HiTrap Lectin Test Kit

INSTRUCTIONS

HiTrap™ Lectin Test Kit consists of four glycoprotein binding columns, HiTrap Con A, HiTrap Lentil Lectin, HiTrap Wheat Germ Lectin and HiTrap Peanut Lectin. The kit is designed to give a wide range of parameters for the separation of glycoproteins. It provides the possibility to screen for the most appropriate medium for a specific separation.

The special design of the columns, together with the matrix, provide fast, simple and easy separations in a convenient format. The columns can be operated with a syringe, peristaltic pump or liquid chromatography system such as ÄKTA™, GradiFrac™ or FPLC™.

Table 1. Content of HiTrap Lectin Test Kit

Code No.	Designation	No. supplied
17-5109-01	HiTrap Lectin Test Kit	
	HiTrap Con A	1 x 1 ml
	HiTrap Lentil Lectin	1 x 1 ml
	HiTrap Wheat Germ Lectin	1 x 1 ml
	HiTrap Peanut Lectin	1 x 1 ml
	Connectors	
	Luerlock female/M6 male	1
	Luerlock female/M6 female	1
	Tubing connector flangeless/M6 male	1
	Tubing connector flangeless/M6 female	1
	Domed nut	5
	Instructions	1

71-5006-13
Edition AC

amersham pharmacia biotech

Description

Gel properties

The lectins are coupled to NHS-activated Sepharose™ High Performance. The base matrix is a rigid, highly cross-linked, beaded agarose with high chemical stability.

The ligands have been chosen to complement each other and to provide a broad spectrum of parameters for the separation of glycoproteins. The specificity of the lectins are shown in the table below.

Table 2. Specificity of lectins

Lectin	Specificity
Mannose/glucose binding lectins	
Con A, Canavalia ensiformis	Branched mannoses, carbohydrates with terminal mannose or glucose (αMan>αGlc>GlcNAc)
Lentil Lectin, Lens culinaris	Branched mannoses with fucose linked α(1,6) to the N-acetyl-glucosamine, (αMan>αGlc>GlcNAc)
N-acetylglucosamine binding lectins	
Wheat Germ Lectin, Triticum vulgare	Chitobiose core of N-linked oligosaccharides, [GlcNAc(β1,4GlcNAc)$_{1-2}$ > βGlcNAc]
N-acetylgalactosamine/galactose binding lectins	
Peanut Lectin, Arachis hypogaea	Terminal β-galactose, (Galβ1,3GalNAc> α and βGal)

Concanavalin A lectin (Con A) is isolated from *Canavalia ensiformis* (jack bean). It is a metalloprotein and contains four metal binding sites. To maintain the binding characteristics of HiTrap Con A, the presence of both Mn^{2+} and Ca^{2+} is essential. These are present in excess in the gel supplied but the protein-metal ion complex remains active and is stable at neutral pH even in the absence of free metal ions.

Application areas for HiTrap Con A include separation, purification and characterization of glycoproteins such as the apolipoprotein H (Ref. 1), acid sphingomyelinase from human placenta (Ref. 2), high-affinity sulfonylurea receptor (Ref. 3), and recombinant human luteinizing hormone (Ref. 4). Other areas where it can be used are purification of glycopeptides such as 21-amino acid glycopeptide prepared from human serum transferrin (Ref. 5), and purification of glycolipids such as lipophosphoglycans from two *Leishmania mexicana* strains (Ref. 6).

Lentil Lectin is isolated from *Lens culinaris* (common lentil). It is a metalloprotein and consists of two isolectins, both tetramers with two dissimilar subunits. Fucose, linked α(1,6) to the glycopeptide core N-acetyl-glucosamine is important for binding activity. To maintain the binding characteristics of HiTrap Lentil Lectin, the presence of both Mn^{2+} and Ca^{2+} is essential. These are present in excess in the gel supplied but the protein-metal ion complex remains active and is stable at neutral pH even in the absence of free metal ions.

HiTrap Lentil Lectin is especially suited for purification of detergent-solubilized membrane glycoproteins as it retains its binding capacity in the presence of detergents.

