



Capto™ adhere ImpRes

Multimodal chromatography resin

Instructions for Use

Capto™ adhere ImpRes is a multimodal BioProcess™ chromatography resin for polishing monoclonal antibodies and other recombinant/native proteins by packed bed chromatography.

The combination of the high-flow agarose used for all Capto products and the small particle size of Capto ImpRes results in good pressure-flow properties as well as an impressive resolution. Capto adhere ImpRes provides:

- a high-resolution chromatography resin based on the well-established Capto ImpRes platform
- innovative selectivity of the multimodal ligand
- efficient design of robust and scalable polishing processes using convenient tools and formats
- high productivity enabling improved process economy

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Read these instructions carefully before using the products.

Safety

For use and handling of the products in a safe way, refer to the Safety Data Sheets.

1 BioProcess chromatography resins

BioProcess chromatography resins are developed and supported for production-scale chromatography. All BioProcess resins are produced with validated methods and are tested to meet the manufacturing requirements. Secure ordering and delivery routines give a reliable supply of resins for production scale. Regulatory Support Files (RSF) are available to assist the process validation and submissions to regulatory authorities. BioProcess resins cover all purification steps from capture to polishing.

2 Resin properties

Multimodal functionality

Capto adhere ImpRes has a ligand with multimodal functionality (see [Fig. 1, on page 4](#)). The multimodal functionality gives a different selectivity compared to traditional ion exchangers and also provides the possibility of operating in different regions with respect to pH and conductivity.

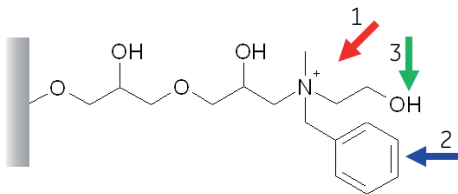


Fig 1. The Capto adhere ImpRes ligand exhibits many functionalities for interaction with a target molecule. The most pronounced are ionic interactions (1), hydrophobic interactions (2), and hydrogen bonding (3). The chromatography resin is designed for polishing, and is based on the high-flow agarose base matrix with a small bead size, which gives good pressure-flow properties and high resolution.

Polishing

Capto adhere ImpRes is designed for post-protein A polishing of monoclonal antibodies. Removal of leached protein A, aggregates, host cell proteins, nucleic acids, and viruses from monoclonal antibodies is performed in either flow-through or bind-elute mode. In flow-through mode the antibody passes directly through the column while the contaminants are bound, while in bind-elute mode the high resolution of the small bead is utilized for separation of antibody monomer from contaminants during elution.

High flow velocities

In spite of the small bead size of Capto adhere ImpRes (average size is 40 μm), the rigid high-flow agarose matrix allows high flow velocities (see [Fig. 2, on page 5](#)).

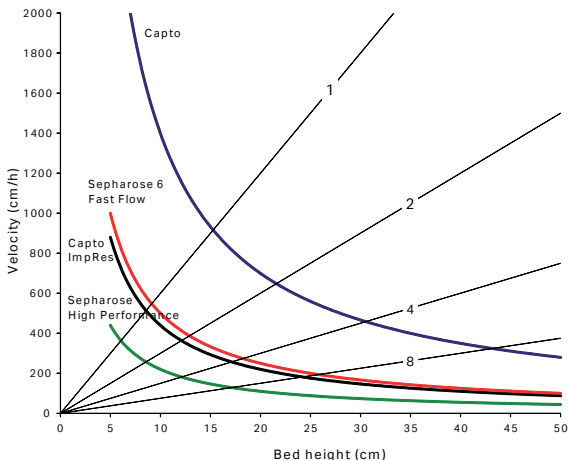


Fig 2. The highly rigid Capto ImpRes matrix allows a large window of operation (area below the curves) at large scale. Data correspond to a 1 m diameter column at 20°C and viscosity equivalent to water. Gray contours (1, 2, 4, 8) give the residence time in the column in minutes.

Characteristics

The highly cross-linked agarose base matrix gives the resin high chemical and physical stability. Characteristics such as capacity, elution behavior, and pressure-flow properties are unaffected by the solutions commonly used in process chromatography and cleaning procedures.

