

MABTrap Kit

MABTrap™ Kit from GE Healthcare is designed for fast and effective purification of monoclonal and polyclonal IgG from ascites fluid, serum and cell culture supernatants.

- Convenient and fast purification of IgG from different sources
- Simple and proven method giving reproducible results
- Recombinant Protein G offering a broad binding specificity
- Pre-made high quality buffer concentrates ensuring optimal binding
- Use with a syringe, no complicated equipment required

Introduction

MABTrap Kit contains a HiTrap™ column prepacked with Protein G Sepharose™ High Performance medium and pre-made Binding, Elution and Neutralizing Buffer concentrates for the purification of monoclonal and polyclonal IgG. Details of the contents of the kit are given in Table 1. MABTrap Kit contains enough material to perform up to 20 purifications using syringe operation. The optimized medium in combination with the prepacked column, provide fast preparative purifications with reproducible results within 10-15 minutes.

Table 1. MABTrap Kit.

Column	HiTrap Protein G HP, 1 ml
Binding Buffer	50 ml, 10 x concentrate
Elution Buffer	15 ml, 10 x concentrate
Neutralizing Buffer	25 ml
Luer adapter, stopper, syringe, instructions.	



Fig 1. MABTrap Kit.

Medium characteristics

Protein G, a cell surface protein of Group G streptococci, is a Type III Fc receptor that binds to the Fc region of IgG by a non-immune mechanism similar to that of protein A of *Staphylococcus aureus*. Protein G and protein A, however, have different IgG binding specificities, dependent on the origin of the IgG. Compared with protein A, protein G binds more strongly to polyclonal IgG from, for example, cow, sheep and horse.

Furthermore, unlike protein A, protein G binds polyclonal rat IgG, human IgG₃ and mouse IgG₁ (Table 2).

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 sample concentration. As a reference, the binding capacity for human IgG is approximately 25 mg IgG/ml medium. GE Healthcare's 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 Sepharose High Performance is produced by coupling protein G to highly cross-linked agarose beads by the N-hydroxysuccinimide activation method. The result is a high capacity medium with high performance chromatographic properties.

Detailed characteristics of HiTrap Protein G HP 1 ml column can be found in Table 3.



Column

The HiTrap column is made of biocompatible polypropylene, which is compatible and noninteractive with biomolecules. The column has porous top and bottom frits which allow high flow rates. Operation is easy, either using the syringe and the Luer adapter provided or, alternatively, using a laboratory pump.

Table 2. Relative binding strengths of 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		++++	+++
Rat	IgG ₁	-	+
	IgG _{2a}	-	++++
	IgG _{2b}	-	++
	IgG ₃	+	++
Sheep		+/-	++

* Purify using HiTrap IgM Purification HP columns.

† Purify using HiTrap IgY Purification HP columns.

++++ = strong binding

++ = medium binding

+/- = weak or no binding

Buffers

MABTrap Kit includes buffer concentrates for binding, elution and neutralization. The buffers have been prepared using the highest quality salts and water, and have been filtered through a 0.22 µm filter.

Table 3. Characteristics of HiTrap Protein G HP.

Bed volume	1 ml
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 bead size	34 µm
Bead structure	Highly cross-linked spherical agarose
Maximum flow rate	4 ml/min (~2 drops/sec)
Chemical stability	All commonly used buffers
pH stability:	
Short term *	2-9
Long term **	3-9
Storage buffer	20% ethanol

* Refers to the pH interval for regeneration, cleaning-in-place and sanitization procedures.

** Refers to the pH interval where the medium is stable over a long period of time without adverse effects on its subsequent chromatographic performance.

Operation

Complete, easy-to-follow instructions, including buffer preparation, sample preparation and purification protocol are included with MABTrap Kit.

Just a few short steps will give you purified IgG. A summary of the method follows.