Application areas for HiTrap Lentil Lectin include separation and purification of glycoproteins, such as human lymphoblast N-acetyl-glusosamine-1-phosphodiester α-N-

acetylglucosaminidase (Ref. 7), chicken oviductal ecto-ATP-diphosphohydrolase (Ref. 8), A/Victoria/3/75 (H3N2) influenza haemaglutinin expressed in insect cells (Ref. 9), synaptic vesicle-binding protein physophilin (Ref. 10), and investigations of the carbohydrate content in the human platelet serotonin transporter (Ref. 11).

Wheat Germ Lectin is isolated from *Triticum vulgare*. It is a dimeric, carbohydrate-free protein composed of two identical subunits. The subunits have a molecular weight of about 20,000. Wheat germ lectin has high affinity to N-acetylglucosamine and reacts strongly with the chitobiose core of N-linked oligosaccharides. It also has affinity to N-acetylneuraminic acid.

Application areas for HiTrap Wheat Germ Lectin include separation and purification of glycoproteins and polysaccharides containing N-acetyl-β-glucosaminyl residues, such as the S fimbria-binding sialoglycoprotein (Ref. 12), the plasmin-sensitive surface protein from *Staphylococcus aureus* (Ref. 13), a 55 kDa zona pellucida glycoprotein expressed in baculovirus expression system (Ref. 14), and concentration of a Ca^{2+}-independent α-latrotoxin-binding protein (Ref. 15), as well as detection of O-GlcNAc moieties on subpopulations of the estrogen receptor (Ref. 16).

Peanut Lectin is isolated from *Arachis hypogaea*. It is a tetrameric, carbohydrate-free protein and it has selectivity for terminal β-galactose and recognizes the T-antigen (Galβ1,3GalNAc). The subunits contain one atom each of Ca^{2+} and Mg^{2+}.

Application areas for HiTrap Peanut Lectin include purification of glycoproteins such as the Gal(β1,3)GalNAc bearing glycoproteins at the nodes of Ranvier in peripheral nerve (Ref. 17), natural human antibodies to dietary lectins (Ref. 18), and

peanut agglutinin-binding glycoproteins from lizard lymphocytes (Ref. 19). Other applications include the characterization of carbohydrate moieties of glyco-proteins such as CBP70 isolated from HL60 cells (Ref. 20), and PCA inhibitory factor present in mouse serum (Ref.21).

The recommended buffers for the different columns are shown in Table 4.

Column

The columns are made of medical grade poly-propylene, which is biocompatible and non-interactive with biomolecules. The top and bottom frits are manufactured from porous polyethylene. The columns are delivered with a stopper on the inlet and a twist-off end on the outlet. Both ends have M6 connections (6 mm metric threads).

The separation can be easily achieved using a syringe together with the supplied luer adaptor, a peristaltic pump, or in a chromatography system such as ÄKTA, GradiFrac, or FPLC.

Note: To prevent leakage it is essential to ensure that the adaptor is tight.

The column cannot be opened or refilled.

The characteristics of the product are summarised below.

Table 3. HiTrap Lectin Test Kit characteristics

Column dimensions i.d. x h	0.7 x 2.5 cm
Column volumes	1 ml
Ligands	Concanavalin A, Lentil Lectin, Wheat Germ Lectin, Peanut Lectin respectively
Ligand concentration	
Con A	12 - 18 mg/ml
Lentil Lectin	2 mg/ml
Wheat Germ Lectin	5 - 8 mg/ml
Peanut Lectin	4 mg/ml
Binding capacity	
Con A	4 mg Transferrin/ml
Lentil Lectin	4 mg Thyroglobulin/ml
Wheat Germ Lectin	4 mg Ovomuroid/ml
Peanut Lectin	3 mg Asialofetuin/ml
Mean particle size	34 µm
Bead structure	Highly cross-linked spherical agarose
Maximum back pressure	0.3 MPa, 3 bar
Maximum flow rate	4 ml/min
Recommended flow rate	0.1 - 1 ml/min
pH stability:	4 - 9
Temperature stability:	
Regular use	4 °C - room temperature
Storage	4 - 8 °C
Storage buffer	
Con A	Binding buffer with 20 % ethanol
Lentil Lectin	Binding buffer with 20 % ethanol
Wheat Germ Lectin	Binding buffer with 20 % ethanol
Peanut Lectin	Binding buffer with 20 % ethanol

Operation

The columns can be operated with a syringe, peristaltic pump or a liquid chromatography system.