Table 1. Characteristics of Capto adhere ImpRes

Matrix	Highly cross-linked agarose
Functional group	Multimodal strong anion exchanger
Particle size (d_{50v})¹	36 to 44 μm
Total ionic capacity	0.08 to 0.11 mmol Cl^-/mL resin
Maximum flow velocity²	At least 220 cm/h in a 1 m diameter column with 20 cm bed height at 20°C using process buffers with the same viscosity as water at <0.3 MPa (3 bar).
Recommended flow velocity²	100 to 300 cm/h (HiScreen™) and 25 to 50 cm/h (HiTrap™)
Maximum flow velocity	300 cm/h
pH stability	
Operational³	3 to 12
CIP⁴	2 to 14
Working temperature⁵	4°C to 30°C
Chemical stability⁶	All commonly used aqueous buffers, 1 M acetic acid, 1 M NaOH
Storage	4°C to 30°C in 20% ethanol

¹ d_{50v} is the average particle size of the cumulative volume distribution.

² The capacity for selective reduction of some key impurities can decrease at high flow velocity.

³ pH range where resin can be operated without significant change in function.

⁴ pH range where resin can be subjected to cleaning- or sanitization-in-place without significant change in function.

⁵ Capto adhere ImpRes can be used under cold-room conditions, but the capacity for some key impurities can decrease.

⁶ Only use 1 M NaOH for cleaning purposes.

3 Method design and optimization

Aim

The aim of designing and optimizing a method to separate biomolecules is to determine the best conditions for purity of the target molecule, maximizing binding capacity, and achieving the highest product yield. For optimization of binding conditions, pH and conductivity (salt concentration) must be screened.

Screening

When using Capto adhere ImpRes, protein binding can be expected at pH values lower than those used with traditional ion exchangers due to the contribution from non-electrostatic interactions. The multimodal nature of the ligand results in different interaction modes, electrostatic *versus* hydrophobic, depending on buffer conditions and target protein characteristics. A thorough screening of conditions is therefore strongly recommended on Capto adhere ImpRes in order to optimize conditions for binding and elution.

Workflow

The recommended workflow is described in the figure below. It starts with the screening of conditions in high-throughput formats, followed by optimization, preferably by applying Design of Experiments (DoE), in small columns and finally scale-up to large columns.



Fig 3. Recommended workflow during process development.

Prepacked chromatography resins

Prefilled PreDictor™ plates or PreDictor RoboColumn units are recommended for condition screening while prepacked HiScreen columns are recommended for optimization. Optimization in columns is preferably done by using DoE. DoE can also be used to evaluate plate data, but often raw data analysis of plate data is enough to find conditions that can be used during optimization in packed bed. For experimental setup, data handling, analysis and evaluation of raw data from plates, the Assist software from Cytiva is useful.

Examples of screening and optimization of bind-elute applications on Capto adhere ImpRes are found in *Application notes CY20571 and CY13429*.

General purification protocol

Flow-through mode	Bind-elute mode
- Adjust pH and conductivity of the sample to target conditions. - Equilibrate the column with starting buffer with the same pH and conductivity as the sample.	- Adjust pH and conductivity of the sample to target conditions. - Equilibrate the column with starting buffer with the same pH and conductivity as the sample. - Load the sample onto the column.

Flow-through mode	Bind-elute mode
• Load the sample onto the column and collect the flow-through fraction (sample in flowthrough). • Wash out unbound material with starting buffer and collect together with the flow-through fraction. • Regenerate the column at low pH¹. • If necessary, perform a CIP of the column.	• Wash out unbound material with starting buffer. • Elute bound material and collect the elution fraction. • Regenerate the column at low pH¹. • If necessary, perform a CIP of the column.

¹ For example, 0.5 M acetic acid.

Sample preparation

Before sample loading, pH and conductivity of the sample must be adjusted to target loading conditions. This is done either by buffer exchange or by direct adjustment of pH and conductivity. Buffers normally used for ion exchange chromatography can also be used for Capto adhere ImpRes ([Table 2, on page 9](#)).

Table 2. Recommended buffers

pH interval	Buffer ^{1,2}	Concentration ³ (mM)
4–5.5	Acetate	20–100
3–6.5	Citrate	20–200
5.5–6.5	Bis-Tris	20–50
6–7.5	Phosphate	20–200
7.5–9	Tris	20–50
8.8–10.6	Glycin-NaOH	20–100

¹ The choice of buffer systems and salts can influence both yield and impurity reduction.

