



# Capto™ SP ImpRes Capto™ Q ImpRes

## Ion exchange chromatography resins

### Instructions for Use

Capto™ SP ImpRes and Capto Q ImpRes resins are strong cation and strong anion exchange BioProcess™ chromatography resins, respectively, for intermediate purification and polishing of a wide range of biomolecules. The combination of the high-flow agarose technique used for all Capto products and the small particle size of Capto ImpRes results in good pressure-flow properties as well as impressive resolution.

Capto SP ImpRes and Capto Q ImpRes resins provide:

- high-resolution intermediate purification and polishing based on the well-established Capto platform with traditional ligands
- flexibility of design – large operational window of flow rates and bed heights
- high-throughput purifications easy to optimize and scale up
- higher manufacturing productivity enables improved process economy
- security of supply and comprehensive regulatory support

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## Important

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 resins

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

## 2 Resin properties

The structures of the well established SP (sulfopropyl group) and Q (quaternary amine group) ligands used for Capto ImpRes resins are shown in Figure 1.

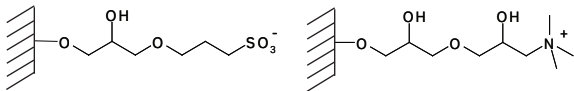


**Fig 1.** The strong ion exchange group of Capto SP ImpRes (left) and Capto Q ImpRes resins (right).

The resins are designed for intermediate purification or polishing. They are based on the high-flow agarose base matrix, which gives good pressure-flow properties ([Fig. 2, on page 4](#)).

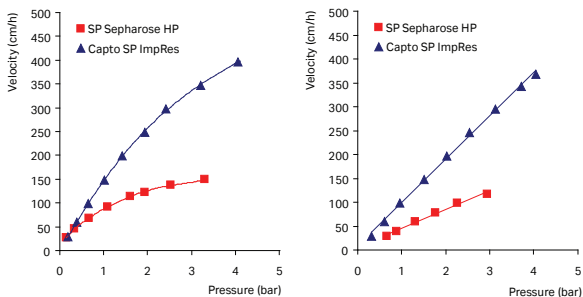


**Fig 2.** Example of pressure-flow curves for Capto SP ImpRes and SP Sepharose™ HP resins in open bed (left) and packed bed (right), respectively. Running conditions: AxiChrom™ 300 chromatography column (30 cm i.d.), 20 cm bed height with packing factor 1.15 in water at 20°C. Pressure contribution from system, tubings, and column is excluded.

The small bead size gives a high resolution. Capto ImpRes resin thus allows for high-resolution purifications at high flow rates. The combination of the well established SP and Q ligands with a small high-flow agarose bead makes the resins ideal for high-throughput intermediate purification and polishing. Further characteristics of the resins are found in [Characteristics of Capto SP ImpRes and Capto Q ImpRes resins on page 5](#).

**Table 1.** Characteristics of Capto SP ImpRes and Capto Q ImpRes resins

|                                                                                          | Capto SP ImpRes                                                                                                                                                | Capto Q ImpRes                               |
|------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------|
| <b>Matrix</b>                                                                            | High-flow agarose                                                                                                                                              | High-flow agarose                            |
| <b>Functional group</b>                                                                  | $-\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_3^-$                                                                                                              | $-\text{CH}_2\text{N}^+(\text{CH}_3)_3$      |
| <b>Total ionic capacity</b>                                                              | 0.13 to 0.16 mmol ( $\text{H}^+$ )/mL resin                                                                                                                    | 0.15 to 0.18 mmol ( $\text{Cl}^-$ )/mL resin |
| <b>Average particle size, <math>d_{50v}</math><sup>1</sup></b>                           | 40 $\mu\text{m}$                                                                                                                                               | 40 $\mu\text{m}$                             |
| <b>Maximum operational flow velocity<sup>2</sup></b>                                     | At least 220 cm/h in a 1 m diameter column with bed height 20 cm at 20°C; measured using process buffers with the same viscosity as water at < 3 bar (0.3 MPa) |                                              |
| <b>Dynamic binding capacity, <math>\text{QB}_{10\%}</math><sup>3</sup> (mg/mL resin)</b> | > 70 mg lysozyme                                                                                                                                               | > 55 mg BSA                                  |
|                                                                                          | > 95 mg BSA                                                                                                                                                    | > 48 mg $\beta$ -lactoglobulin               |
| <b>pH stability<sup>4</sup></b>                                                          |                                                                                                                                                                |                                              |
| Operational                                                                              | 4 to 12                                                                                                                                                        | 2 to 12                                      |
| Cleaning-in-place                                                                        | 3 to 14                                                                                                                                                        | 2 to 14                                      |
| <b>Working temperature</b>                                                               | 4°C to 30°C                                                                                                                                                    | 4°C to 30°C                                  |
| <b>Chemical stability</b>                                                                | Stable in commonly used aqueous buffers: 1 M sodium hydroxide <sup>5</sup> , 8 M urea, 6 M guanidine hydrochloride, 30% isopropanol, and 70% ethanol           |                                              |
| <b>Avoid</b>                                                                             | Oxidizing agents, cationic detergents                                                                                                                          | Oxidizing agents, anionic detergents         |
| <b>Storage</b>                                                                           | 20% ethanol, 0.2 M sodium acetate                                                                                                                              | 20% ethanol                                  |

