

# HiTrap IgY Purification HP

## AFFINITY CHROMATOGRAPHY

HiTrap™ IgY Purification HP is a 5 ml prepacked column designed for fast and easy purification of IgY from egg yolk. This ready to use column enables convenient purification of IgY.

Separation can be easily achieved using a syringe, a pump, an ÄKTA™ system, or other chromatography systems.

- Fast and easy purification of IgY from egg yolk
- Good purity and recovery
- Convenient to use

## Medium characteristics

HiTrap IgY Purification HP columns are packed with a thiophilic adsorption medium, 2-mercaptopyridine coupled to Sepharose™ High Performance. Thiophilic adsorption is promoted by water-structuring salts. The interaction between protein and ligand is considered 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 is a rigid, highly cross-linked, beaded agarose with good flow properties and great physical and chemical stability.

The main application of HiTrap IgY Purification HP is purification of IgY from egg yolk.

## Column characteristics

HiTrap IgY Purification HP is a 5 ml column made of polypropylene, a material which is biocompatible and does not interact with biomolecules. The top and bottom frits are manufactured from porous polyethylene. The column is delivered with a stopper on the inlet and a twist-off end on the outlet.

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



Fig 1. Prepacked 5 ml HiTrap IgY Purification HP.

Table 1. HiTrap IgY Purification HP characteristics

Column volume	5 ml
Column dimensions, i.d. × h	1.6 × 2.5 cm
Ligand	2-mercaptopyridine
Ligand concentration	3 mg/ml
Binding capacity	100 mg pure IgY/column 1/4 egg yolk/column
Average particle size	34 µm
Bead structure	highly cross-linked spherical agarose
Recommended flow rate	5 ml/min
Maximum flow rate <sup>1</sup>	20 ml/min
Column hardware pressure limit	5 bar (0.5 MPa, 70 psi)
pH stability <sup>2</sup>	
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

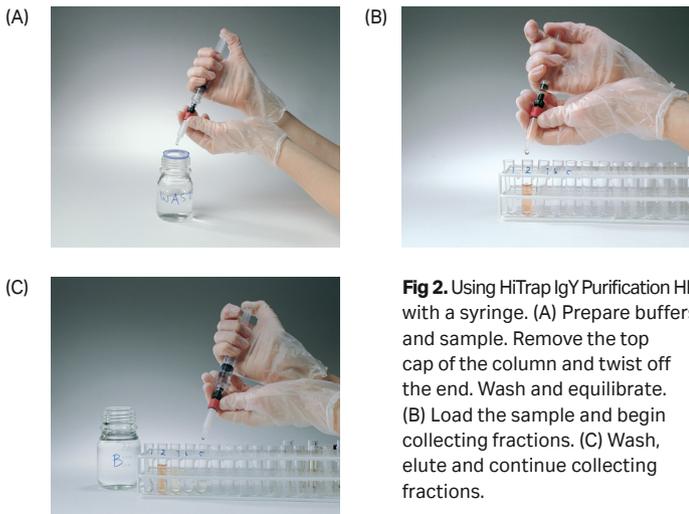
<sup>1</sup> Room temperature, aqueous buffers

<sup>2</sup> 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.

# One-step separation of IgY

Purifying IgY is a fast and easy procedure with HiTrap IgY Purification HP — prepare the sample, apply the sample to the column, wash and elute the IgY. See Figure 2 for usage with a syringe. For larger samples, several columns can easily be connected in series (backpressure will increase).

For a fast and simple start, easy-to-follow instructions covering both sample preparation and method optimization are supplied with the column.