Buffer preparation

Water and chemicals used for buffer preparation should be of high purity. It is recommended to filter the buffers by passing them through a 0.45 µm filter before use.

Table 4. Recommended buffers for HiTrap Lectin Test Kit columns

	Binding buffer	Elution buffer	Detergent
HiTrap Con A	20 mM Tris™-HCl, 0.5 M NaCl, 1 mM $MnCl_2$, 1 mM $CaCl_2$, pH 7.4	0.5 M methyl-α-D-glucopyranoside, 20 mM Tris-HCl, 0.5 M NaCl, pH 7.4	Affects the binding of glycoproteins
HiTrap Lentil Lectin	20 mM Tris-HCl, 0.5 M NaCl, 1 mM $MnCl_2$, 1mM $CaCl_2$, pH 7.4	0.3 M methyl-α-D-mannopyranoside, 20 mM Tris-HCl, 0.5 M NaCl, pH 7.4	Retains binding capacity in the presence of detergents
HiTrap Wheat Germ Lectin	20 mM Tris-HCl, 0.5 M NaCl, pH 7.4	0.5 M N-acetylglucos-amine (GlcNAc), 20 mM Tris-HCl, 0.5 M NaCl, pH 7.4	Retains binding capacity in the presence of detergents
HiTrap Peanut Lectin	10 mM sodium phosphate, 0.15 M NaCl, 0.5 mM $MgCl_2$, 0.5 mM $CaCl_2$, pH 7.4	0.5 M β-lactose, 10 mM sodium phosphate, 0.15 M NaCl, 0.5 mM $MgCl_2$, 0.5 mM $CaCl_2$, pH 7.4	Retains binding capacity in the presence of some detergents

Sample preparation

The sample should be adjusted to the composition of the binding buffer. This can be done by either diluting the sample with binding buffer or by buffer exchange using HiTrap Desalting or PD-10 columns. The sample should be filtered through a 0.45 µm filter or centrifuged before it is applied to the column.

Purification on HiTrap Con A

The recommended flow rate for HiTrap Con A is 1 ml/min, unless another flow rate is stipulated.

Note: 1ml/min corresponds to approximatley 30 drops/min when the column is operated with a syringe.

1. Fill the syringe or pump tubing with binding buffer. Remove the stopper and connect the column to the syringe (with the provided adaptor), or pump tubing, ”drop to drop” to avoid introducing air into the column.
2. Remove the twist-off end.
3. Wash the column with 10 column volumes of binding buffer.
4. Apply the sample, using a syringe fitted to the luer adaptor or by pumping it onto the column. For best results use a low flow rate, 0.1 - 0.5 ml/min, during sample application.
5. Wash with 5-10 column volumes of binding buffer or until no material appears in the effluent.
6. Elute with 5-10 column volumes of elution buffer.

Note: Before reuse the column has to be regenerated by washing with 10 column volumes of 20 mM Tris-HCl, 1 M NaCl, pH 8.5 followed by reequilibration with binding buffer. The reuse of HiTrap Con A depends on the nature of the sample and should only be performed with identical samples to prevent cross-contamination.

Binding

The most important parameter affecting the binding of glycoproteins to the immobilised lectin is the flow rate. It is important to keep the flow rate as low as 0.1 - 0.5 ml/min during sample application for maximum binding capacity. This is especially important for samples containing detergents as the binding activity decreases in the presence of detergents.

When applying the sample with a syringe an alternative to keeping the flow rate low is to apply 1 ml sample at the time and let it bind for a couple of minutes. This is also possible when using a chromatography system or peristaltic pump where the sample volume exceeds the column volume (1 ml). Repeat this procedure until all the sample is applied to the column.

To preserve binding activity below pH 5, excess Mn^{2+} and Ca^{2+} (1 mM) must be present.