<sup>1</sup>  $d_{50v}$  is the median particle size of the cumulative volume distribution.

<sup>2</sup> Flow velocity stated in the Table is dependent on the column used.

<sup>3</sup> Dynamic binding capacity at 10% breakthrough measured at a residence time of 4 minutes (150 cm/h) in a Tricorn™ 5/100 column with 10 cm bed height. Capto SP ImpRes: 20 mM sodium phosphate, pH 7.2 (lysozyme) and 50 mM Tris, pH 8.0 (BSA) Capto Q ImpRes: 50 mM sodium acetate, pH 4.75 (BSA and  $\beta$ -lactoglobulin).

<sup>4</sup> Operational: pH range where resin can be operated without significant change in function.

Cleaning-in-place: pH stability where the resin can be subjected to cleaning-in-place without significant change in function.

<sup>5</sup> 1 M NaOH should only be used for cleaning purposes.

### 3 Method optimization

The aim of designing and optimizing an ion exchange separation process is to identify conditions that promote binding of the highest amount of target molecule, in the shortest possible time with highest possible product recovery and purity. The design of the method is at laboratory scale.

For certain proteins, dynamic binding capacities increase at increased conductivity and this is pH dependent. Therefore, scouting of both pH and conductivity for optimal dynamic binding conditions on Capto SP ImpRes and Capto Q ImpRes resins is recommended. Flow velocity can also be included in the scouting, even though the effect of flow should be small for Capto ImpRes resin.

Elution of protein can either be done by use of salt, pH or a combination of both. For optimization of the elution, sample load, flow velocity and gradient volume should be considered. The three factors are interrelated and best results will be obtained using:

- maximized sample load with respect to dynamic binding capacity
- maximized flow velocity with respect to system constraints and resin rigidity
- the gradient volume that provides the best resolution with maximized sample load and maximized flow velocity

The use of PreDicator™ plates is preferably included in the method development. The PreDicator plates are 96-well filter plates prefilled with chromatography resins, which can be used for rapid screening of chromatographic conditions at a small scale. The suggested workflow with PreDicator plates is shown in [Fig. 4, on page 26](#), where a large design space can be explored prior to further experiments in packed column formats, such as prepacked HiScreen™ columns.



**Fig 3.** The recommended workflow is described in the figure. It starts with 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.

**Table 2.** The experimental conditions to consider when designing and optimizing the process

| Phases                                            | Activity                                         | Conditions to consider                                                                                                                                                                  |
|---------------------------------------------------|--------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1. Equilibration of column and sample preparation | Equilibration of column and adjustment of sample | <ul style="list-style-type: none"> <li>• pH</li> <li>• Conductivity</li> <li>• Column volume</li> <li>• Column bed height</li> <li>• Particle content</li> <li>• Temperature</li> </ul> |

