Instructions 28-9475-75 AC

HiTrap™ Albumin & IgG Depletion, 1 ml

HiTrap Albumin & IgG Depletion is a prepacked column designed for depletion of albumin and IgG from human plasma or serum. Application of a sample volume of up to 150 µl undiluted human plasma results in more than 95% albumin depletion and more than 90% IgG depletion. The design of the HiTrap column together with the Sepharose™ High Performance matrix, provides rapid and easy processing in a convenient format.

HiTrap Albumin & IgG Depletion columns can be operated with a syringe, peristaltic pump, or liquid chromatography systems such as ÄKTA™.

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Please read these instructions carefully before using HiTrap columns.

Intended use

HiTrap columns are intended for research use only, and shall not be used in any clinical or *in vitro* procedures for diagnostic purposes.

Safety

For use and handling of the product in a safe way, please refer to the Safety Data Sheet.

1 Introduction

HiTrap Albumin & IgG Depletion is a fast and efficient sample preparation tool prior to proteomics analysis, such as 1D- and 2D-electrophoresis and mass spectrometry.

Depletion of albumin and IgG removes more than 60% of the total protein content in human plasma, whereby proteins normally obscured by albumin and IgG during analysis can be visualized. This also allows for a relatively higher load of less abundant proteins during analysis, enabling detection of an increased number of proteins.

2 Product description

HiTrap column characteristics

The columns are made of biocompatible polypropylene that does not interact with biomolecules.

The columns are delivered with a stopper at the inlet and a snap-off end at the outlet. Table 2 lists the characteristics of HiTrap columns.

Fig 1. HiTrap, 1 ml column.

Note: *HiTrap columns cannot be opened or refilled.*

Note: *Make sure that the connector is tight to prevent leakage.*

Table 1. Characteristics of HiTrap columns.

Column volume (CV)	1 ml
Column dimensions	0.7 × 2.5 cm
Column hardware pressure limit	5 bar (0.5 MPa)

Note: *The pressure over the packed bed varies depending on a range of parameters such as the characteristics of the*

chromatography medium, sample/liquid viscosity and the column tubing used.

Supplied Connector kit with HiTrap column

Connectors supplied	Usage	No. supplied
Union 1/16" male/ luer female	For connection of syringe to HiTrap column	1
Stop plug female, 1/16"	For sealing bottom of HiTrap column	2, 5 or 7

Chromatography medium properties

HiTrap Albumin & IgG Depletion 1 ml column is prepacked with a mixture of anti-HSA Sepharose High Performance and Protein G Sepharose High Performance. The mixed chromatography media consists of highly cross-linked agarose beads with covalently immobilized affinity ligands. The average bead size is 34 µm.

The ligand of anti-HSA Sepharose High Performance is based on a single domain antibody fragment with high specificity and capacity for human serum albumin.

The ligand of Protein G Sepharose High Performance is derived from the IgG binding regions of Protein G, a cell surface protein of *Streptococcus* bacteria. The Protein G ligand binds human IgG_1, IgG_2, IgG_3 and IgG_4. It is also effective in removing IgG from rat and mouse plasma.

The characteristics of the HiTrap Albumin & IgG Depletion column are summarized in Table 2.

Table 2. HiTrap Albumin & IgG Depletion characteristics.

Matrix	Highly cross-linked 6% agarose
Average particle size	34 µm
Ligands	Recombinant Protein G fragment and recombinant protein binding HSA.
Recommended flow rate	1 ml/min
Maximum flow rate	4 ml/min
pH stability	
- short term (2 hours)	2 to 9
- long term (one week)	3 to 9
Storage	20% ethanol at 4ºC to 8ºC

3 General considerations

HiTrap Albumin & IgG Depletion is designed for a sample volume of up to 150 µl human plasma.

A load of 150 µl undiluted plasma containing normal levels of albumin and IgG (approximately 40 mg HSA/ml, approximately 15 mg IgG/ml) results in >95% albumin depletion and >90% IgG depletion.