1. Allow column and buffers to warm to room temperature.
2. Dilute buffers and prepare sample.
3. Connect the syringe to the column through the Luer adapter.
4. Equilibrate the column with 5 ml distilled H₂O and 3 ml diluted binding buffer (Fig 2a).
5. Apply the sample (Fig 2b).
6. Wash with 5-10 ml diluted binding buffer until no material appears in the effluent.
7. Elute with 3-5 ml diluted elution buffer. Collect fractions into tubes containing neutralizing buffer (Fig 2c).
8. Re-equilibrate the column with 5 ml diluted binding buffer.
9. Store the column in 20% ethanol at 4°C to 8°C.
10. Store MABTrap Kit at 4°C to 8°C.



Fig 2. Syringe operation of HiTrap Protein G HP 1 ml.

Storage

When not in use, HiTrap Protein G HP 1 ml should be washed with 10 ml 20% ethanol and stored in this solution. The Binding Buffer and Neutralizing Buffer contain 20% ethanol as a preservative. Store the entire MAbTrap Kit at 4°C to 8°C.

Applications

MAbTrap Kit is designed for fast purification of polyclonal IgG from serum, monoclonal antibodies from cell culture supernatants and ascites fluid, as well as the isolation of immune complexes. The applications shown below illustrate the use of MAbTrap Kit for the isolation of mouse monoclonal antibodies from cell culture supernatant.

Purification of mouse monoclonal IgG₁ from cell culture supernatant

In this application IgG was purified using MAbTrap Kit operated both with a syringe (Fig 2) and a peristaltic pump (Fig 3). In both cases the same purification procedure was used.

Equilibration:	5 ml diluted binding buffer
Sample application:	10 ml mouse monoclonal cell supernatant
Washing:	7 ml diluted binding buffer
Elution:	5 ml diluted elution buffer.
Neutralization:	75 µl neutralizing buffer added per ml fraction
Re-equilibration:	5 ml diluted binding buffer
Analysis of fractions:	SDS-electrophoresis on PhastSystem™ using PhastGel™ Gradient 10–15 and silver staining

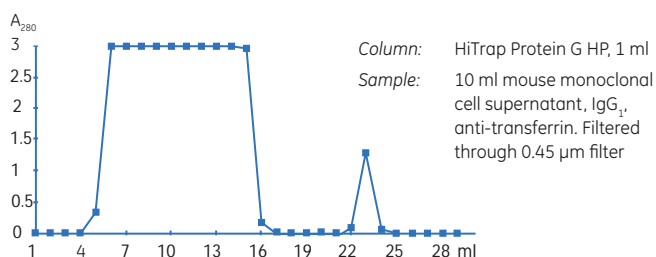


Fig 3. Purification of mouse monoclonal IgG₁ from cell culture supernatant with syringe operation.

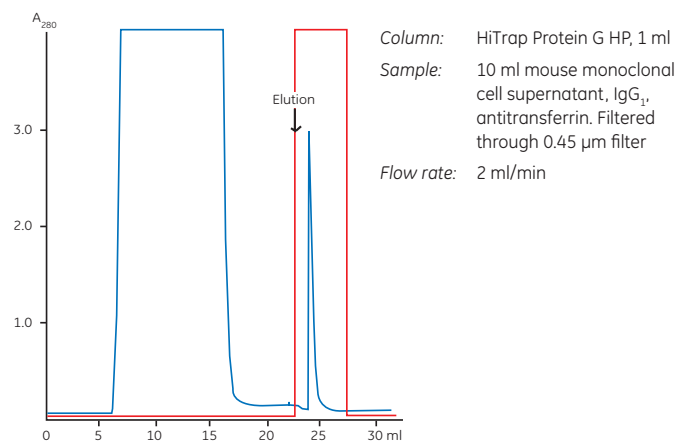


Fig 4. Purification of mouse monoclonal IgG₁ from cell culture supernatant with pump operation.

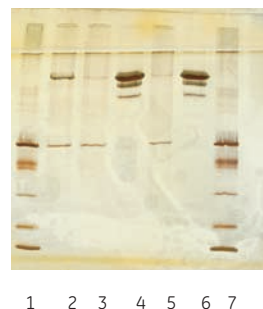


Fig 5. SDS-electrophoresis on Phast System using PhastGel Gradient 10-15 and silver staining of fractions from mouse IgG₁ purification on MAbTrap Kit.