| Phases                | Activity                                                                         | Conditions to consider                                                                                                                                                |
|-----------------------|----------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 2. Sample application | Manual or automatic application onto the column                                  | <ul style="list-style-type: none"> <li>• Flow rate</li> <li>• Sample pH</li> <li>• Sample conductivity</li> <li>• Upward/downward flow</li> </ul>                     |
| 3. Wash               | Wash out unbound material with clean binding buffer                              | <ul style="list-style-type: none"> <li>• Flow rate</li> <li>• Upward/downward flow</li> <li>• Buffer choice (normally same as column equilibration buffer)</li> </ul> |
| 4. Elution            | Elute the material from the column either with adding salt or by changing the pH | <ul style="list-style-type: none"> <li>• Sample load</li> <li>• pH</li> <li>• Conductivity</li> <li>• Flow rate</li> <li>• Upward/downward flow</li> </ul>            |

For more information about method development and optimization, refer to the handbook *Ion exchange Chromatography: Principles and methods (CY13983)*.

## 4 Scale-up

### Introduction

After optimizing the method at laboratory scale, the process can be scaled up. Scale-up is typically performed by keeping bed height and flow velocity constant while increasing bed diameter and volumetric flow rate. However, since optimization is preferably performed with small column volumes, in order to save sample and buffer, some parameters such as the dynamic binding capacity can be optimized using shorter bed heights than those being used at the final scale. As long as the residence time is kept constant, the binding capacity for the target molecule remains the same.

Other factors, such as clearance of critical impurities, can change when column bed height is modified and must be validated using the final bed height. The residence time is calculated as the bed volume (mL) divided by the volumetric flow rate (mL/min) applied during sample loading.

### Procedure

| Step | Action |
|------|--------|
|------|--------|

- |   |                                                                                                                    |
|---|--------------------------------------------------------------------------------------------------------------------|
| 1 | Select a bed volume according to required binding capacity. Keep sample concentration and gradient slope constant. |
| 2 | Select a column diameter to obtain the bed height (10 to 40 cm) from method optimization.                          |

**Note:**

*The rigidity of the high-flow base matrix allows for flexibility in choice of bed heights.*

| Step | Action |
|------|--------|
|------|--------|

- |   |                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|---|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 3 | The use of larger equipment might cause deviations from the method optimized at small scale. In such cases, check the buffer delivery system and monitoring system for time delays or volume changes. Different lengths and diameters of outlet tubing can cause zone spreading on larger systems. Also check the compatibility of the hardware and chromatography resin pressure limits with expected pressure during packing and operation. |
|---|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

## 5 Column packing

### 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 the Data file *Tricorn - Empty High Performance Columns (CY17144)*.

### Materials

- Capto SP ImpRes or Capto Q ImpRes
- plastic spoon or spatula
- glass filter 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

## Equipment

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

Equilibrate all materials to room temperature.

## Washing the chromatography resin

Mount the glass filter funnel onto the filter flask. Suspend the resin by shaking and pour into the funnel and wash according to the following instructions:

| Step | Action                                                                                         |
|------|------------------------------------------------------------------------------------------------|
| 1    | Wash 5 times with 1–2 mL 10 mM NaCl/mL chromatography resin.                                   |
| 2    | Gently stir with a spatula between additions.                                                  |
| 3    | Transfer the washed resin to a beaker and add 10 mM NaCl to obtain a 50% slurry concentration. |

## Preparing the packing slurry

Check the slurry concentration after settling overnight in a measuring cylinder or use the method for slurry concentration measurement described in application note CY13253. Tricorn columns can be packed with an excess of resin to be removed after packing.

## Packing procedure

### Main features

**Table 3.** Main features of the packing method

|                         | Tricorn 5/100 | Tricorn 10/100 |
|-------------------------|---------------|----------------|
| Slurry packing solution | 10 mM NaCl    | 10 mM NaCl     |
| Slurry concentration    | 50%           | 50%            |
| <b>Phase 1</b>          |               |                |
| 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          |
| <b>Phase 2</b>          |               |                |
| 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         |