² Normally, buffers in the pH interval 5.5–8 are the most efficient for impurity reduction.

³ Conductivity can be adjusted by addition of salt or by varying the buffer concentration.

Buffer exchange

For preparation of well-defined samples for optimization of loading conditions at laboratory scale, buffer exchange can easily be performed on HiPrep™ 26/10 Desalting columns, using sample volumes up to 15 mL per column.

Conductivity and pH adjustment

For larger volumes of sample, sample preparation is preferably performed by diafiltration or directly by adjustment of pH and conductivity.

Equilibration of packed column

After packing, and before a purification run, equilibrate with at least five column volumes (CV) of starting buffer or until the column effluent shows stable conductivity and pH values.

4 Column packing

Recommended columns

Column	Inner diameter (mm)	Bed volume ¹	Maximum bed height (cm)
Laboratory-scale			
Tricorn™ 5/100	5	2 mL	10
Tricorn 10/100	10	8 mL	10
HiScale™ 10/40	10	8 to 20 mL ²	25 ²
HiScale 16/20	16	20 to 40 mL	20
HiScale 16/40	16	20 to 70 mL	35
HiScale 26/20	26	53 to 106 mL	20
HiScale 26/40	26	53 to 186 mL	35
HiScale 50/20	50	196 to 393 mL	20
HiScale 50/40	50	196 to 687 mL	35
Production-scale			
AxiChrom™	50 to 200	0.2 to 12.5 L	40
AxiChrom ³	300 to 2000	7 to 1256 L	40
BPG ⁴	100 to 300	1 to 28 L	40
Chromaflow™ ⁵	400 to 600	12 to 151 L	30

¹ Bed volume range calculated from 10 cm bed height to maximum bed height.

² Packing methods for bed heights up to 25 cm are provided.

³ Maximum bed height for AxiChrom 1000 is 20 cm.

⁴ Note that the pressure rating for BPG 450 is too low for Capto adhere ImpRes resin.

⁵ Chromaflow columns with inner diameters > 600 mm are not recommended for packing with Capto adhere ImpRes resin. Maximum flow velocity for Chromaflow 600 is approximately 150 cm/h with 20 cm bed height at 20°C using process buffers with the same viscosity as water.

All large-scale columns can be supplied as variable bed height columns.

For more details about packing HiScale columns, refer to instructions *HiScale columns (10, 16, 26, 50) and accessories (28967470)*. For information on packing of process-scale columns, including appropriate packing solutions, contact your local Cytiva representative.

Packing Tricorn columns

The following instructions are for packing Tricorn 5/100 and Tricorn 10/100 columns with a 10 cm bed height.

For more details about packing Tricorn columns, refer to *Instructions 28409488*.

Packing materials

- Capto adhere ImpRes
- plastic spoon or spatula
- glass filter funnel G4
- vacuum suction equipment
- filter flask
- measuring cylinder
- thin capillary
- 10 mM NaCl
- Tricorn 5/100 column, Tricorn Glass Tube 5/100 (to be used as packing tube), and Tricorn Packing Connector 5-5, or
- Tricorn 10/100 column, Tricorn Packing Equipment 10/100, which includes the 10-mm packing connector, 100-mm glass tube (to be used as packing tube)

- bottom unit with filter holder, cap, and stop plug

Packing equipment

Depending on the flow rate required, an ÄKTA™ system or a stand-alone pump such as Pump P-900, can be used for packing.

Equilibrate all materials to room temperature.

Washing the resin

Mount the glass filter funnel onto the filter flask. Suspend the resin by shaking it, pour the resin into the funnel, and wash it as follows:

Step	Action
1	Wash the resin five times with 1 to 2 mL of 10 mM NaCl/mL chromatography resin.
2	Gently stir with a spatula between additions.
3	Transfer the washed resin in the filter funnel to a beaker and add 10 mM NaCl to obtain a 50% slurry concentration.

Preparing the packing slurry

Check the slurry concentration after letting the slurry settle overnight in a measuring cylinder or use the Slurry Concentration Kit (29096100). Tricorn columns can be packed with an excess of resin to be removed after packing.

