# MAbTrap Kit

AFFINITY CHROMATOGRAPHY

MAbTrap™ Kit from Cytiva 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 premade 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 to 15 minutes.

**Table 1.** MAbTrap Kit

| | |
|---|---|
| Column | HiTrap Protein G HP, 1 ml |
| Binding Buffer | 50 ml, 10 × concentrate |
| Elution Buffer | 15 ml, 10 × 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 $IgG_3$ and mouse $IgG_1$ (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. Cytiva'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 | varible | — |
| | 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

# 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) |
| Column hardware pressure limit | 5 bar (0.5 MPa, 70 psi) |
| Chemical stability | All commonly used buffers |
| pH stability: | |
| Working* | 3 to 9 |
| Cleaning† | 2 to 9 |
| Storage buffer | 20% ethanol |

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

# 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 $H_2O$ and 3 ml diluted binding buffer (Fig 2A).
5. Apply the sample (Fig 2B).
6. Wash with 5 to 10 ml diluted binding buffer until no material appears in the effluent.
7. Elute with 3 to 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.

(A)

(B)

(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 $IgG_1$ 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 |
| *Column:* | HiTrap Protein G HP, 1 ml |
| *Sample:* | 10 ml mouse monoclonal cell supernatant, $IgG_1$, anti-transferrin. Filtered through 0.45 µm filter |

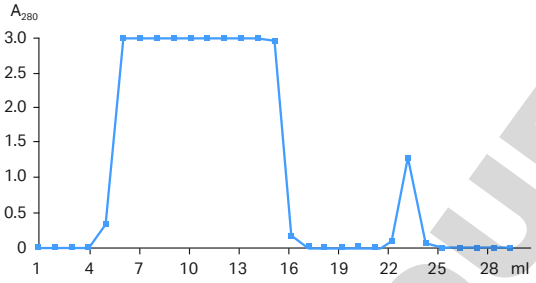



**Fig 3.** Purification of mouse monoclonal $IgG_1$ from cell culture supernatant with syringe operation.

| | |
|---|---|
| *Column:* | HiTrap Protein G HP, 1 ml |
| *Sample:* | 10 ml mouse monoclonal cell supernatant, $IgG_1$, antitransferrin. Filtered through 0.45 µm filter |
| *Flow rate:* | 2 ml/min |

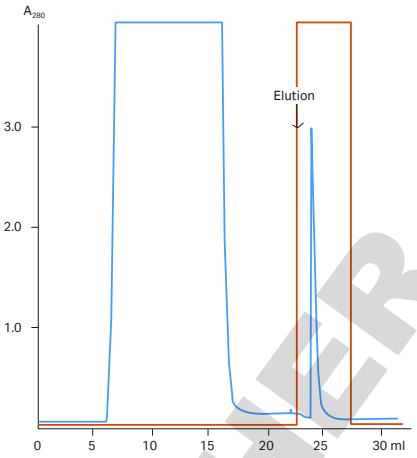
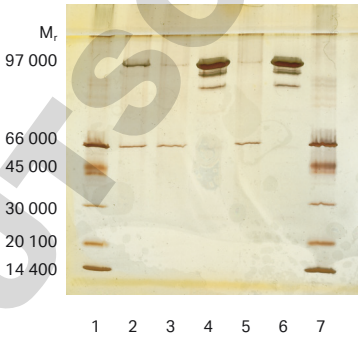



**Fig 4.** Purification of mouse monoclonal $IgG_1$ from cell culture supernatant with pump operation.




| | |
|---|---|
| Lane 1: | Low Molecular Markers |
| Lane 2: | Crude cell culture supernatant, mouse $IgG_1$ diluted 1:11. |
| Lane 3: | Flowthrough, using a P-1 pump, diluted 1:10. |
| Lane 4: | Eluted mouse $IgG_1$, using a P-1 pump. |
| Lane 5: | Flowthrough, using a syringe, diluted 1:10. |
| Lane 6: | Eluted mouse $IgG_1$ using a syringe. |
| Lane 7: | Low Molecular Markers |

**Fig 5**. SDS-electrophoresis on PhastSystem using PhastGel Gradient 10-15 and silver staining of fractions from mouse $IgG_1$ purification on MAbTrap Kit.