## Preparing for packing

| Step | Action |
|------|--------|
|------|--------|

- |   |                                                                                                                                                                                                                                              |
|---|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1 | Assemble the column according to the column instructions <i>Tricorn Empty High Performance Columns (28409488)</i> . For additional information, visit Technical support at <a href="https://www.cytiva.com/tricorn">cytiva.com/tricorn</a> . |
|---|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

| Step | Action |
|------|--------|
|------|--------|

- |   |                                                                                                                                                                                                                                                           |
|---|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 2 | Put 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. |
| 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

| Step | Action |
|------|--------|
|------|--------|

- |   |                                                                                                                                                                                                                                                                                                         |
|---|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1 | Pack the resin at 7.4 mL/min (Tricorn 5/100) or 29.4 mL/min (Tricorn 10/100) for 3 minutes.                                                                                                                                                                                                             |
| 2 | When the resin is packed, switch off the pump, attach the stop plug to the bottom of the column tube, disconnect the pump and remove the packing tube and packing connector. If necessary, remove excess resin by re-suspending the top of the packed bed and remove with a Pasteur pipette or spatula. |
| 3 | Fill up the column with the same solution that was used for packing the column.                                                                                                                                                                                                                         |

| Step | Action |
|------|--------|
|------|--------|

- |   |                                                                                                                              |
|---|------------------------------------------------------------------------------------------------------------------------------|
| 4 | Place a pre-wetted 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 SP ImpRes or Capto Q ImpRes.*

- |   |                                                                                                                                                                                                   |
|---|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 5 | 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.                                                               |
| 6 | Wet the O-ring on the adapter unit by dipping it into water or buffer.                                                                                                                            |
| 7 | Screw the adapter unit onto the column tube and make 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. |
| 8 | Screw the adapter down to approximately 1 mm above the surface of the bed.                                                                                                                        |
| 9 | Connect the pump to the adapter unit. Remove the stop plug in the bottom of the column.                                                                                                           |

## Phase 2

| Step | Action |
|------|--------|
|------|--------|

- |   |                                                                                                              |
|---|--------------------------------------------------------------------------------------------------------------|
| 1 | Pack the resin at 7.4 mL/min (Tricorn 5/100) or 29.4 mL/min (Tricorn 10/100) for 20 minutes.                 |
| 2 | Turn the adapter down to the bed surface (make sure not to compress the bed).                                |
| 3 | Switch off the pump, attach the stop plug into the bottom of the column tube and disconnect the pump slowly. |
| 4 | Press the adapter lock down into the locked position.                                                        |
| 5 | Screw a stop plug into the adapter unit. The column is now ready to be used.                                 |

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

## Testing the packed column

See section [Chapter 6 Evaluation of packed column](#), on page 24.

# Packing HiScale columns

## Introduction

The following instructions are for 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, see instructions *HiScale columns (10, 16, 26, 50) and accessories (28967470)*.

## Materials

- Capto SP ImpRes or Capto Q ImpRes
- HiScale column
- HiScale packing tube (depending on bed height)
- plastic spoon or spatula
- glass filter G4
- vacuum suction equipment
- filter flask
- measuring cylinder
- 10 mM NaCl

## Equipment

A chromatography system, such as ÄKTA system, or a stand-alone pump such as Pump P-900, depending on the flow rate required, 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:

| Parameter            | Description                                                                                 |
|----------------------|---------------------------------------------------------------------------------------------|
| $L_{\text{settled}}$ | Settled bed height (cm)<br>Bed height measured after settling by gravity                    |
| $L_{\text{cons}}$    | Consolidated bed height (cm)<br>Bed height measured after settling at a given flow velocity |
| $L_{\text{packed}}$  | 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 ( $\text{cm}^2$ )                                        |
| $V_C$                | Column volume, $V_C = L_{\text{packed}} \times A_C$ (mL)                                    |
| $C_{\text{slurry}}$  | Slurry concentration (%)                                                                    |

## Preparing the slurry

To measure the slurry concentration, let the resin settle in 20% ethanol at least overnight in a measuring cylinder or use the method for slurry concentration measurement described in application note CY13253. This method can also be used for HiScale columns. For HiScale 10/40, a slurry concentration of 63% is recommended.