Main packing features

Table 3. Main features of the packing method

	Tricorn 5/100	Tricorn 10/100
Slurry packing solution	10 mM NaCl	
Slurry concentration	50%	50%
Phase 1		
Packing flow velocity	2250 cm/h	2250 cm/h
Packing flow rate	7.4 mL/min	29.4 mL/min
Packing time	3 min	3 min
Phase 2		
Packing flow velocity	2250 cm/h	2250 cm/h
Packing flow rate	7.4 mL/min	29.4 mL/min
Packing time	20 min	20 min

Packing procedure

Step	Action
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- | | |
|---|---|
| 1 | Assemble the column according to the column instructions (<i>Tricorn Empty High Performance Columns (28409488)</i>). For additional information, visit Technical support at cytiva.com/tricorn . |
| 2 | Place a stop plug in the bottom of the column tube and pour the suspended resin slurry (50%) into the top of the packing tube, filling both column tube and packing tube. Avoid formation of air bubbles in the resin by pouring it along a thin capillary. |

Step	Action
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| 3 | Attach an extra bottom unit or an adapter unit to the top of the packing tube. Place a beaker beneath the column tube and connect a pump to the top of the packing unit. Remove the stop plug from the bottom of the column tube. |
|---|---|

Phase 1

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|---|--|
| 4 | Pack the resin at 7.4 mL/min (Tricorn 5/100) or 29.4 mL/min (Tricorn 10/100) for 3 minutes. |
| 5 | When the resin is packed, switch off the pump, attach the stop plug into the bottom of the column tube, disconnect the pump, and remove the packing tube and packing connector. If necessary, remove excess resin by resuspending the top of the packed bed and remove with either a Pasteur pipette or a spatula. |
| 6 | Fill up the column with the same solution that was used for packing the column. |
| 7 | Place a pre-wet filter on top of the solution in the column and gently push it into the column tube with the filter tool. |

Note:

Do not use a coarse filter with Capto adhere ImpRes.

- | | |
|---|---|
| 8 | Prepare the adapter unit by screwing the guiding ring inside the adapter unit out to its outer rim and then turn it back 1.5 turns. |
| 9 | Wet the O-ring on the adapter unit by dipping it into water or buffer. |

Step	Action
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| 10 | Screw the adapter unit onto the column tube, making sure that the inner part of the guiding ring fits into the slot on the column tube threads. Make sure that there are no trapped air bubbles. |
| 11 | Screw the adapter down to approximately 1 mm above the surface of the bed. |
| 12 | Connect the pump to the adapter unit. Remove the stop plug from the bottom of the column. |

Phase 2

- | | |
|----|--|
| 13 | Pack the resin at 7.4 mL/min (Tricorn 5/100) or 29.4 mL/min (Tricorn 10/100) for 20 minutes. |
|----|--|

Note:

It is important to keep the time 20 minutes.

- | | |
|----|--|
| 14 | Turn the adapter down to the bed surface, making sure not to compress the bed. |
| 15 | Switch off the pump, attach the stop plug to the bottom of the column tube and disconnect the pump slowly. |
| 16 | Press the adapter lock down into the locked position. |
| 17 | Screw a stop plug into the adapter unit. |

The column is now ready for testing.

Note: *It is recommended to perform CIP after packing, as column packing involves open handling of the resin.*

Testing the packed column

See [Chapter 5 Evaluation of column packing, on page 25](#).

Packing HiScale columns

Introduction

The following instructions apply to packing HiScale 10/40, 16/20, 16/40 and HiScale 26/20, 26/40, 50/20 and 50/40 with 10, 20 and 35 cm bed heights. For HiScale 10/40, packing methods are provided for bed heights 10, 20, and 25 cm.

For more details about packing HiScale columns, refer to Instructions *HiScale columns (10, 16, 26, 50) and accessories (28967470)*.

Packing materials

- Capto adhere ImpRes
- HiScale column
- HiScale packing tube (depending on bed height)
- plastic spoon or spatula
- glass filter funnel G4
- vacuum suction equipment
- filter flask
- measuring cylinder
- 20% ethanol with 0.4 M NaCl

Packing equipment

Depending on the flow rate required, an ÄKTA system or a stand-alone pump such as Pump P-900, can be used for packing.