When applying plasma containing albumin and IgG above normal levels it is recommended to use a lower sample volume (100 µl to 125 µl) to obtain the same depletion efficiency.

4 Operation

Buffer preparation

Water and chemicals used for buffer preparation should be of high purity. It is recommended to filter buffers through a 0.22 µm or a 0.45 µm filter before use to remove any potential debris.

Recommended buffers

- Binding buffer: 20 mM sodium phosphate, 0.15 M NaCl, pH 7.4.
- Elution buffer: 0.1 M glycin-HCl, pH 2.7.

Sample preparation

No dilution of the human plasma is required. Filter the human plasma through a 0.45 or 0.22 µm filter shortly before applying it to the column.

Depletion procedure

A flow rate of 1 ml/min is recommended for the entire depletion procedure.

1. Fill the pump tubing with binding buffer. Remove the stopper and the snap-off end from the column and connect it to the pump tubing 'drop-to-drop' to avoid introducing air into the system.
2. Wash the column with 5 ml binding buffer to remove the 20% ethanol storage solution.
3. Equilibrate with 10 ml of binding buffer.
4. Apply 150 µl filtrated human plasma and wash with at least 5 ml binding buffer until the absorbance reaches a steady baseline. Collect the flow through during sample application and wash. The flow through contains the depleted sample.
5. *Optional:* Elute and collect the bound proteins (albumin and IgG) with 10 ml elution buffer.

   Note: *Step 5 should be performed if the column is to be re-used or if the bound Albumin & IgG fraction is to be analyzed.*

For manual depletion procedure (without using a pump), the syringe is connected to the column by the provided luer connector. Be careful to use a flow rate of approximately 1 ml/min. Note that too high flow rate will damage the packing of the chromatography medium and cause high back pressure.

5 Cleaning-in-place (CIP)

The column can be used for a limited number of depletion runs. If cross-contamination between samples needs to be prevented a cleaning-in-place can be performed. Wash with 5 ml 70% ethanol and re-equilibrate with 10 ml binding buffer.

6 Adjusting pressure limits in chromatography system software

Pressure generated by the flow through a column affects the packed bed and the column hardware, see Fig 2. Increased pressure is generated when running/using one or a combination of the following conditions:

- High flow rates
- Buffers or sample with high viscosity
- Low temperature
- A flow restrictor

Note: *Exceeding the flow limit (see Table 2) may damage the column.*

Fig 2. Pre-column and post-column measurements..

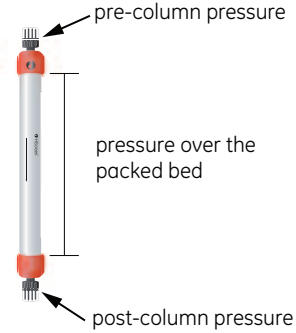

Fig 3. Pre-column and post-column measurements.

ÄKTA avant and ÄKTA pure

The system will automatically monitor the pressures (pre-column pressure and pressure over the packed bed, Δp). The pre-column pressure limit is the column hardware pressure limit (see Table 1).

The maximum pressure the packed bed can withstand depends on media characteristics and sample/liquid viscosity. The measured value also depends on the tubing used to connect the column to the instrument.

ÄKTAexplorer, ÄKTApurifier, ÄKTAFPLC and other systems with pressure sensor in the pump

To obtain optimal functionality, the pressure limit in the software may be adjusted according to the following procedure:

1. Replace the column with a piece of tubing. Run the pump at the maximum intended flow rate. Note the pressure as *total system pressure*, P1.
2. Disconnect the tubing and run the pump at the same flow rate used in step 1. Note that there will be a drip from the column valve. Note this pressure as P2.
3. Calculate the new pressure limit as a sum of P2 and the column hardware pressure limit (see Table 1). Replace the pressure limit in the software with the calculated value.

