**Instructions 71-5004-38 AL**

# HiTrap™ IgY Purification HP, 5 ml

HiTrap IgY Purification HP is a prepacked, ready to use, column for purification of IgY from egg yolk. The special design of the column, together with the matrix, provides fast, simple and easy separations in a convenient format. The column can be operated with a liquid chromatography system such as ÄKTA™. It can also be used with a peristaltic pump or a simple 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, 5 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) | 5 ml |
| Column dimensions | 1.6 x 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.*

### Supplied Connector kit with HiTrap column

| Connectors supplied | Usage | No. supplied |
|---|---|---|
| Union 1/16" male/ luer female | For connection of syringe to HiTrap column | 1 |
| Stop plug female, 1/16" | For sealing bottom of HiTrap column | 2, 5 or 7 |

## Medium properties

HiTrap IgY Purification HP is a thiophilic adsorption medium with 2-mercaptopyridine coupled to Sepharose™ High Performance.

Thiophilic adsorption was first described by Porath et al. (*FEBS Lett., 1985, 185, 306–310*) and 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 hydrophilichydrophobic interaction.

The base-matrix is a rigid, highly cross-linked, beaded agarose with high chemical stability.

The main application area for HiTrap IgY Purification HP is purification of IgY from chicken egg yolk, but it can also be used for purification of other immunoglobulins.

**Table 2.** HiTrap IgY Purification HP characteristics

| | |
|---|---|
| Ligand | 2-mercaptopyridine |
| Ligand concentration | 3 mg/ml |
| Binding capacity[1] | 100 mg pure IgY per 5 ml column<br>1/4 egg yolk per 5 ml column |
| Mean particle size | 34 µm |
| Bead structure | Highly cross-linked spherical agarose |
| Maximum flow rate | 20 ml/min |
| Recommended flow rate | 5 ml/min |
| pH stability[2] | |
| Working range | 3 to 11 |
| Cleaning-in-place | 2 to 13 |
| Storage | 4°C to 30°C in 20% ethanol |

[1] Running conditions according to the recommendations found in Section 2 Operation.
[2] Working range pH: pH interval where the medium can be operated without significant change in function.
Cleaning-in-place pH: pH stability where the medium can be subjected to cleaning- or sanitization-in-place without significant change in function.

# 2 Operation

## Buffer preparation

Water and chemicals used for buffer preparation should be of high purity. It is recommended to filter the buffers by passing them through a 0.45 µm filter before use.

**Binding buffer:** 20 mM sodium phosphate, 0.5 M $K_2SO_4$, pH 7.5.

**Elution buffer:** 20 mM sodium phosphate, pH 7.5.

**Cleaning buffer:** 20 mM sodium phosphate, pH 7.5 with 30% isopropanol

## Sample preparation

As many of the egg yolk lipids as possible must be removed before purification. These can be precipitated using different methods, for example with water or PEG. Precipitation with water is described below.

Separate the egg yolk from the egg white. To one part egg yolk add nine parts of distilled water. Mix and stir slowly for 6 hours at 4°C. Centrifuge at 10 000 × g, at 4°C for 25 minutes to precipitate the lipids. Collect the supernatant containing the IgY.

While stirring slowly, add $K_2SO_4$ to the sample to a final concentration of 0.5 M. Adjust the pH to 7.5. Pass the sample through a 0.45 µm filter immediately before applying to the HiTrap IgY Purification HP column.

## Purification

The purification capacity for HiTrap IgY Purification HP is approximately 1/4 of an egg yolk per 5 ml column. This capacity can be increased by connecting two or more columns in series (back pressure will increase). The recommended flow rate for HiTrap IgY Purification HP is 5 ml/min.

