Con A Sepharose™ 4B
HiTrap™ Con A 4B

Lectins are proteins that interact specifically and reversibly with certain sugar residues. Immobilized lectins are invaluable tools for isolating and separating glycoproteins, glycolipids, polysaccharides, subcellular particles and cells, and for purifying detergent-solubilized cell membrane components. They are also useful for assessing changes in levels or composition of surface glycoproteins during cell development and in malignant or virally transformed variants.

Con A Sepharose 4B is a chromatography medium for separation and purification of glycoproteins, polysaccharides, and glycolipids.

HiTrap Con A 4B are ready-to-use columns prepacked with Con A Sepharose 4B. The design of the prepacked HiTrap column provides simple and easy separations in a convenient format. A complete instruction for use is delivered with each package together with a variety of connectors for easy connection to a syringe, peristaltic pumps, and chromatography systems such as ÄKTA™ design. Prepacked HiTrap Con A 4B columns offer convenience, save time, and ensure reproducible results.

Con A Sepharose 4B is also available in 5 ml and 100 ml bulk packs.

Fig 1. HiTrap Con A 4B 1 ml and 5 ml columns for convenient purification of glycoproteins, polysaccharides, and glycolipids. Con A Sepharose 4B is also available in 5 ml and 100 ml bulk packs.

Description

Chromatography medium characteristics

Con A Sepharose is an affinity medium with concanavalin A (Con A) coupled to Sepharose 4B by the cyanogen bromide method. Table 1 summarizes the characteristics of Con A Sepharose 4B.

Con A is a tetrameric metalloprotein isolated from *Canavalia ensiformis* (jack bean). Con A binds to molecules containing α-D-mannopyranosyl, α-D-glucopyranosyl, and sterically related residues. To maintain the binding characteristics of Con A Sepharose 4B, the presence of both Mn^{2+} and Ca^{2+} is essential. These ions are present in large excess in the delivered medium/columns. The Con A-metal ion complex remains active and is stable at neutral pH even in the absence of free metal ions. However, to preserve the binding activity below pH 5, excess Mn^{2+} and Ca^{2+} (1 mM) must be present in the buffer solutions. This will ensure an active Con A-metal complex.

Table 1. Characteristics of Con A Sepharose 4B

Matrix	4% agarose
Average particle size	90 µm
Ligand	Con A
Ligand concentration	10 to 15 mg Con A/ml medium
Binding capacity	20 to 45 mg porcine thyroglobulin/ml medium
Maximum flow rate[1]	75 cm/h
Chemical stability	Stable in all commonly used aqueous buffers. Chelating agents such as EDTA, 8 M urea, or solutions having pH below 3 should be avoided as these conditions result in removal of manganese from the lectin with loss of activity as a result.
pH stability	4 to 9
Storage	4ºC to 8ºC in 20% ethanol containing 0.1 M acetate buffer pH 6, 1 M NaCl, 1 mM $CaCl_2$, 1 mM $MnCl_2$, and 1 mM $MgCl_2$.

[1] Aqueous buffer, room temperature, 1.6 mm i.d. column, 5 cm bed height

HiTrap Con A 4B characteristics

HiTrap Con A 4B belongs to the HiTrap family of prepacked columns. These 1 ml and 5 ml columns are made of biocompatible polypropylene that does not interact with biomolecules. Prepacked HiTrap Con A 4B columns provide simple and easy separations in a convenient format. They are delivered with a stopper on the inlet and a snap-off end on the outlet. HiTrap Con A 4B can be operated with a syringe, a peristaltic pump, or a liquid chromatography system such as ÄKTA design. Note that HiTrap columns cannot be opened or refilled. Table 2 summarizes the characteristics of prepacked HiTrap Con A 4B columns.

Table 2. Characteristics of HiTrap Con A 4B

Column volume	1 ml or 5 ml
Column dimensions	0.7 × 2.5 cm (1 ml) 1.6 × 2.5 cm (5 ml)
Recommended flow rates	0.1 to 1 ml/min (1 ml) 0.5 to 5 ml/min (5 ml)
Maximum flow rates	4 ml/min (1 ml) 20 ml/min (5 ml)
Maximum back pressure[1]	0.3 MPa/3 bar

[1] H_2O at room temperature

Application

Enrichment of glycoproteins from human plasma

Glycoproteins from human plasma were enriched on a HiTrap Con A 4B 1 ml column connected to ÄKTAexplorer™ 100. The resulting chromatogram is shown in Figure 2. Analysis by Coomassie™ stained SDS-PAGE (non-reducing conditions), showed that unfractionated plasma, flowthrough, and wash fractions all had an intensive band corresponding to a relative molecular mass (M_r) of 67 000 (Fig 3). This band corresponds to high-abundant, non-glycosylated serum albumin, which was not detected in the eluate.