Results

Operating HiTrap Protein G HP 1 ml with a syringe resulted in an IgG₁ pool of 3 ml with an absorbance of 0.44 (A_{280}) and a corresponding yield of 0.9 mg pure mouse monoclonal IgG₁. A similar experiment in which the column was operated with a P-1 pump resulted in an IgG₁ pool of 2 ml with an absorbance of 0.60 (A_{280}), corresponding to a total yield of 0.9 mg pure mouse monoclonal IgG₁. The result of the electrophoretic analysis of both separations is shown in Figure 5.

Purification of mouse monoclonal IgG_{2b} from cell culture supernatant

Mouse IgG_{2b} was purified from 10 ml cell culture supernatant (Fig 6) using the same procedure outlined for the purification of mouse IgG₁ (Fig 4).

Results

Operating HiTrap Protein G HP 1 ml with a P-1 pump resulted in an IgG_{2b} pool of 2 ml with an absorbance of 1.48 (A_{280}), corresponding to a total yield of 2.1 mg pure mouse monoclonal IgG_{2b}. A similar experiment in which the column was operated with a syringe resulted in an IgG_{2b} pool of 2 ml with an absorbance of 1.52 (A_{280}), with a corresponding yield of 2.2 mg pure mouse monoclonal IgG_{2b}. The result of the electrophoretic analysis of both separations is shown in Figure 7.

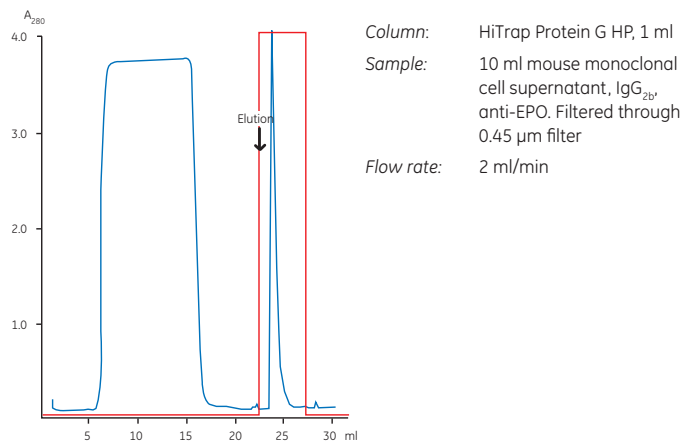


Fig 6. Purification of mouse monoclonal IgG_{2b} from cell culture supernatant with pump operation.

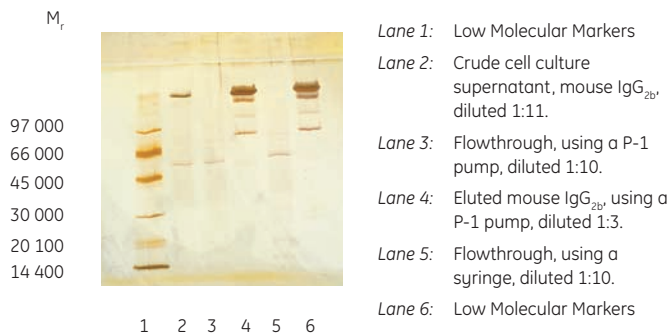


Fig 7. SDS-electrophoresis on PhastSystem using PhastGel Gradient 10-15 and silver staining of fractions from mouse IgG_{2b} purification on MAbTrap Kit.

Ordering Information

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

Related products	Quantity	Code No.
HiTrap Desalting	5 × 5 ml	17-1408-01
HiPrep™ 26/10 Desalting	1 (53 ml)	17-5087-01
	4 (53 ml)	17-5087-02

Related literature	Quantity	Code No.
Antibody Purification Handbook	1	18-1037-46
Affinity Chromatography Handbook, Principles and Methods	1	18-1022-29
Affinity Columns and Media, Selection Guide	1	18-1121-86
Convenient Protein Purification, HiTrap Column Guide	1	18-1129-81

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