Equilibrate all materials to room temperature.

Definitions

The height of a bed that has settled by gravity differs from the height of a bed that has settled at a given flow velocity (consolidated). Therefore, the compression factor (CF) must be separated from the packing factor (PF). The parameters for calculating the bed height are described below:

L_{settled}	Settled bed height (cm) Bed height measured after settling by gravity
L_{cons}	Consolidated bed height (cm) Bed height measured after settling at a given flow velocity
L_{packed}	Packed bed height (cm)
CF	Compression factor, $CF = L_{\text{settled}} / L_{\text{packed}}$
PF	Packing factor, $PF = L_{\text{cons}} / L_{\text{packed}}$
A_C	Cross-sectional area of the column (cm^2)
V_C	Column volume, $V_C = L_{\text{packed}} \times A_C$ (mL)
C_{slurry}	Slurry concentration (%)

Preparation of the slurry

To measure the slurry concentration, let the resin settle in 20% ethanol at least overnight in a measuring cylinder or use the Slurry Concentration Kit (29096100). This method can also be used for HiScale columns.

Washing the resin

Mount a glass filter funnel onto a filter flask. Suspend the resin by shaking it, pour it into the filter funnel, and wash it as follows:

Step Action

- 1 Wash the resin five times with 1 to 2 mL 20% ethanol, 0.4 M NaCl/mL chromatography resin.
- 2 Gently stir with a spatula between additions.
- 3 Transfer the washed resin from the filter funnel to a beaker and add 20% ethanol, 0.4 M NaCl to obtain a 50% slurry concentration.

Note:

HiScale 10/40 is packed using a slurry concentration of 63%.

Packing parameters

Table 4. Main packing parameters for HiScale 10/40

Parameter	HiScale 10/40		
Bed height (cm)	10	20	25
Slurry/packing solution	20% ethanol with 0.4 M NaCl		
Slurry concentration (%)	63	63	63
Packing factor ¹ (PF)	1.05	1.05	1.05
Packing flow velocity (cm/h)	783	783	783
Packing flow rate (mL/min)	10.3	10.3	10.3
Conditioning flow velocity (cm/h)	1108	1108	1108
Conditioning flow rate (mL/min)	14.5	14.5	14.5

¹ When packing HiScale 10/40 with Capto Adhere ImpRes, the compression of the resin to target bed height is done with a combination of flow and mechanical compression. The packing factor in the table refers to the mechanical compression of the bed.

Note: *Bed heights up to 20 cm can be packed without using a packing tube.*

Table 5. Main packing parameters for HiScale 16/20 and HiScale 16/40

Parameter	HiScale 16/20	HiScale 16/40	
Bed height (cm)	10	20	35
Slurry/packing solution	20% ethanol with 0.4 M NaCl		
Slurry concentration (%)	50	50	50
Packing factor (PF)	1.12	1.12	1.09
Packing flow velocity (cm/h)	600	500	350
Packing flow rate (mL/min)	20.1	16.7	11.7
Conditioning flow velocity (cm/h)	600	500	350
Conditioning flow rate (mL/min)	20.1	16.7	11.7

Table 6. Main packing parameters for HiScale 26/20 and HiScale 26/40

Parameter	HiScale 26/20	HiScale 26/40	
Bed height (cm)	10	20	35
Slurry/packing solution	20% ethanol with 0.4 M NaCl		
Slurry concentration (%)	50	50	50
Packing factor (PF)	1.12	1.12	1.09
Packing flow velocity (cm/h)	600	500	350
Packing flow rate (mL/min)	53.0	44.2	31.0

Table 7. Main packing parameters for HiScale 50/20 and HiScale 50/40

Parameter	HiScale 50/20	HiScale 50/40	
Bed height (cm)	10	20	35
Slurry/packing solution	20% ethanol with 0.4 M NaCl		
Slurry concentration (%)	50	50	50
Packing factor (PF)	1.12	1.12	1.09
Packing flow velocity (cm/h)	600	500	350
Packing flow rate (mL/min)	196.3	163.5	114.5

Packing procedure

For HiScale 10/40 columns, compression is performed with a combination of flow compression and mechanical compression. The packing factor in [Table 4, on page 19](#) refers to mechanical compression of the bed.

