HiTrap IgM Purification HP

AFFINITY CHROMATOGRAPHY

HiTrap™ IgM Purification HP is a 1 ml column designed for fast and efficient purification of monoclonal IgM from hybridoma cell cultures. The prepacked columns are easily connected with a syringe, a pump, an ÄKTA™ system, or other chromatography systems. With this column, IgM purification becomes a simple, one-step elution procedure.

Key characteristics of HiTrap IgM Purification HP columns include:

- Fast and easy purification of monoclonal IgM
- High purity with retained activity
- Convenient use

Medium characteristics

HiTrap IgM Purification HP columns are packed with a thiophilic adsorption medium with 2-mercaptopyridine coupled to Sepharose™ High Performance. Thiophilic adsorption is promoted by water-structuring salts. The interaction between protein and ligand has been suggested to result from a combined electron donating and accepting action of the ligand, or alternatively as a mixed mode hydrophilic-hydrophobic interaction.

The base matrix, Sepharose High Performance, has good flow properties as well as high physical and chemical stability. The main application of HiTrap IgM Purification HP is purification of IgM from hybridoma cell cultures.

Column characteristics

HiTrap columns are made of polypropylene, which is biocompatible with biomolecules. Top and bottom frits are manufactured from porous polyethylene. The columns are delivered with a stopper on the inlet and a snap-off end on the outlet.

The characteristics of HiTrap IgM Purification HP columns are shown in Table 1.

Fig 1. HiTrap IgM Purification HP 1 ml column.

Table 1. Characteristics of HiTrap IgM Purification HP columns

Column volume	1 ml
Column dimensions	0.7 × 2.5 cm
Ligand	2-mercaptopyridine
Ligand concentration	2 mg/ml medium
Binding capacity	5 mg human IgM/ml medium
Mean particle size	34 µm
Matrix	Highly cross-linked spherical agarose
Recommended flow rate	1 ml/min
Maximum flow rate[1]	4 ml/min
Column hardware pressure limit	5 bar (0.5 MPa, 70 psi)
pH stability[2]	
Working	3 to 11
Cleaning	2 to 13
Temperature stability	
Regular use	4°C to room temperature
Storage	4°C to 8°C
Storage buffer	20% ethanol

[1] Room temperature, aqueous buffers

[2] The ranges given are estimates based on our knowledge and experience. Please note the following:

pH stability, working 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, cleaning refers to the pH interval for regeneration, cleaning-in-place and sanitization procedures.

Fast and easy purification of IgM

Purifying IgM is a fast and easy procedure with HiTrap IgM Purification HP. Simply attach the column to the selected equipment, apply sample, wash, and then elute the bound IgM in a single step. See Figure 2 for an example of use with a syringe. Complete, easy to follow "step-by-step" instructions for fast start up and method optimization, together with recommendations for sample preparation, are included with the columns.

For large sample amounts, several columns can be easily connected in series. The columns cannot be opened and repacked.

Operation

As for all HiTrap columns, HiTrap IgM Purification HP is convenient to use. A set of connectors supplied with the column enables easy connection to a syringe, a peristaltic pump, or a liquid chromatography system.

(A)

(B)

(C)

Fig 2. Using HiTrap IgM Purification HP with a syringe. (A) Prepare buffers and sample. Remove the top cap of the column and snap off the end. Wash and equilibrate. (B) Load the sample and begin collecting fractions. (C) Wash, elute and continue collecting fractions.

High purity

Results from the purification of monoclonal α-Shigella IgM from hybridoma cell culture supernatant show that the eluted IgM is more than 80% pure and retains its high activity (see Applications).

Applications

Monoclonal α-Shigella IgM from a hybridoma cell culture supernatant was purified on HiTrap IgM Purification HP. After sample loading the column was washed and IgM was eluted with sodium phosphate. Remaining impurities were eluted with 30% isopropanol (Fig 3).