## Results

Operating HiTrap Protein G HP 1 ml with a syringe resulted in an $IgG_1$ pool of 3 ml with an absorbance of 0.44 ($A_{280}$) and a corresponding yield of 0.9 mg pure mouse monoclonal $IgG_1$. A similar experiment in which the column was operated with a P-1 pump resulted in an $IgG_1$ pool of 2 ml with an absorbance of 0.60 ($A_{280}$), corresponding to a total yield of 0.9 mg pure mouse monoclonal $IgG_1$. 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 $IgG_1$ (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.

*Column:* HiTrap Protein G HP, 1 ml
*Sample:* 10 ml mouse monoclonal cell supernatant, $IgG_{2b}$, anti-EPO. Filtered through 0.45 µm filter
*Flow rate:* 2 ml/min

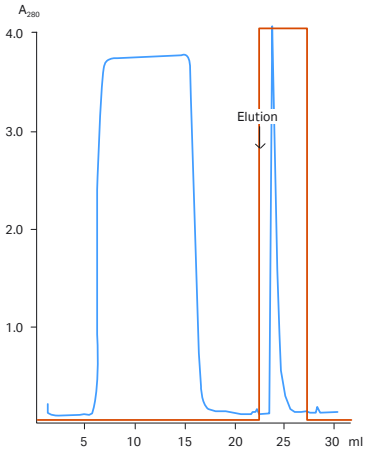
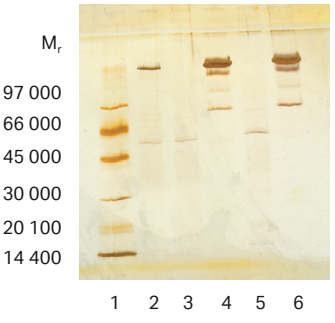



**Fig 6.** Purification of mouse monoclonal $IgG_{2b}$ from cell culture supernatant with pump operation.




Lane 1: Low Molecular Markers
Lane 2: Crude cell culture supernatant, mouse $IgG_{2b}$, diluted 1:11.
Lane 3: Flowthrough, using a P-1 pump, diluted 1:10.
Lane 4: Eluted mouse $IgG_{2b}$, using a P-1 pump, diluted 1:3.
Lane 5: Flowthrough, using a syringe, diluted 1:10.
Lane 6: Low Molecular Markers

**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 number |
|---|---|---|
| MAbTrap Kit | 1 | 17-1128-01 |

| Related products | Quantity | Code number |
|---|---|---|
| HiTrap Desalting | 5 × 5 ml | 17-1408-01 |
| HiTrap Desalting | 1 × 5 ml | 29-0486-84 |
| HiTrap Desalting | 100 × 5 ml* | 11-0003-29 |
| HiPrep™ 26/10 Desalting | 1 × 53 ml | 17-5087-01 |
| HiPrep 26/10 Desalting | 4 × 53 ml | 17-5087-02 |

* Pack size available by special order.

| Accessories | Quantity | Code number |
|---|---|---|
| 1/16" male/luer female* | 2 | 18-1112-51 |
| Tubing connector flangeless/M6 female | 2 | 18-1003-68 |
| Tubing connector flangeless/M6 male | 2 | 18-1017-98 |
| Union 1/16" female/M6 male | 6 | 18-1112-57 |
| Union M6 female /1/16" male | 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"† | 5 | 11-0004-64 |
| Fingertight stop plug, 1/16"‡ | 5 | 11-0003-55 |

* One connector included in each HiTrap package.
† Two, five, or seven stop plugs female included in HiTrap packages depending on products.
‡ One fingertight stop plug is connected to the top of each HiTrap column at delivery.

| Related literature | Code number |
|---|---|
| Antibody Purification Handbook | 18-1037-46 |
| Affinity Chromatography Handbook, Principles and Methods | 18-1022-29 |
| Affinity Chromatography Columns and Media Product Profile | 18-1121-86 |
| Convenient Protein Purification, HiTrap Column Guide | 18-1129-81 |

**cytiva.com/hitrap**
**cytiva.com/protein-purification**



CY14295-11Sep20-DF