Step	Action
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|---|--|
| 1 | Assemble the column according to the column instructions <i>HiScale columns (10, 16, 26, 50) and accessories (28967470)</i> . |
| 2 | Attach the column tube to a stand. |
| 3 | Connect the bottom adapter unit to the pump or a syringe and prime the bottom net with a slow flow of packing solution. This is easiest done if the nets are dry, but if air is trapped under the net it can be removed by a light suction with a syringe. |
| 4 | Attach the bottom adapter unit in the bottom of the column tube and tighten the O-ring firmly. |

Step	Action
5	Fill the column with approximately 1 cm packing liquid using the pump/syringe. Disconnect the pump/syringe and place a stop plug in the outlet.
6	Attach the packing tube on top of the column tube if needed to achieve the requested bed height.
7	Connect the top adapter to the pump and prime the pump with a slow downward flow. The net needs to face up. If air is trapped under the net it can be removed by light suction with a syringe
8	Fill the column with slurry suspended in packing solution. If needed, top up the slurry with extra packing solution so the top adapter dips into the slurry to avoid air under the net.
9	Attach the top adapter unit to the top of the packing tube. Tighten the O-ring firmly and remove the bottom stop plug.
10	Start a downward flow with packing flow velocity according to the tables in Section <i>Packing parameters</i> .
11	Let the flow run until the bed has consolidated.
12	Use the graduation markings on the column to measure the bed height. There might be a buildup of resin at the column wall after the bed has consolidated. To easier see where the top of the bed is, a light source can be used.

Step	Action
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|----|---|
| 13 | Calculate the final bed height by dividing the consolidated bed height with the recommended packing factor. |
|----|---|

$L_{\text{packed}} = L_{\text{cons}} / \text{PF}$. See Tables in Section *Packing parameters*.

For HiScale 10/40, proceed to step 21.

- | | |
|----|--|
| 14 | Turn off the flow and place a stop plug in the bottom. |
| 15 | Remove the top adapter from the packing tube. |
| 16 | Over a beaker or a sink, detach the packing tube from the column. |
| 17 | Re-attach the top adapter in the column tube. Make sure that no air is trapped under the net and lower the adapter down to 1 to 2 cm above the bed, making sure that the surface is not disturbed. |
| 18 | Tighten the O-ring on the adapter. Remove the bottom stop plug and carefully start turning the end cap down. While spilling out liquid through the bottom, proceed turning until the calculated final bed height is reached. |
| 19 | Make sure that any pressure peaks when turning the end knob down do not exceed the pressure specifications of the resin. |

Result:

HiScale 26 or HiScale 50 columns are now ready to be tested.

Step	Action
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|----|---|
| 20 | HiScale 16: Start a downflow to condition the bed. The flow rate is shown in Table 5, on page 20 in Section <i>Packing parameters</i> . Let the flow run for about 30 minutes. |
|----|---|

Result:

The HiScale 16 column is now ready to be tested.

- | | |
|----|--|
| 21 | HiScale 10/40: Increase the flow to compress and condition the bed. The flow is shown in Table 4. Continue this phase for 5 CV. At the end of the phase, mark the gel height while keeping up the flow. |
|----|--|

- | | |
|----|---|
| 22 | HiScale 10/40: Turn off the flow and untighten the top adapter slightly (release the pressure on the O-ring) which facilitates adjustment of the adapter without "twisting" the O-ring. This procedure must be performed with the bottom tubing open which makes it possible to monitor the pressure. The pressure that is generated from turning the adapter down must not exceed the pressure during the conditioning phase. |
|----|---|

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|----|--|
| 23 | HiScale 10/40: Before turning the adapter down into the bed (according to calculation in step 13), the O-ring must be firmly tightened. |
|----|--|

- | | |
|----|---|
| 24 | HiScale 10/40: Continue the conditioning phase with 10 CV, with the conditioning flow according to Table 4, on page 19 . |
|----|---|

Result:

The HiScale 10/40 column is now ready to be tested.