Elution

The recovery of glycoproteins can sometimes be improved by pausing the flow for 2 minutes during elution. For complex samples containing glycoproteins with different affinity for the lectin, a continuous gradient or step elution is recommended with 0 - 0.5 M methyl-α-D-glucopyranoside or methyl-α-D-mannopyranoside in elution buffer. A continuous gradient can be achieved by use of a chromatography system, such as GradiFrac, FPLC or ÄKTA, or by use of a pump and gradient mixer. Tightly bound substances can be eluted by lowering the pH, but not below pH 4.

Borate is known to form complexes with cis-diols on sugar residues and thus act as an competing eluent. For elution with borate, use 0.1 M sodium borate buffer, pH 6.5. Recovery on HiTrap Con A is decreased in the presence of detergents, while

HiTrap Lentil Lectin and HiTrap Wheat Germ Lectin retain their activity. If the glycoprotein of interest needs the presence of detergent and has affinity for either of the above mentioned columns, the recovery might be improved using an alternative HiTrap column.

Storage

Store the column at 4 - 8 °C in binding buffer with 20 % ethanol.

Purification on HiTrap Lentil Lectin

Purification of detergent-solubilized proteins requires buffers with higher pH as the recommended detergent, sodium deoxycholate, is not soluble below pH 8.0. The column should be equilibrated with the binding buffer mentioned above before starting to ensure that the column is saturated with Mn^{2+} and Ca^{2+}.

For separations without detergent see Table 4 for recommended buffers.

Binding buffer: 20 mM Tris-HCl, 0.5 M NaCl, 0.5 % sodium deoxycholate pH 8.3.

Elution buffer: 0.3 M methyl-α-D-mannopyranoside, 20 mM Tris-HCl, 0.5 M NaCl, 0.5 % sodium deoxycholate, pH 8.3.

The recommended flow rate for HiTrap Lentil Lectin is 1 ml/min, unless another flow rate is stipulated.

Note: 1 ml/min corresponds to approximately 30 drops/min, when the column is operated with a syringe.

1. Fill the syringe or pump tubing with binding buffer. Remove the stopper and connect the column to the syringe (with the provided adaptor), or pump tubing, ”drop to drop” to avoid introducing air into the column.

2. Remove the twist-off end.
3. Wash the column with 10 column volumes of binding buffer.
4. Apply the sample, using a syringe fitted to the luer adaptor or by pumping it onto the column. For best results use a low flow rate, 0.1 - 0.5 ml/min, during sample application.
5. Wash with 5-10 column volumes of binding buffer or until no material appears in the effluent.
6. Elute with 5-10 column volumes of elution buffer.

Note: Before reuse the column has to be regenerated by washing with 10 column volumes of 20 mM Tris-HCl, 1 M NaCl, pH 8.5 followed by reequilibration with binding buffer. The reuse of HiTrap Lentil Lectin depends on the nature of the sample and should only be performed with identical samples to prevent cross-contamination.

Binding

The most important parameter affecting the binding of glycoproteins to the immobilised lectin is the flow rate. It is important to keep the flow rate as low as 0.1 - 0.5 ml/min during sample application for maximum binding capacity.
When applying the sample with a syringe an alternative to keeping the flow rate low is to apply 1 ml sample at the time and let it bind for a couple of minutes. This is also possible when using a chromatography system or peristaltic pump where the sample volume exceeds the column volume (1 ml). Repeat this procedure until all the sample is applied to the column.

To preserve binding activity below pH 5, excess Mn^{2+} and Ca^{2+} (1 mM) must be present.

Elution

The recovery of glycoproteins can sometimes be improved by pausing the flow for a few minutes during elution. For complex

samples containing glycoproteins with different affinity for the lectin, a continuous gradient or step elution is recommended with 0 - 0.5 M methyl-α-D-glucopyranoside or methyl-α-D-mannopyranoside in elution buffer. A continuous gradient can be achieved by use of a chromatography system, such as GradiFrac, FPLC or ÄKTA, or by use of a pump and a gradient mixer.

Tightly bound substances can be eluted by lowering the pH, but not below pH 4. In some cases strongly bound substances can be eluted with detergent, for example 1.0 % deoxycholate.
Borate is known to form complexes with cis-diols on sugar residues and thus act as an competing eluent. For elution with borate, use 0.1 M sodium borate buffer, pH 6.5.

