Instructions 71-5003-43 AK

MAbTrap™ Kit

MAbTrap Kit is an affinity chromatography kit for fast and effective purification of monoclonal and polyclonal IgG from ascites fluid, serum and cell culture supernatants. MAbTrap Kit contains everything necessary to perform a complete one step purification without using complicated equipment.

It also eliminates time consuming buffer preparation and column packing. The kit includes one HiTrap™ column, prepacked with Protein G Sepharose™ High Performance. The column can be operated with a syringe, peristaltic pump or liquid chromatography system such as ÄKTA™.

Kit Contents

Code No.	Product	No. supplied
17-1128-01	HiTrap Protein G HP	1 × 1 ml
	1/16" male/luer female	1
	Stop plug female	1
	Syringe, 5 ml	1
	Binding Buffer, 50 ml, 10 × concentrate containing 20% ethanol as a preservative	1
	Elution Buffer, 15 ml, 10 × concentrate	1
	Neutralizing Buffer, 25 ml, containing 20% ethanol as a preservative	1
	Instructions	1

MAbTrap Kit contains enough material to perform up to 20 purifications when operated with a syringe.

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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 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 1 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.*

Chromatography medium properties

Protein G Sepharose High Performance is produced by coupling protein G to highly cross-linked agarose beads by the N-hydroxysuccinimide activation method.

The recombinant protein G, M_r 17 000, is produced in *E. coli* and contains two IgG binding regions. The albumin binding region of native protein G has been genetically deleted, thereby avoiding undesirable cross-reactions with albumin.

Protein G is similar to protein A but has different IgG binding specificities, dependent on the origin of the IgG. These differences are illustrated in Table 3. The binding capacity of the matrix-bound protein G for IgG depends on the source species of the immunoglobulin, as well as other factors such as the flow rate during sample application, and the sample concentration. As a reference, the binding capacity for human IgG is approximately 25 mg IgG/ml medium.

The characteristics of HiTrap Protein G HP are summarized in Table 2.

Table 2. Protein G HP characteristics.

Ligand	Recombinant protein G lacking the albumin-binding region M_r ~ 17 000, pI ~ 4.1
Degree of substitution	~ 2 mg protein G/ml medium
Binding capacity	> 25 mg human IgG/ml medium
Mean particle size	34 µm
Bead structure	Highly cross-linked spherical agarose
Maximum flow rate	4 ml/min (~ 2 drops/sec)
Recommended flow rate	1 ml/min
Chemical stability	All commonly used buffers
pH stability[1]	
Short term	2 to 9
Long term	3 to 9
Storage	2°C to 8°C in 20% ethanol

[1] The ranges given are estimates based on our knowledge and experience. Please note the following:
pH stability, long term refers to the pH interval where the medium is stable over a long period of time without adverse effects on its subsequent chromatographic performance.
pH stability, short term refers to the pH interval for regeneration, cleaning-in-place and sanitization procedures.
pH below 3 is sometimes required to elute strongly bound IgG species. However protein ligands may hydrolyze at very low pH.

Table 3. Relative binding strengths for protein A and protein G.

Species	Subclass	Protein A binding	Protein G binding
Human	IgA	variable	–
	IgD	–	–
	IgE	–	–
	IgG_1	++++	++++
	IgG_2	++++	++++
	IgG_3	–	++++
	IgG_4	++++	++++
	IgM*	variable	–
Avian egg yolk	IgY†	–	–
Cow		++	++++
Dog		++	+
Goat		–	++
Guinea pig	IgG_1	++++	++
	IgG_2	++++	++
Hamster		+	++
Horse		++	++++
Koala		–	+
Llama		–	+
Monkey (rhesus)		++++	++++
Mouse	IgG_1	+	++++
	IgG_{2a}	++++	++++
	IgG_{2b}	+++	+++
	IgG_3	++	+++
	IgM*	variable	–
Pig		+++	+++
Rabbit	no distinction	++++	+++
Rat	IgG_1	–	+
	IgG_{2a}	–	++++
	IgG_{2b}	–	++
	IgG_3	+	++
Sheep		+/–	++

* Purified using HiTrap IgM Purification HP columns.
† Purified using HiTrap IgY Purification HP columns.
++++ = strong binding
++ = medium binding
– = weak or no binding

2 Buffers

The buffers have been prepared using the highest quality buffers salts and water and have been filtered through a 0.45 µm filter.

Note: *The high salt concentration in the buffer stock solution may cause salt crystals to form at low temperature. These crystals will dissolve at room temperature. We therefore recommend that the buffer stock solutions be allowed to reach room temperature before use. The formation of salt crystals that dissolve at room temperature does not affect the performance of the product.*

3 Preparation

Allow the column and buffers to warm to room temperature. The column is delivered with a top-cap on the inlet and a snap off end on the outlet. The medium is supplied in 20% ethanol.

1 Buffer preparation, for one purification, using a syringe

Dilute the 10 × buffer concentrates as follows:

a 2.5 ml binding buffer concentrate + 22.5 ml high quality water to a total volume of 25 ml.

b 0.5 ml elution buffer concentrate + 4.5 ml high quality water to a total volume of 5 ml.

2 Tube preparation

Prepare collection tubes by adding 60–200 µl of neutralizing buffer per ml of fraction to be collected. This allows for immediate renaturing of the purified IgG to preserve the activity of labile IgGs.

3 Sample preparation

The sample should be centrifuged and filtered (0.45 µm filter) if there are particles present or the appearance is cloudy. If the sample is serum or ascites fluid dilute the sample 1:1 with prepared binding buffer. Serum and ascites fluid samples may also be filtered through glass wool to increase the clarification and prevent back pressure problems.