Note: *It is recommended to perform CIP after packing, as column packing involves open handling of the resin.*

Testing the packed column

See [Chapter 5 Evaluation of column packing, on page 25](#).

5 Evaluation of column packing

Introduction

The packing quality needs to be checked by column efficiency testing. Perform column efficiency testing:

- after packing
- at regular intervals during the working life of the column
- when separation performance deteriorates

Column efficiency testing

The best method of expressing the efficiency of a packed column is in terms of the height equivalent to a theoretical plate (HETP) and the asymmetry factor (A_s). The values are easily determined by applying a test sample such as 1% acetone solution or sodium chloride to the column.

Note: *Use a concentration of 0.8 M NaCl in water as sample and 0.4 M NaCl in water as eluent.*

The calculated plate number depends on the test conditions and must only be used as a reference value. It is important that the test conditions and the equipment are the same so that the results are comparable.

Note: Changes in solute, solvent, eluent, sample volume, flow velocity, liquid pathway, temperature, chromatography system, etc., affect the results.

For more information about column efficiency testing, refer to *Application note (CY13149)*.

Sample volume and flow velocity

For optimal column efficiency results, the sample volume must be approximately 1% of the column volume and the flow velocity 30 cm/h. If an acceptance limit is defined in relation to column performance, the column plate number can be used as one of the acceptance criteria for column packing.

Method for measuring HETP and A_s

Calculate HETP and A_s from the UV curve (or conductivity curve) as follows:

$$\text{HETP} = \frac{L}{N}$$

L = bed height (cm)

N = number of theoretical plates

V_R = retention volume, volume eluted from the start of sample application to the peak maximum

$$N = 5.54 \times \left(\frac{V_R}{W_h} \right)^2$$

W_h = peak width measured as the width of the recorded peak at half of the peak height

V_R and W_h are in the same units

The concept of reduced plate height is often used for comparing column performance.

The reduced plate height (h) is calculated as follows:

$$h = \frac{\text{HETP}}{d_{50v}}$$

d_{50v} = median particle size of the cumulative volume distribution (cm)

As a guideline, a value of < 3 for (h) is very good.

The peak must be symmetrical, and the asymmetry factor as close to 1 as possible. A typical acceptable range could be $0.8 < A_s < 1.5$.

A change in the shape of the peak is usually the first indication of bed deterioration due to excessive use.

Peak asymmetry factor calculation:

$$A_s = \frac{b}{a}$$

a = ascending part of the peak width at 10% of peak height

b = descending part of the peak width at 10% of peak height

The figure below shows a UV trace for acetone in a typical test chromatogram from which the HETP and A_s values are calculated.

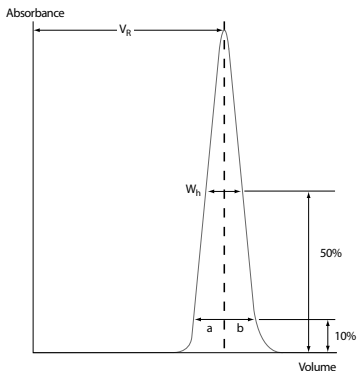


Fig 4. A typical test chromatogram showing the parameters used for HETP and A_s calculations.

6 Maintenance

For best performance of Capto adhere ImpRes over a long working lifetime, follow the procedures described below.

Equilibration

After packing, and before a chromatographic run, equilibrate with loading buffer by washing with at least 5 bed volumes, or until the column effluent shows stable conductivity and pH values.

Regeneration

After each step, elute any reversibly bound material with low pH (e.g., 3 CV of 0.5 M acetic acid). Regenerate the resin by washing in reversed flow direction until the column effluent shows stable conductivity and pH values.

Cleaning-In-Place

Cleaning-In-Place (CIP) is a procedure that removes impurities or contaminants such as lipids, endotoxins, nucleic acids, and precipitated or denatured proteins that remain in the packed column after regeneration. Regular CIP prevents the buildup of impurities or contaminants in the resin bed and helps to maintain the capacity, flow properties, and general performance of Capto adhere ImpRes. A specific CIP protocol must be designed for each process according to the type of impurities or contaminants present.

It is recommended to perform a CIP:

- before first-time use – especially after packing the column – or after long-term storage
- between cycles
- when an increase in back pressure or a reduction in column performance is observed
- to prevent potential cross-contamination or carry-over, when the same column is used for purification of different proteins or protein lots and batches.