1. Remove the stopper.
2. Fill the syringe or pump tubing with binding buffer. Using the connector provided, connect the column "drop to drop" to the syringe, or pump tubing, to avoid introducing air into the column.
3. Remove the snap-off end at the column outlet.
4. Wash the column with at least 5 column volumes (CV) of each buffer: Binding, elution and cleaning buffers.
5. Equilibrate the column with 5 CV of binding buffer.
6. Apply the sample, using a syringe fitted to the luer adapter or by pumping it onto the column.
7. Wash with at least 10 CV of binding buffer or until no material appears in the effluent.

**8** Elute the IgY with 10 CV of elution buffer.

**9** Regenerate the column with 8 CV of cleaning buffer.

**10** Re-equilibrate the column with 5 CV of binding buffer.

**Note:** *The reuse of HiTrap IgY Purification HP depends on the nature of the sample. To prevent cross-contamination, it should only be reused with identical samples.*

**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.*

### Binding

To improve recovery of total IgY or a specific IgY antibody the 0.5 M $K_2SO_4$ in the binding buffer can be replaced with 0.6 to 0.8 M $Na_2SO_4$. The sample should have the same concentration of $Na_2SO_4$ as the binding buffer. An increase in salt concentration will, however, adversely affect the purity of the eluted IgY.

### Elution

The purity of the eluted IgY may be improved by using gradient elution with, for example, a linear gradient 0–100% elution buffer over 10 column volumes, followed by elution at 100% elution buffer for a few column volumes. Gradients can be achieved by use of a liquid chromatography system such as ÄKTA system.

# 3 Scaling up

For quick scale-up of purification, two or three HiTrap columns can be connected in series (back pressure will increase).

# 4 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.*

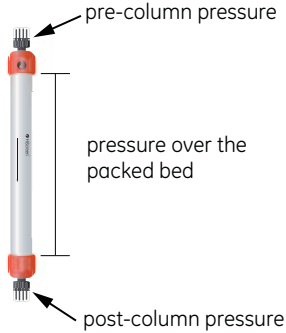


**Fig 2.** Pre-column and post-column measurements.

### ÄKTA avant and ÄKTA pure

The system will automatically monitor the pressures (pre-column pressure and pressure over the packed bed, $\Delta$p). 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 ($\Delta$p) will during run be equal to actual measured pressure - *total system pressure* (P1).

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

# 5 Storage

Store the column at 4°C to 30°C in 20% ethanol.

# 6 Ordering Information

| Product | Quantity | Code No. |
|---|---|---|
| HiTrap IgY Purification HP | 1 × 5 ml | 17-5111-01 |

| Related products | Quantity | Code No. |
|---|---|---|
| HiTrap Desalting | 1 × 5 ml | 29-0486-84 |
| | 5 × 5 ml | 17-1408-01 |
| PD-10 Desalting Column | 30 | 17-0851-01 |

| Accessories | Quantity | Code No. |
|---|---|---|
| 1/16" male/luer female<br>*(For connection of syringe to top of HiTrap column)* | 2 | 18-1112-51 |
| Tubing connector flangeless/M6 female<br>(*For connection of tubing to bottom of HiTrap column)* | 2 | 18-1003-68 |
| Tubing connector flangeless/M6 male<br>*(For connection of tubing to top of HiTrap column)* | 2 | 18-1017-98 |
| Union 1/16" female/M6 male<br>*(For connection to original FPLC System through bottom of HiTrap column)* | 6 | 18-1112-57 |
| Union M6 female /1/16" male<br>*(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"<br>*(For sealing bottom of HiTrap column)* | 5 | 11-0004-64 |
| Fingertight stop plug, 1/16" | 5 | 11-0003-55 |

| Literature | Quantity | Code No. |
|---|---|---|
| Affinity Chromatography Handbook, Principles and Methods | 1 | 18-1022-29 |
| Affinity Chromatography Columns and Media, Selection Guide | 1 | 18-1121-86 |
| Prepacked chromatography columns for ÄKTA systems, Selection guide | 1 | 28-9317-78 |

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71-5004-38 AL 04/2014