Column: HiTrap IgM Purification HP 1 ml
Sample: 75 ml of cell culture supernatent containing α-Shigella IgM, filtered through a 0.45 µm filter
Binding buffer: 20 mM sodium phosphate buffer, 0.5 M potassium sulphate, pH 7.5
Elution buffer: 20 mM sodium phosphate buffer, pH 7.5
Cleaning buffer: 20 mM sodium phosphate buffer, 30% isopropanol, pH 7.5
Flow rate: 1 ml/min
System: ÄKTAexplorer 10S

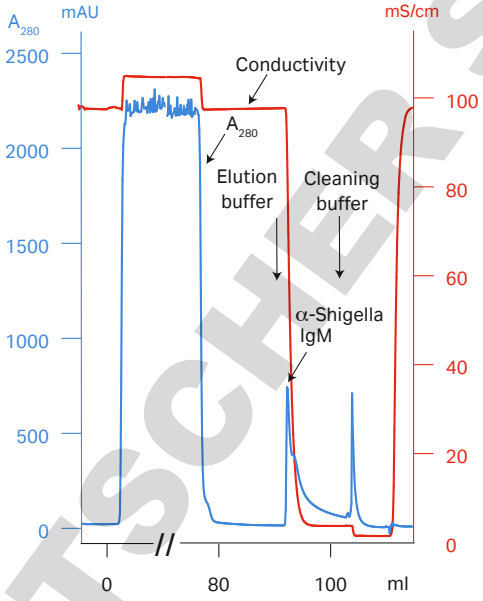


Fig 3. Purification of α-Shigella IgM on HiTrap IgM Purification HP.

ELISA detection of α-Shigella IgM

Results from ELISA showed that active protein was eluted and that all IgM included in the large sample volume bound to the column, Table 2.

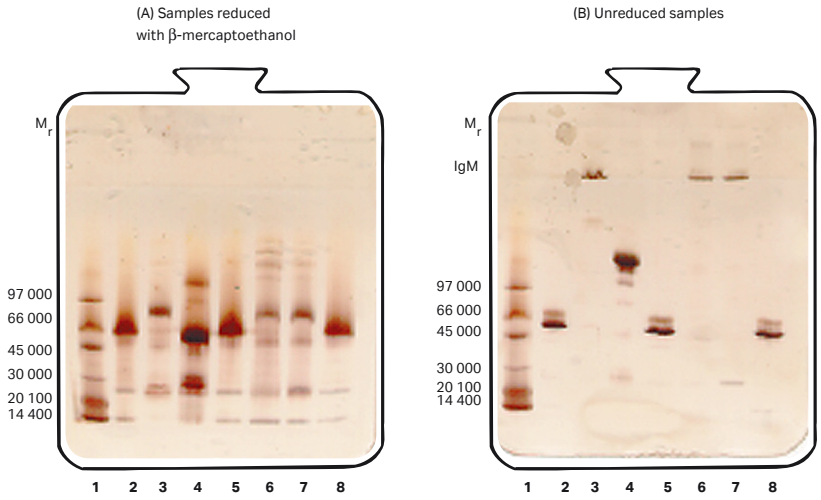
Table 2. Activity of α-Shigella IgM measured by ELISA

Sample	α-Shigella IgM conc. (mg/ml)	Volume (ml)	Recovery (%)
Cell culture supernantant	0.01	100	100
Flow-through pool, 0 to 90 ml	n.d.[1]	90	0
Flow-through pool, 90 to 100 ml	n.d.	10	0
Eluate	0.1 to 0.3	9	> 100%
Cleaning eluate	approx. 0.1	1	approx. 10

[1] n.d. = not detectable

Determination of purity

The identity and purity of the eluted IgM fractions were measured by SDS-PAGE electrophoresis and by analytical gel filtration (see Figures 4a, 4b, and 5). The results show excellent purity, > 80%, of the collected IgM fractions.