Recommended protocols for removing specific contaminants or impurities are described below.

CIP protocols

Precipitated hydrophobically bound proteins or lipoproteins	Wash with at least 3 CV of 1.0 M NaOH at 150 cm/h with reversed flow direction. Contact time at least 15 min.
Ionically bound proteins	Wash with 0.5 to 2 CV of 2 M NaCl with reversed flow direction.
Lipids and very hydrophobic proteins	Wash with 1% to 5% 1-propanol or 5% to 30% isopropanol with reversed flow direction. 1-propanol has a higher flash point and might be preferred in an industrial environment.
Nucleic acids	Wash with 0.1 M acetate, pH 3, for 2 to 5 CV followed by a buffer at neutral pH for 1 to 2 CV. Then wash with at least 3 CV of 1.0 M NaOH at a low flow velocity with reversed flow direction. Contact time 15 min.

Sanitization

To reduce microbial contamination in the packed column, sanitization using 0.5 to 1 M NaOH with a contact time of 1 hour is recommended. The CIP protocol for precipitated, hydrophobically bound proteins or lipoproteins removes bound impurities and sanitizes the chromatography resin effectively. For more information about the use of NaOH for sanitization, refer to *Application note (CY13951)*.

Storage

Store chromatography resin in its container at a temperature of 4°C to 30°C. Make sure that the screw top is fully tightened. Packed columns must be equilibrated with 20% ethanol to prevent microbial growth. After storage, equilibrate with at least 5 CV of starting buffer before use.

7 Ordering information

Product	Quantity	Product code
Capto adhere ImpRes	25 mL	17371501
	100 mL	17371502
	1 L	17371503
	5 L ¹	17371504
	10 L ¹	17371505

¹ Pack sizes available upon request.

Related product	Quantity	Product code
HiTrap Capto adhere ImpRes	1 × 1 mL	29400464
	5 × 1 mL	17371510
HiScreen Capto adhere ImpRes	1 × 4.7 mL	17371520
PreDicator Capto adhere ImpRes, 6 µL	4 x 96-well filter plates	17371530
PreDicator Capto adhere ImpRes, 20 µL	4 x 96-well filter plates	17371531
PreDicator RoboColumn Capto adhere ImpRes, 200 µL	One row of eight columns	17371540
PreDicator RoboColumn Capto adhere ImpRes, 600 µL	One row of eight columns	17371541
Assist 1.7 Software		29206558

Related prepacked product	Quantity	Product code
HiTrap MabSelect SuRe™	5 × 1 mL	11003493
HiTrap MabSelect SuRe	5 × 5 mL	11003495
HiPrep 26/10 Desalting	1 × 53 mL	17508701
HiPrep 26/10 Desalting	4 × 53 mL	17508702

Related empty column	Quantity	Product code
Tricorn 5/100 column	1	28406410
Tricorn 10/100 column	1	28406415
HiScale 10/40	1	29360550
HiScale 16/20	1	28964441
HiScale 16/40	1	28964424
HiScale 26/20	1	28964514
HiScale 26/40	1	28964513
HiScale 50/20	1	28964445
HiScale 50/40	1	28964444

Accessories	Quantity	Product code
Tricorn Glass Tube 5/100	1	18115306
Tricorn Packing Connector 5-5	1	18115321
Tricorn Packing Equipment 10/100	1	18115325
Packing tube 20 (HiScale 10)	1	29360551
Packing tube 20 (HiScale 16)	1	28986816
Packing tube 40 (HiScale 16)	1	28986815
Packing tube 20 (HiScale 26)	1	28980383
Packing tube 40 (HiScale 26)	1	28964505
Packing tube 20 (HiScale 50)	1	28980251
Packing tube 40 (HiScale 50)	1	28964506

Related literature		Reference
Data file	Capto adhere ImpRes	CY11775
Application note	Polishing of monoclonal antibodies using Capto adhere ImpRes in bind and elute mode	CY13429
	mAb polishing step development using Capto adhere ImpRes in bind-elute mode	CY20571
	Use of sodium hydroxide for cleaning and sanitization of chromatography resins and systems	CY13951

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