4 Purification

1. Fill the syringe or pump tubing with distilled water. Remove the top-cap and connect the column and the syringe through the luer connector (or to a pump). Make the connection "drop to drop" to avoid introducing air into the column.
2. Remove the snap-off end at the column outlet.
3. Wash out the ethanol preservative with 5 ml of distilled water at ~ 1 drop/sec (~ 2 ml/min).
4. Equilibrate the column with at least 3 ml of binding buffer.
5. Apply the sample, using a syringe or by pumping onto the column.
6. Wash with 5–10 ml binding buffer or until no material appears in the effluent. Excessive washing should be avoided if the interaction between the protein of interest and the ligand is weak, since this may decrease the yield.
7. Elute with elution buffer. 3–5 ml is usually sufficient, but larger volumes will be required if the interaction is difficult to break.
8. The purified IgG fractions can be desalted using a HiTrap Desalting or PD-10 Desalting column if necessary.
9. Recondition the column with 5 ml binding buffer.

Note: *The column may also be attached to a chromatography system like ÄKTA design.*

Note: *Note: If a P1-pump is used a max flow rate of 1–3 ml/min can be run on a HiTrap 1 ml column packed with Sepharose High Performance media.*

5 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.*

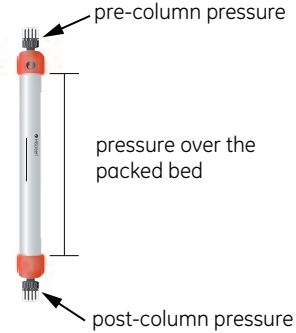

Fig 2. Pre-column and post-column measurements.

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).

Note: *Repeat the procedure each time the parameters are changed.*

6 Storage

Store the entire MAbTrap Kit at 2°C to 8°C. If the column is going to be stored after use, equilibrate it with 10 ml 20% ethanol and close the column with the included stoppers. The equilibration is easily done with the supplied syringe. For further information visit *www.gelifesciences.com/hitrap.* Useful handbooks are available, *see* Ordering information.

7 Applications

Purification of mouse monoclonal IgG_1 from cell culture supernatant using MAbTrap Kit and a syringe.

Purification procedure

Equilibration:	5 ml distilled water (to waste) plus 3 ml prepared binding buffer
Sample:	10 ml mouse monoclonal cell supernatant, IgG_1, anti IgE. Filtered through 0.45 µm filter
Washing:	7 ml prepared binding buffer
Elution:	5 ml prepared elution buffer
Neutralization:	75 µl neutralizing buffer added per ml fraction
Column:	HiTrap Protein G HP 1 ml

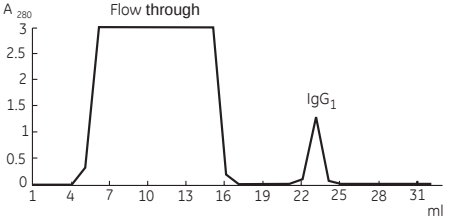

Fig 3. Purification of mouse monoclonal IgG_1 from cell culture supernatant with syringe operation.

Results:

Operating the system with a syringe resulted in an IgG_1 pool of 2 ml with an absorbance of 0.643 (A_{280}) and a corresponding yield of 0.9 mg pure mouse monoclonal IgG_1, as judged by SDS-PAGE and silver staining.

Purification of mouse monoclonal IgG_1 from ascites fluid using MAbTrap Kit and a syringe.

Purification procedure

Equilibration:	5 ml distilled water (to waste) plus 3 ml prepared binding buffer
Sample:	1 ml mouse monoclonal IgG_1 from ascites fluid. Filtered through 0.45 µm filter, mixed 1:1 with binding buffer
Washing:	7 ml prepared binding buffer
Elution:	5 ml prepared elution buffer
Neutralization:	75 µl neutralizing buffer added per ml fraction
Column:	HiTrap Protein G HP 1 ml

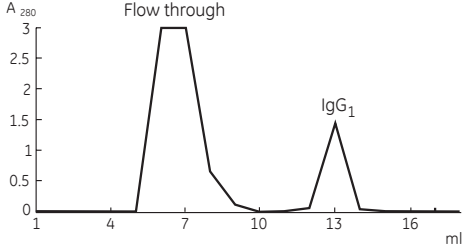

Fig 4. Purification of mouse monoclonal IgG_1 from ascites fluid with syringe operation.

Results:

Operating the system with a syringe resulted in an IgG_1 pool of 1 ml with an absorbance of 1.46 (A_{280}) and a corresponding yield of 1.1 mg pure mouse monoclonal IgG_1, as judged by SDS-PAGE and silver staining.

8 Ordering information

Product	No. supplied	Code No.
MAbTrap Kit	1	17-1128-01

Related products	No. supplied	Code No.
HiTrap Protein G HP	2 × 1 ml	17-0404-03
	5 × 1 ml	17-0404-01
	1 × 5 ml	17-0405-01
	5 × 5 ml	17-0405-03
HiTrap Desalting	1 × 5 ml	29-0486-84
	5 × 5 ml	17-1408-01
PD-10 Desalting column	30	17-0851-01

Related literature	Code No.
Antibody Purification Handbook	18-1037-46
Solutions for Antibody Purifications, Selection Guide	28-9351-97
Affinity Chromatography Handbook, Principles and Methods	18-1022-29
Affinity Chromatography Columns and Media, Selection Guide	18-1121-86

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

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