Column:	HiTrap Con A 4B 1 ml
Sample:	0.5 ml human plasma diluted to 5 ml with binding buffer
Flow rates:	Sample load: 0.2 ml/min Elution: 0.1 ml/min (with pause 4 min)
Binding buffer:	20 mM Tris, 500 mM NaCl, 1 mM $MnCl_2$, 1 mM $CaCl_2$, pH 7.4
Elution buffer:	20 mM Tris, 500 mM NaCl, 300 mM methyl-D-glucoside, pH 7.4
System:	ÄKTAexplorer 100

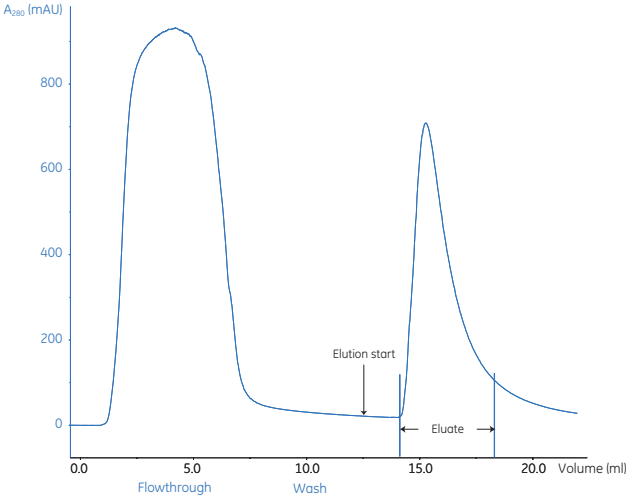

Fig 2. Chromatographic enrichment of glycoproteins from human plasma using HiTrap Con A 4B 1 ml.

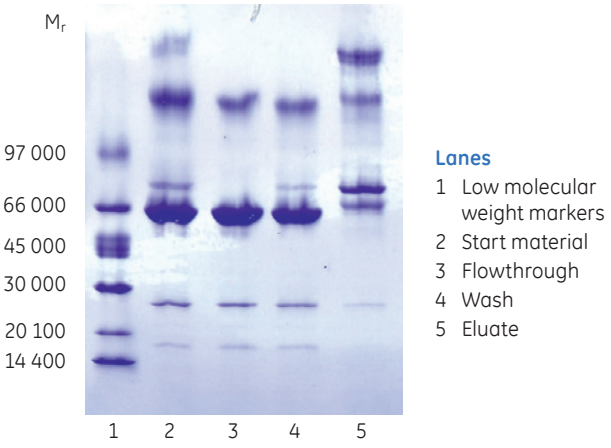

Fig 3. SDS-PAGE anaysis with Coomassie stained ExcelGel™ 8–18 Gradient gel (non-reduced conditions) of fractions from enrichment of glycoproteins from human plasma using HiTrap Con A 4B 1 ml.

Starting material and eluate were labeled with different fluorescent CyDye™ and run in parallel on a 2-D gel, to visualize the qualitative differences in protein abundances (Fig 4). Multicolor detection was performed using a Typhoon™ scanner. Proteins in the plasma sample are visualized as green spots. Con A-enriched proteins are seen as red spots. The protein spots were identified by comparison with standard plasma electrophoresis 2-D gels (Swiss-Prot database). To confirm these results, analysis with LC-MS was performed (data not shown). Both LC-MS and 2-D electrophoresis identified the same glycosylated proteins in the eluate. The results showed that Con A 4B is a reliable tool for enrichment of glycosylated proteins.

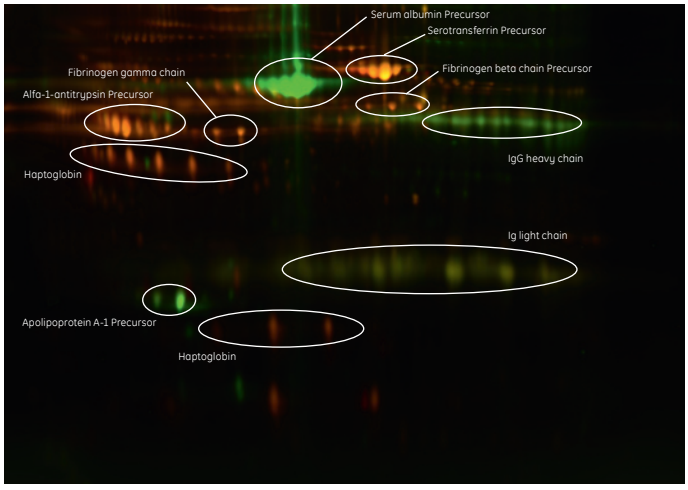

Fig 4. 2-D-image of CyDye labeled human plasma (green spots) and CyDye labeled Con A-enriched glycosylated proteins (red spots). The glycosylated apolipoprotein A-1 precusor did not bind to Con A, due to its different glycosylation pattern.

Ordering information

Product	Quantity	Code no.
HiTrap Con A 4B	5 × 1 ml	28-9520-85
	5 × 5 ml	28-9520-96
Con A Sepharose 4B	5 ml	17-0440-03
	100 ml	17-0440-01

HiTrap accessories	No. supplied	Code no.
1/16" male/Luer female[1]	2	18-1112-51
Tubing connector flangeless/M6 female[1]	2	18-1003-68
Tubing connector flangeless/M6 male[1]	2	18-1017-98
Union 1/16" female/M6 male[1]	6	18-1112-57
Union M6 female /1/16" male[1]	5	18-3858-01
Union Luerlock female/M6 female	2	18-1027-12
HiTrap/HiPrep™, 1/16" male connector for ÄKTA design	8	28-4010-81
Stop plug female, 1/16"	2	11-0004-64
Fingertight stop plug, 1/16"[2]	5	11-0003-55

[1] One connector included in each HiTrap package
[2] One fingertight stop plug is connected to the top of each HiTrap column at delivery

Related literature	Code no.
Affinity Chromatography Handbook, Principles and Methods	18-1022-29
Affinity Chromatography, Columns and Media, Selection Guide	18-1121-86
HiTrap Column Guide	18-1129-81
Prepacked chromatography columns for ÄKTA design systems, Selection Guide	28-9317-78

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