Storage

Store the column at 4 - 8 C in binding buffer with 20 % ethanol

Purification on Wheat Germ Lectin

The recommended flow rate for HiTrap Wheat Germ Lectin is 1 ml/min.

Note: 1 ml/min corresponds to approximately 30 drops/min, when the column is operated with a syringe.

1. Fill the syringe or pump tubing with binding buffer. Remove the stopper and connect the column to the syringe (with the provided adaptor), or pump tubing, ”drop to drop” to avoid introducing air into the column.
2. Remove the twist-off end.
3. Wash the column with 10 column volumes of binding buffer.
4. Apply the sample, using a syringe fitted to the luer adaptor or by pumping it onto the column.
5. Wash with at least 5-10 column volumes of binding buffer or until no material appears in the effluent.
6. Elute with 5-10 column volumes of elution buffer.

Note: Before reuse the column has to be regenerated by washing with 5–10 column volumes of 20 mM Tris-HCl, 1 M NaCl, pH 8.5 followed by reequilibration with binding buffer. The reuse of HiTrap Wheat Germ Lectin depends on the nature of the sample and should only be performed with identical samples to prevent cross-contamination.

Binding

HiTrap Wheat Germ Lectin can be used with detergents, such as 1 % deoxycholate or 0.5 % Triton™ X-100.
When the sample volume exceeds the column volume (1 ml), apply 1 ml sample at the time and let it bind for a couple of minutes. A lower flow rate than 1 ml/min may enhance binding of some glycoproteins. Other buffers with neutral pH can be used as binding buffers, for example sodium phosphate.

Elution

The recovery of glycoproteins can sometimes be improved by pausing the flow for 2 minutes during elution.

For complex samples containing glycoproteins with different affinity for the lectin, a continious gradient or step elution is recommended with 0 - 0.5 M N-acetylglucosamine (GlcNAc), 20 mM Tris-HCl, 0.5 M NaCl, pH 7.4. A continious gradient can be achieved by use of a chromatography system, such as GradiFrac, FPLC or ÄKTA, or by use of a pump and a gradient mixer.

Tightly bound substances can also be eluted with 20 mM acetate buffer, pH 4.5 or with an alternative sugar for example triacetylchitotriose.

Storage

Store the column at 4 - 8 °C in binding buffer with 20 % ethanol.

Purification on HiTrap Peanut Lectin

The recommended flow rate for HiTrap Peanut Lectin is 1 ml/min.

Note: 1 ml/min corresponds to approximately 30 drops/min, when the column is operated with a syringe.

1. Fill the syringe or pump tubing with binding buffer. Remove the stopper and connect the column to the syringe (with the provided adaptor), or pump tubing, ”drop to drop” to avoid introducing air into the column.
2. Remove the twist-off end.
3. Wash the column with 10 column volumes of binding buffer.
4. Apply the sample, using a syringe fitted to the luer adaptor or by pumping it onto the column. For best result use a low flow rate, 0.1 - 0.5 ml/min, during sample application.
5. Wash with 5-10 column volumes of binding buffer or until no material appears in the effluent.
6. Elute with 5-10 column volumes of elution buffer.

Note: Before reuse the column has to be regenerated by washing with 10 column volumes of 1 M NaCl followed by requilibration with at least 25 column volumes of binding buffer. The reuse of HiTrap Peanut Lectin depends on the nature of the sample and should only be performed with identical samples to prevent cross-contamination.

Binding

The most important parameter affecting the binding of glycoproteins to the matrix, is the flow rate. It is important to keep the flow rate as low as 0.1 - 0.5 ml/min during sample application for maximum binding capacity.

To preserve binding activity excess Mg^{2+} and Ca^{2+} (0.5 mM) must be present.

Elution

The recovery of glycoproteins can sometimes be improved by pausing the flow for a few minutes during elution.
For complex samples containing glycoproteins with different affinity for the lectin, a continuous gradient of 0 - 0.5 M β-lactose, or a step gradient of galactose followed by β-lactose is recommended. A continuous gradient can be achieved by use of a chromato-graphy system, such as GradiFrac, FPLC, ÄKTA, or by use of a pump and a gradient mixer.

