub>2</sub> O (MW 197.91 g/mol)	
200 mM Lactose	7.21 g Lactose (MW 360.31 g/mol)	
Adjust pH to 6.0 using HCl and starile filter (0.2 or 0.45 µm)		

Adjust pH to 6.0 using HCl and sterile filter (0.2 or 0.45  $\mu$ m).

#### Buffers to use with AIL and PNA cartridges

#### Binding Buffer (1 Liter):

10 mM HEPES	2.38 g HEPES (MW 238.3 g/mol)	
150 mM NaCl	8.77 g NaCl (MW 58.44 g/mol)	
1 mM CaCl <sub>2</sub> ·2 H <sub>2</sub> O	0.147 g CaCl <sub>2</sub> ·2 H <sub>2</sub> O (MW 147.01 g/mol)	
Adjust pH to 8.0 using NaOH and sterile filter (0.2 or 0.45 $\mu$ m).		

#### Elution buffer for AIL (100 ml):

10 mM HEPES	0.24 g HEPES (MW 238.3 g/mol)	
150 mM NaCl	0.88 g NaCl (MW 58.44 g/mol)	
1 mM CaCl <sub>2</sub> ·2 H <sub>2</sub> O	0.01 g CaCl <sub>2</sub> ·2 H <sub>2</sub> O (MW 147.01 g/mol)	
200 mM Methylgalactose	3.88 g Methylgalactose (MW 194.18 g/mol)	
Adjust pH to 8.0 using NaOH and sterile filter (0.2 or 0.45 $\mu$ m).		

#### Elution buffer for PNA (100 ml):

10 mM HEPES	0.24 g HEPES (MW 238.3 g/mol)	
150 mM NaCl	0.88 g NaCl (MW 58.44 g/mol)	
1 mM CaCl <sub>2</sub> ·2 H <sub>2</sub> O	0.01 g CaCl <sub>2</sub> ·2 H <sub>2</sub> O (MW 147.01 g/mol)	
200 mM Galactose	3.60 g Galactose (MW 180.16 g/mol)	
Adjust pH to 8.0 using NaOH and sterile filter (0.2 or 0.45 $\mu$ m).		

### **Appendix B: Cleaning Lectin Cartridges**

#### Cleaning-in-place protocol

If an increase in back pressure or significant contamination of the resin is observed, a cleaning-in-place procedure, which usually fully restores performance, can be performed.

#### Procedure

- 1. Wash cartridge with 20 column volumes of Cleaning Buffer (appropriate Binding Buffer containing 0.1% [w/v] Triton X-100)\*.
- 2. Re-equilibrate with 60 column volumes of the appropriate Binding Buffer (flow rate 1 ml/min).

The cartridge is now ready for use. Store cartridge in Binding Buffer supplemented with 0.05% (w/v) sodium azide.\*

### Appendix C: 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.

<sup>\*</sup> When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

## **Ordering Information**

Contents	Cat. no.
For specific enrichment of glycoproteins with mannose-rich glycan moieties: 3 FPLC-compatible cartridges prefilled with 1 ml ConA lectin resin	38003
For specific enrichment of glycoproteins with sialic acid-rich glycan moieties: 3 FPLC-compatible cartridges prefilled with 1 ml WGA lectin resin	38013
able on demand — please inquire*	
For specific enrichment of glycoproteins with mannose-rich glycan moieties	38001 38005
For specific enrichment of glycoproteins with sialic acid-rich glycan moieties	38011 38015
For specific enrichment of glycoproteins with sialic acid-rich glycan moieties	38021 38025
For specific enrichment of glycoproteins with sialic acid-rich glycan moieties	38031 38035
For specific enrichment of glycoproteins with mannose-rich glycan moieties	38041 38045
For specific enrichment of glycoproteins with mannose-rich glycan moieties	38051 38055
For specific enrichment of glycoproteins with O-glycan moieties	38061 38065
For specific enrichment of glycoproteins with O-glycan moieties	38071 38075
	For specific enrichment of glycoproteins with mannose-rich glycan moieties: 3 FPLC-compatible cartridges prefilled with 1 ml ConA lectin resin For specific enrichment of glycoproteins with sialic acid-rich glycan moieties: 3 FPLC-compatible cartridges prefilled with 1 ml WGA lectin resin <b>able on demand — please inquire*</b> For specific enrichment of glycoproteins with mannose-rich glycan moieties For specific enrichment of glycoproteins with sialic acid-rich glycan moieties For specific enrichment of glycoproteins with mannose-rich glycan moieties For specific enrichment of glycoproteins with op-glycan moieties For specific enrichment of glycoproteins with O-glycan moieties

\* For specific lectin glycan-binding specificities, refer to Table 3, page 10.

Product	Contents	Cat. no.
Qproteome Spin Colum proteins	nn Kits — for isolation of glycosylated	
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: AlL and PNA Lectin Spin Columns (3 each); Buffers; Protease Inhibitor Solution; Collection Tubes (6 x 2 ml)	37571

#### **QIAGEN** Companies

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

### **QIAGEN** Distributors and Importers

For a complete, up-to-date list of QIAGEN distributors and importers and contact information, visit <u>www.qiagen.com/contact/qiagenworldwide.aspx</u>

#### www.qiagen.com

Australia = Orders 03-9840-9800 = Fax 03-9840-9888 = Technical 1-800-243-066

- Austria = Orders 0800/28-10-10 = Fax 0800/28-10-19 = Technical 0800/28-10-11
- Belgium = Orders 0800-79612 = Fax 0800-79611 = Technical 0800-79556
- Canada = Orders 800-572-9613 = Fax 800-713-5951 = Technical 800-DNA-PREP (800-362-7737)
- China = Orders 021-51345678 = Fax 021-51342500 = Technical 021-51345678
- Denmark = Orders 80-885945 = Fax 80-885944 = Technical 80-885942
- Finland = Orders 0800-914416 = Fax 0800-914415 = Technical 0800-914413
- France = Orders 01-60-920-926 = Fax 01-60-920-925 = Technical 01-60-920-930 = Offers 01-60-920-928
- Germany = Orders 02103-29-12000 = Fax 02103-29-22000 = Technical 02103-29-12400
- Hong Kong = Orders 800 933 965 = Fax 800 930 439 = Technical 800 930 425
- Ireland = Orders 1800-555-049 = Fax 1800-555-048 = Technical 1800-555-061
- Italy = Orders 02-33430411 = Fax 02-33430426 = Technical 800-787980
- Japan = Telephone 03-5547-0811 = Fax 03-5547-0818 = Technical 03-5547-0811
- Luxembourg = Orders 8002-2076 = Fax 8002-2073 = Technical 8002-2067
- The Netherlands = Orders 0800-0229592 = Fax 0800-0229593 = Technical 0800-0229602
- **Norway** = Orders 800-18859 = Fax 800-18817 = Technical 800-18712
- South Korea = Orders 1544 7145 = Fax 1544 7146 = Technical 1544 7145
- Sweden = Orders 020-790282 = Fax 020-790582 = Technical 020-798328
- Switzerland = Orders 055-254-22-11 = Fax 055-254-22-13 = Technical 055-254-22-12
- **UK** = Orders 01293-422-911 = Fax 01293-422-922 = Technical 01293-422-999
- USA = Orders 800-426-8157 = Fax 800-718-2056 = Technical 800-DNA-PREP (800-362-7737)



# Sample & Assay Technologies