Lane 1: Low Molecular Marker, LMW
Lane 2: Cell culture supernatant, starting material, dil. 20×
Lane 3: Human IgM (standard)
Lane 4: IgG (standard)
Lane 5: Flow-through pool, dil. 20×
Lane 6: Eluted IgM, fraction 8, dil. 8×
Lane 7: Eluted IgM, fraction 9, dil. 8×
Lane 8: Washing out unbound material, pool dil. 3×

Fig 4. SDS-PAGE on PhastSystem™, using PhastGel™ 4–15, silver staining.

Column: Superdex™ 200, 10 × 300 mm

Sample: 250 µl of pooled α-Shigella IgM eluate from HiTrap IgM Purification HP

Buffer: 20 mM sodium phosphate buffer, 0.15 M sodium chloride, pH 7.2

Flow rate: 0.5 ml/min

System: ÄKTAexplorer 10S

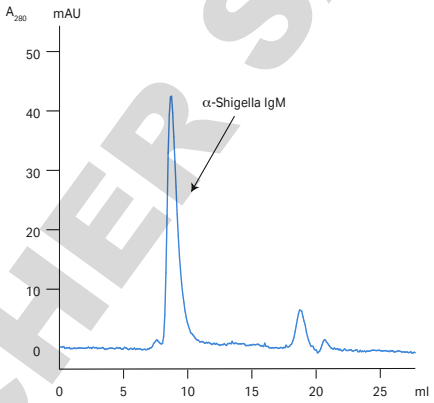


Fig 5. Gel filtration on Superdex 200, 10 × 300 mm column.

Table 3. Physio-chemical properties of human immunoglobulins

Immunoglobulin	Heavy chain	Light chain	Sedimentation coefficient	Mol. Wt (M_r)	M_r heavy chain	Carbohydrate content (%)	A_{280nm}	pI
IgG_1	λ_1	κ, λ	7S	146 000	50 000	2–3	13.8	5.0–9.5
IgG_2	λ_1	κ, λ	7S	146 000	50 000	2–3		5.0–8.5
IgG_3	λ_1	κ, λ	7S	170 000	60 000	2–3		8.2–9.0
IgG_4	λ_1	κ, λ	7S	146 000	50 000	2–3		5.0–6.0
IgM	μ	κ, λ	19S	900 000	68 000	12	12.5	5.1–7.8
IgA_1	α_1	κ, λ	7S	160 000	56 000	7–11	13.4	5.2–6.6
IgA_2	α_2	κ, λ	7S	160 000	52 000	7–11		5.2–6.6
IgA_s	α_1, α_2	κ, λ	11S	370 000	52–56 000	11		4.7–6.2
IgD	δ	κ, λ	7S	184 000	68 000	12	17.0	–
IgE	ε	κ, λ	8S	190 000	72 000	12	15.3	–

Table 4. Physio-chemical properties of mouse immunoglobulins

Immunoglobulin	Heavy chain	Light chain	Sedimentation coefficient	Mol. Wt (M_r)	M_r heavy chain	Carbohydrate content (%)	pI
IgG_1	λ_1	κ, λ	7S	150 000	50 000	2–3	7.0–8.5
IgG_{2a}	λ_{2a}	κ, λ	7S	150 000	50 000	2–3	6.5–7.5
IgG_{2b}	λ_{2b}	κ, λ	7S	150 000	50 000	2–3	5.5–7.0
IgG_3	λ_3	κ, λ	7S	150 000	50 000	2–3	–
IgM	μ	κ, λ	19S	900 000	80 000	12	4.5–7.0
IgA	α	κ, λ	7S	170 000	70 000	7–11	4.0–7.0
IgD	δ	κ, λ	7S	180 000	68 000	12–14	–
IgE	ε	κ, λ	8S	190 000	80 000	12	–

Ordering information

Products	Quantity	Code number
HiTrap IgM Purification HP	5 × 1 ml	17-5110-01

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

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 female	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 ÄKTAdesign	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, Principle and Methods	18-1022-29
Convenient Protein Purification, HiTrap Column Guide	18-1129-81

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