## Washing the resin

Mount a glass filter funnel onto a filter flask. Shake the resin to suspend it, pour it into the funnel, and wash it as follows:

---

**Step Action**

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- 1 Wash 5 times with 1 to 2 mL 10 mM NaCl/mL resin.
  - 2 Gently stir with a spatula between additions.
  - 3 Transfer the washed resin to a beaker and add 10 mM NaCl to obtain a 50% slurry concentration.
- 

## 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 (PF)               | 1.05 <sup>1</sup>           | 1.05 <sup>1</sup> | 1.04 <sup>1</sup> |
| 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              |

<sup>1</sup> When packing HiScale 10 with Capto SP ImpRes or Capto Q 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 a packing tube.*

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

| Parameter                         | HiScale<br>16/20 | HiScale 16/40 |            |
|-----------------------------------|------------------|---------------|------------|
| Bed height (cm)                   | 10               | 20            | 35         |
| Slurry/packing solution           | 10 mM NaCl       | 10 mM NaCl    | 10 mM NaCl |
| Slurry concentration (%)          | 50               | 50            | 50         |
| Packing factor (PF)               | 1.12             | 1.12          | 1.09       |
| Packing flow velocity (cm/h)      | 1200             | 1000          | 700        |
| Packing flow rate (mL/min)        | 40               | 33            | 23         |
| Conditioning flow velocity (cm/h) | 1200             | 1000          | 700        |
| Conditioning flow rate (mL/min)   | 40               | 33            | 23         |

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

| Parameter                    | HiScale<br>26/20 | HiScale 26/40 |            |
|------------------------------|------------------|---------------|------------|
| Bed height (cm)              | 10               | 20            | 35         |
| Slurry/packing solution      | 10 mM NaCl       | 10 mM NaCl    | 10 mM NaCl |
| Slurry concentration (%)     | 50               | 50            | 50         |
| Packing factor (PF)          | 1.12             | 1.12          | 1.09       |
| Packing flow velocity (cm/h) | 1200             | 1000          | 700        |
| Packing flow rate (mL/min)   | 106              | 88            | 62         |

**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      | 10 mM NaCl    | 10 mM NaCl    | 10 mM NaCl |
| Slurry concentration (%)     | 50            | 50            | 50         |
| Packing factor (PF)          | 1.12          | 1.12          | 1.09       |
| Packing flow velocity (cm/h) | 1200          | 1000          | 700        |
| Packing flow rate (mL/min)   | 393           | 327           | 229        |

## Packing procedure

For HiScale 10/40 columns, the compression is done with a combination of flow and mechanical compression. The packing factor in [Table 4, on page 18](#) refers to the mechanical compression of the bed.

| Step | Action |
|------|--------|
|------|--------|

- |   |                                                                                                                                                                                                                                                            |
|---|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 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 lab 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 to the bottom of the column tube and tighten the O-ring.                                                                                                                                                                    |

| Step | Action                                                                                                                                                                                                                        |
|------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 5    | Fill the column with approximately 1 cm packing liquid using the pump/syringe. Disconnect the pump/syringe and screw a stop plug in the outlet.                                                                               |
| 6    | Mount 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 it with a slow downward flow. The net needs to be facing up as this is done. If air is trapped under the net, it can be removed by a 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 <a href="#">Packing parameters, on page 18</a> .                                                                                                  |
| 11   | Let the flow run until the bed has consolidated.                                                                                                                                                                              |
| 12   | Use the graduation marks 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 |
|------|--------|
|------|--------|

- |    |                                                                                                             |
|----|-------------------------------------------------------------------------------------------------------------|
| 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 the tables in [Packing parameters, on page 18](#).

**For HiScale 10/40, proceed to step 21.**

- |    |                                                                                                                                                                                                                                   |
|----|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 14 | Turn off the flow and screw a stop plug in the bottom.                                                                                                                                                                            |
| 15 | Disconnect 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 to 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 draining liquid through the bottom, keep turning the end cap until the calculated final bed height is reached. |
| 19 | Make sure that any pressure peaks while 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 |
|------|--------|
|------|--------|

- |    |                                                                                                                                                                              |
|----|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 20 | <b>HiScale 16:</b> Start a downward flow to flow condition the bed. The flow rate is shown in <a href="#">Table 5, on page 19</a> . Let the flow run for approx. 30 minutes. |
|----|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

*Result:*

The HiScale 16 column is now ready to be tested.