   The actual pressure over the packed bed (Δp) will during run be equal to actual measured pressure - *total system pressure* (P1).

Repeat the procedure each time the parameters are changed.

7 Storage

Store at 4°C to 8°C in 20% ethanol.

8 Troubleshooting

The following tips may be of assistance. If you have further questions about HiTrap Albumin & IgG Depletion columns, please visit *www.gelifesciences.com/sampleprep* or contact technical support or your local representative.

Event	Possible cause	Action
Less efficient depletion of albumin and/or IgG	The sample may contain albumin and IgG above the normal levels	Use a lower sample volume, ex 100 µl, for the next depletion run
	The buffer composition is incorrect	Check pH and composition of the binding buffer
	The column has not been sufficiently equilibrated	Equilibrate the column according to the Depletion procedure
	The column has been used for a number of repeated runs	Clean the column according to Cleaning-in-place procedure or exchange column
The protein of interest is not found in the collected flow-through fraction	The protein may be associated with albumin	Collect the fraction eluted with 0.1 M glycine-HCl

9 Ordering Information

Product	Quantity	Code No.
HiTrap Albumin & IgG Depletion	2 × 1 ml	28-9466-03

Related products	Quantity	Code No.
2-D Quant Kit	500 assays	80-6483-56
Nuclease Mix	0.5 ml	80-6501-42
Protease Mix	1 ml	80-6501-23
2D-Clean-Up kit	50 samples	80-6484-51
SDS-PAGE Clean-Up Kit	50 samples	80-6484-70
Vivaspin™ 500, 3kDa MWCO PES	25 samples	28-9322-18
Vivaspin 500, 5kDa MWCO PES	25 samples	28-9322-23
Vivaspin 500, 10kDa MWCO PES	25 samples	28-9322-25
Vivaspin 500, 30kDa MWCO PES	25 samples	28-9322-35
Vivaspin 500, 50kDa MWCO PES	25 samples	28-9322-36
Vivaspin 500, 100kDa MWCO PES	25 samples	28-9322-37

Additional Vivaspin formats are available, please see www.gelifesciences.com/sampleprep

Accessories	Quantity	Code No.
1/16" male/luer female *(For connection of syringe to top of HiTrap column)*	2	18-1112-51
Tubing connector flangeless/M6 female *(For connection of tubing to bottom of HiTrap column)*	2	18-1003-68
Tubing connector flangeless/M6 male *(For connection of tubing to top of HiTrap column)*	2	18-1017-98
Union 1/16" female/M6 male *(For connection to original FPLC System through bottom of HiTrap column)*	6	18-1112-57
Union M6 female /1/16" male *(For connection to original FPLC System through top of HiTrap column)*	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" *(For sealing bottom of HiTrap column)*	5	11-0004-64
Fingertight stop plug, 1/16"	5	11-0003-55

Related literature	Code No.
2-D Electrophoresis; Principles and Methods	80-6429-60
Ettan DIGE System User Manual	18-1173-17
Recombinant Protein Purification Handbook, Principles and Methods	18-1142-75
Affinity Chromatography Handbook, Principles and Methods	18-1022-29
Affinity Chromatography Columns and Media Selection Guide	18-1121-86

For local office contact information, visit
www.gelifesciences.com/contact

GE Healthcare Bio-Sciences AB
Björkgatan 30
751 84 Uppsala
Sweden

www.gelifesciences.com/sampleprep

GE Healthcare Europe GmbH
Munzinger Strasse 5,
D-79111 Freiburg,
Germany

GE Healthcare UK Ltd
Amersham Place
Little Chalfont
Buckinghamshire, HP7 9NA
UK

GE Healthcare Bio-Sciences Corp
800 Centennial Avenue
P.O. Box 1327 Piscataway,
NJ 08855-1327
USA

GE Healthcare Bio-Sciences KK
Sanken Bldg.
3-25-1, Hyakunincho
Shinjuku-ku, Tokyo 169-0073
Japan

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