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

## Operation

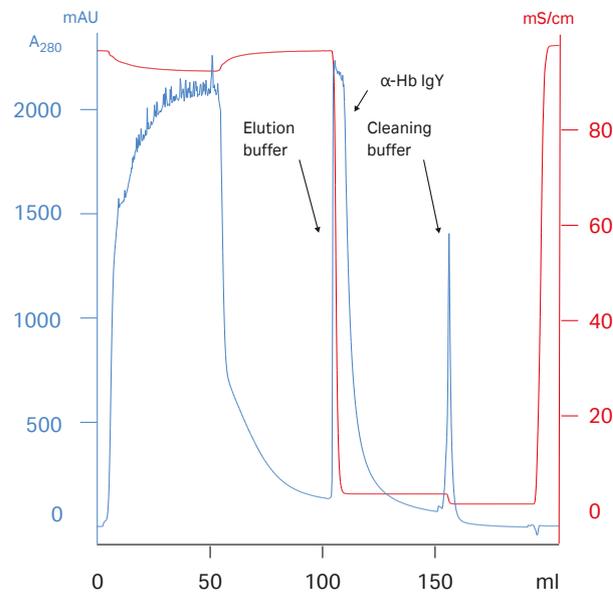
As for all HiTrap columns, HiTrap IgY Purification HP is convenient to use. Instructions and connectors are included with each pack of columns.

Columns cannot be opened and repacked.

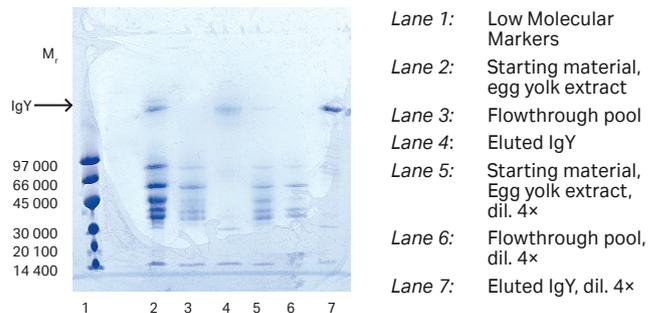
## High purity and recovery

The purity of the IgY obtained after a single-step separation on HiTrap IgY Purification HP is usually more than 70%. As an example, the recovery of  $\alpha$ -Hb IgY was approximately 80%, see Figures 3 and 4.

**Sample:** 45 ml of egg yolk extract (corresponding to 1/4 of an egg yolk) containing  $\alpha$ -Hb IgY, filtered through a 0.45  $\mu$ m filter  
**Column:** HiTrap IgY Purification HP 5 ml  
**Binding buffer:** 20 mM sodium phosphate buffer, 0.5 M potassium sulfate, 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:** 5 ml/min  
**Instrumentation:** ÄKTAexplorer 10S



**Fig 3.** Purification of IgY on HiTrap IgY Purification HP.



**Fig 4.** SDS-PAGE of unreduced samples on PhastSystem™, using PhastGel™ 4% to 15%, Coomassie™ Blue staining.

## Applications

The lipids from an egg yolk were removed by precipitation with water and centrifugation at 4°C.

One quarter of an egg yolk was applied to HiTrap IgY Purification HP, the column was washed and IgY was eluted in a single step with sodium phosphate buffer. Remaining lipids and other impurities were washed out with 30% isopropanol, see Figure 3.

Purity of the IgY was checked with SDS-PAGE and the results show a purity of more than 70%, see Figure 4.

Recovery of the  $\alpha$ -Hb IgY applied to the column was high, approximately 80%, as shown in Table 2.

**Table 2.** Recovery of  $\alpha$ -Hb IgY from HiTrap IgY Purification HP

Sample	$\alpha$ -Hb IgY conc. ( $\mu$ g/ml)	Volume (ml)	Recovery (%)
Egg yolk extract	3.3	45	100
Flow-through pool	n.d. <sup>1</sup>	60	n.d.
Eluate	5.8	20	78

<sup>1</sup> n.d. = not detectable

## Ordering information

Products	Quantity	Code number
HiTrap IgY Purification HP	1 × 5 ml	17-5111-01
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" <sup>†</sup>	5	11-0004-64
Fingertight stop plug, 1/16" <sup>‡</sup>	5	11-0003-55

\* One connector included in each HiTrap package

<sup>†</sup> Two, five, or seven stop plugs female included in HiTrap packages depending on products.

<sup>‡</sup> 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, Selection guide	18-1121-86
Convenient Protein Purification, HiTrap Column guide	18-1129-81

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