- |    |                                                                                                                                                                                                                                                             |
|----|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 21 | <b>HiScale 10/40:</b> Increase the flow to compress and condition the bed. The flow is shown in <a href="#">Table 4, on page 18</a> . Continue this step for 5 column volumes (CV). At the end of this step, mark the bed height while keeping up the flow. |
|----|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

- |    |                                                                                                                                                                                                                                                                                                                                                                                                                                      |
|----|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 22 | <b>HiScale 10/40:</b> 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 step. |
|----|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

- |    |                                                                                                                                                                     |
|----|---------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 23 | <b>HiScale 10/40:</b> Before turning the adapter down into the bed (according to the calculation in <a href="#">step 13</a> ), the O-ring must be firmly tightened. |
|----|---------------------------------------------------------------------------------------------------------------------------------------------------------------------|

- |    |                                                                                                                                           |
|----|-------------------------------------------------------------------------------------------------------------------------------------------|
| 24 | <b>HiScale 10/40:</b> Perform the conditioning step for 10 CV with a conditioning flow according to <a href="#">Table 4, on page 18</a> . |
|----|-------------------------------------------------------------------------------------------------------------------------------------------|

*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.*

**Note:** *For packing Axichrom, BPG, and Chromaflow™ columns, refer to How to pack Capto ImpRes resins using verified packing methods available on [cytiva.com](https://www.cytiva.com).*

## Testing the packed column

See the following chapter to evaluate column packing.

# 6 Evaluation of packed column

## Introduction

The packing quality needs to be checked by column efficiency testing. The test must be performed after packing, at regular intervals during the column lifespan, and also when separation performance has deteriorated.

## 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:** Changing the solute, solvent, eluent, sample volume, flow velocity, liquid pathway, temperature, chromatography system, etc., influences the results.

For more information about column efficiency testing, refer to the application note CY13149.

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 the column use.

## 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

$V_R$  = 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.

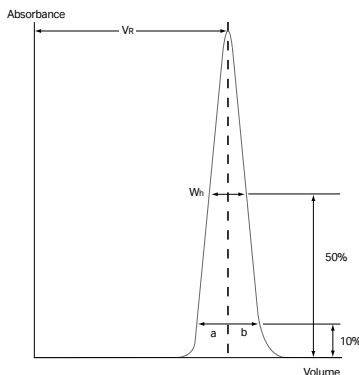


**Fig 4.** A typical test chromatogram showing the parameters used for HETP and  $A_s$  calculations.

## 7 Maintenance

For best performance from Capto SP ImpRes and Capto Q ImpRes and to maximize the resin lifespan, follow the procedures described below.

### Equilibration

After packing and CIP, and before a chromatographic run, equilibrate with start buffer by washing with at least five bed volumes or until the column effluent shows stable conductivity and pH values. The equilibration step can be shortened by first washing with a high concentration buffer to obtain approximately the target pH value and then washing with start buffer until the conductivity and pH values are stable.

### Regeneration

After each separation, elute any reversibly bound material with a high ionic strength solution (e.g., 1 to 2 M NaCl in elution buffer). Regenerate the resin by washing with at least five bed volumes of equilibration buffer or until the column effluent shows stable conductivity and pH values.

### Cleaning-in-place (CIP)

Regular CIP prevents the buildup of impurities or contaminants in the resin bed and helps to maintain the capacity, flow properties, and general performance. 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.

|                                                              |                                                                                                                                                                                                                                                          |
|--------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Precipitated, hydrophobically bound proteins or lipoproteins | Wash with at least 3 CV of 1 M NaOH solution with reversed flow direction. Contact time 15 to 30 min, dependent on sample. Use 1 M NaOH with 1 M NaCl if the results are not satisfactory with NaOH alone.                                               |
| Ionically bound proteins                                     | Wash with 0.5 to 2 CV of 2 M NaCl with reversed flow direction. Contact time 10 to 15 minutes.                                                                                                                                                           |
| Lipids and very hydrophobic proteins                         | Wash with 2 to 4 CV of up to 70% ethanol <sup>1</sup> or 30% isopropanol with reversed flow direction. Contact time 1 to 2 h. Alternatively, wash with 2 to 4 CV of 0.1% to 0.5% nonionic detergent with reversed flow direction. Contact time 1 to 2 h. |

<sup>1</sup> Consult local safety regulations to verify if safety precautions are required for the use of 70% ethanol or 30% isopropanol.

