

# HiTrap Affinity columns

## HiTrap Wheat Germ Lectin, 1 ml

## INSTRUCTIONS

HiTrap™ Wheat Germ Lectin is a pre-packed, ready-to-use, column for preparative affinity chromatography. The special design of the column, together with the matrix, provides fast, simple and easy separations in a convenient format.

The column can be operated with a syringe, peristaltic pump or liquid chromatography system such as ÄKTA™, GradiFrac™ or FPLC™.

**Table 1.** Content of HiTrap Wheat Germ Lectin

Code No.	Designation	No. supplied
17-5107-01	HiTrap Wheat Germ Lectin	5x1 ml
<b>Connectors</b>		
	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

71-5004-33

Edition AC



*amersham pharmacia biotech*

## Description

### Gel properties

Wheat Germ Lectin is isolated from *Triticum vulgare* and coupled to NHS-activated Sepharose™ High Performance. The base matrix is a rigid, highly cross-linked, beaded agarose with high chemical stability. The specificity of Wheat Germ Lectin can be found in the table below.

**Table 2.** Specificity of lectins

Lectin	Specificity
Mannose/glucose binding lectins Con A, <i>Canavalia ensiformis</i>	Branched mannoses, carbohydrates with terminal mannose or glucose (aMan>aGlc>GlcNAc)
Lentil Lectin, <i>Lens culinaris</i> fucose linked a(1,6) to the	Branched mannoses with N-acetyl-glucosamine, (aMan>aGlc>GlcNAc)
N-acetylglucosamine binding lectins Wheat Germ Lectin, <i>Triticum vulgare</i>	Chitobiose core of N-linked oligosaccharides, [GlcNAc(b1,4GlcNAc) <sub>1-2</sub> > bGlcNAc]
N-acetylgalactosamine/galactose binding lectins Peanut Lectin, <i>Arachis hypogaea</i>	Terminal b-galactose, (Galb1,3GalNAc > a and bGal)

Wheat Germ Lectin 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- $\beta$ -glucosaminyl residues, such as the S fimbria-binding sialoglycoprotein (Ref. 1), the plasmin-sensitive surface protein from *Staphylococcus aureus* (Ref. 2), a 55 kDa zona pellucida glycoprotein expressed in baculovirus expression system (Ref. 3), and concentration of a  $\text{Ca}^{2+}$ -independent  $\alpha$ -latrotoxin-binding protein (Ref. 4), as well as detection of O-GlcNAc moieties on subpopulations of the estrogen receptor (Ref. 5).

### **Column**

The column is made of medical grade polypropylene, which is biocompatible and non-interactive with biomolecules. The column has porous polyethylene top and bottom frits. It is 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 Wheat Germ Lectin characteristics

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Column dimensions i.d. x h	0.7 x 2.5 cm
Column volume	1 ml
Ligand	Wheat Germ Lectin
Ligand concentration	4.6 - 7.6 mg/ml
Binding capacity	4 mg Ovomuroid/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	Binding buffer with 20 % ethanol

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## Operation

The column can be operated with a syringe, peristaltic pump or a 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.

Binding of glycoproteins occurs at neutral pH. Recommended buffers: **Binding buffer:** 20 mM Tris<sup>TM</sup>-HCl, 0.5 M NaCl, pH 7.4. **Elution buffer:** 0.5 M N-acetylglucosamine (GlcNAc), 20 mM Tris-HCl, 0.5 M NaCl, pH 7.4

## 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 column. The sample should be filtered through a 0.45  $\mu\text{m}$  filter or centrifuged before it is applied to the column.

## Purification

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

*Note:* 1 ml/min corresponds to approximately 30 drops/sec, 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 5-10 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 column volumes of binding buffer or until no material appears in the effluent.
6. Elute with 5 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 for 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 continuous 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 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 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.

## References

1. Identification and characterization of S fimbria-binding sialoglycoproteins on brain microvascular endothelial cells.

*Infect.Immun.* 65 (1997) 2852-2860. Prasaderao NV, Wass CA and Kim KS.

2. Purification and characterization of a plasmin-sensitive surface protein of *Staphylococcus aureus*. *Eur. J. Biochem.* 236 (1996)

904-910. Hilden P, Savolainen K, Tynnelä J, Vuento M and Kuusela P.

3. Evaluating zona pellucida structure and function using antibodies to rabbit 55 kDa ZP protein expressed in baculovirus expression system. *Mol. Reprod. Dev.* 43 (1996) 519-529. Prasad SV, Wilkins B, Skinner SM and Dunbar BS.

4. Isolation and biochemical characterization of a Ca<sup>2+</sup>-independent  $\alpha$ -latrotoxin-binding protein. *J. Biol. Chem.* 271 (1996) 23239-23245. Davletov BA, Shamotienko OG, Lelianova VG, Grishin EV and Ushkaryov YA.

5. A subpopulation of estrogen receptors are modified by O-linked N-acetylglucosamine. *J. Biol. Chem.* 272 (1997) 2421-2428. Jiang M-S and Hart GW.

## Ordering Information

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 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
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 Blue, 1 ml	5x1 ml	17-0412-01
HiTrap Blue, 5 ml	1x5 ml	17-0413-01
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 Disposable Column	30	17-0851-01



## Accessories

Designation	No. Supplied	Code No.
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

## **Important Information**

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