Storage

Store the column at 4 - 8 °C in binding buffer with 20 % ethanol.

Ordering information

The columns included in HiTrap Lectin Test Kit can be ordered separately under the following Code numbers:

Designation	No. Supplied	Code No.
GSTrap™, 1 ml	5x1 ml	17-5130-01
GSTrap, 1 ml	2x1 ml	17-5130-02
GSTrap, 5 ml	1x5 ml	17-5131-01
HiTrap Con A, 1 ml	5x1 ml	17-5105-01
HiTrap Lentil Lectin, 1 ml	5x1 ml	17-5106-01
HiTrap Wheat Germ Lectin,1ml	5x1 ml	17-5107-01
HiTrap Peanut Lectin, 1 ml	5x1 ml	17-5108-01
HiTrap Lectin Test Kit, 1 ml	4x1 ml	17-5109-01
HiTrap Blue, 1 ml	5x1 ml	17-0412-01
HiTrap Blue, 5 ml	1x5 ml	17-0413-01
HiTrap NHS-activated, 1 ml	5x1 ml	17-0716-01
HiTrap NHS-activated, 5 ml	1x5 ml	17-0717-01
HiTrap Desalting, 5 ml	5x5 ml	17-1408-01
HiTrap SP, 1 ml	5x1 ml	17-1151-01
HiTrap SP, 5 ml	5x5 ml	17-1152-01
HiTrap Q, 1 ml	5x1 ml	17-1153-01

Designation	No. Supplied	Code No.
HiTrap Q, 5 ml	5x5 ml	17-1154-01
HiTrap IEX test kit, 1 ml	4x1 ml	17-6001-01
HiTrap rProtein A, 1 ml	2x1 ml	17-5079-02
HiTrap rProtein A, 1 ml	5x1 ml	17-5079-01
HiTrap rProtein A, 5 ml	1x5 ml	17-5080-01
HiTrap Protein A, 1 ml	2x1 ml	17-0402-03
HiTrap Protein A, 1 ml	5x1 ml	17-0402-01
HiTrap Protein A, 5 ml	1x5 ml	17-0403-01
HiTrap Protein G, 1 ml	2x1 ml	17-0404-03
HiTrap Protein G, 1 ml	5x1 ml	17-0404-01
HiTrap Protein G, 5 ml	1x5 ml	17-0405-01
MAbTrap™ G II kit	1 kit	17-1128-01
HiTrap Heparin, 1 ml	5x1 ml	17-0406-01
HiTrap Heparin, 5 ml	1x5 ml	17-0407-01
HiTrap Chelating, 1 ml	5x1 ml	17-0408-01
HiTrap Chelating, 5 ml	1x5 ml	17-0409-01
HiTrap IgM Purification, 1 ml	5x1 ml	17-5110-01
HiTrap IgY Purification, 5 ml	1x5 ml	17-5111-01
HiTrap Streptavidin, 1 ml	5x1 ml	17-5112-01
HisTrap™	1 kit	17-1880-01
HiTrap HIC Test Kit, 1 ml	1 kit	17-1349-01
PD-10 Disposible Column	30	17-0851-01

Accessories

Domed nut*	4	18-2450-01
Union Luerlock		
female/M6 female*	2	18-1027-12
female/M6 male*	2	18-1027-62
Tubing connector		
flangeless/M6 female*	2	18-1003-68
flangeless/M6 male*	2	18-1017-98
Union female /1/16"male (to connect columns with M6 connections to ÄKTAdesign)	5	18-3858-01

* included in HiTrap package

References

HiTrap Con A

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HiTrap Peanut Lectin

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Important Information

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Amersham Pharmacia Biotech AB
SE-751 84 Uppsala
Sweden

Amersham Pharmacia Biotech UK Limited
Amersham Place, Little Chalfont
Buckinghamshire HP7 9NA
England

Amersham Pharmacia Biotech Inc
P.O. Box 1327
Piscataway, NJ 08855
USA

Amersham Pharmacia Biotech Europe GmbH
Postfach 5480
D-79021 Freiburg
Germany

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