## Sanitization

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

## Storage

Store unused resin in its container at a temperature of 4°C to 30°C. Make sure that the cap is tightened fully. It is recommended to store packed resin and bulk resin in 0.2 M sodium acetate in 20% ethanol (Capto SP ImpRes resin) or in 20% ethanol (Capto Q ImpRes resin).

After storage, equilibrate with at least five CV of start buffer.

# 8 Ordering information

| Product         | Quantity | Product code |
|-----------------|----------|--------------|
| Capto SP ImpRes | 25 mL    | 17546810     |
|                 | 100 mL   | 17546802     |
|                 | 1 L      | 17546803     |
|                 | 5 L      | 17546804     |
|                 | 10 L     | 17546805     |
| Capto Q ImpRes  | 25 mL    | 17547010     |
|                 | 100 mL   | 17547002     |
|                 | 1 L      | 17547003     |
|                 | 5 L      | 17547004     |
|                 | 10 L     | 17547005     |

Capto SP ImpRes resin is supplied in suspension in 20% ethanol containing 0.2 M sodium acetate. Capto Q ImpRes resin is supplied in suspension in 20% ethanol. For additional information, contact your local Cytiva representative.

## Related products

| Product                                       | Quantity                  | Product code |
|-----------------------------------------------|---------------------------|--------------|
| PreDicator Capto SP ImpRes, 6 µL              | 4 × 96-well filter plates | 17546816     |
| PreDicator Capto SP ImpRes, 20 µL             | 4 × 96-well filter plates | 17546817     |
| PreDicator RoboColumn Capto SP ImpRes, 200 µL | One row of 8 columns      | 28997449     |
| PreDicator RoboColumn Capto SP ImpRes, 600 µL | One row of 8 columns      | 28997450     |
| HiTrap™ Capto SP ImpRes                       | 5 × 1 mL                  | 17546851     |
| HiTrap Capto SP ImpRes                        | 5 × 5 mL                  | 17546855     |
| HiScreen Capto SP ImpRes                      | 1 × 4.7 mL                | 17546815     |
| PreDicator Capto Q ImpRes, 6 µL               | 4 × 96-well filter plates | 17547016     |
| PreDicator Capto Q ImpRes, 20 µL              | 4 × 96-well filter plates | 17547017     |
| PreDicator RoboColumn Capto Q ImpRes, 200 µL  | One row of 8 columns      | 28996918     |
| PreDicator RoboColumn Capto Q ImpRes, 600 µL  | One row of 8 columns      | 28997391     |
| HiTrap Capto Q ImpRes                         | 5 × 1 mL                  | 17547051     |
| HiTrap Capto Q ImpRes                         | 5 × 5 mL                  | 17547055     |
|                                               | 1 × 4.7 mL                | 17547015     |
| 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     |

| Product       | Quantity | Product code |
|---------------|----------|--------------|
| HiScale 26/20 | 1        | 28964514     |
| HiScale 26/40 | 1        | 28964513     |
| HiScale 50/20 | 1        | 28964445     |
| HiScale 50/40 | 1        | 28964444     |

## Accessories

| Product                          | 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     |

## Literature

| Product                                                                                    | Reference |
|--------------------------------------------------------------------------------------------|-----------|
| <b>Application notes</b>                                                                   |           |
| Column efficiency testing                                                                  | CY13149   |
| Use of sodium hydroxide for cleaning and sanitization of chromatography resins and systems | CY13951   |
| Packing Capto S, Capto Q, and Capto DEAE in production-scale columns                       | CY13253   |
| <b>Data files</b>                                                                          |           |
| Capto SP ImpRes and Capto Q ImpRes                                                         | CY12674   |
| Tricorn - Empty high performance columns                                                   | CY17144   |
| <b>Handbooks</b>                                                                           |           |
| Ion Exchange Chromatography: Principles and methods                                        | CY13983   |
| High-throughput process development with PreDicator plates: Principles and methods         | CY16051   |
| <b>Instructions</b>                                                                        |           |
| Tricorn Empty High Performance Columns                                                     | 28409488  |
| HiScale columns (10, 16, 26, 50) and accessories                                           | 28967470  |

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