Capto™ Phenyl ImpRes
Capto™ Butyl ImpRes

Hydrophobic interaction chromatography resins

Instructions for Use

Capto™ Phenyl ImpRes and Capto Butyl ImpRes are hydrophobic interaction chromatography (HIC) resins for the intermediate and polishing steps in a downstream protein purification process.

The Capto HIC ImpRes products are based on a highly cross-linked and spherical agarose matrix for good pressure/flow properties. It combines high flow properties with a small particle size of 40 µm for increased resolution.

HIC resins from Cytiva are produced as a series of hydrophobic resins based on alkyl or aryl ligands attached to a hydrophilic base matrix. Ligand type and concentration cover the range of protein hydrophobicity in a biological extract, varying from weak to moderate to strong hydrophobic proteins. This strategy results in HIC resins for all occasions where the emphasis is on high recovery, purity and reduced risk of denaturation of the target proteins in a biological extract.

Capto HIC resins offer the following benefits:

- improved productivity and process economy in downstream operations
- high flow rates and large sample volume processing
- good chemical stability

1 Introduction

Important

Read these instructions carefully before using the product.

Safety

Refer to the Safety Data sheet for safe use and handling of the product.

2 BioProcess 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 manufacturing requirements. Secure ordering and delivery routines give a reliable supply of resins for production-scale.

Regulatory Support Files (RSF) are available to assist in process validation and submissions to regulatory authorities. BioProcess resins cover all purification steps from capture to polishing.

3 Product description

3.1 Applications

Capto HIC ImpRes resins can be used for the intermediate and polishing steps in downstream purification. The products can be used in combination with:

- ion exchange chromatography (IEX)
- affinity chromatography (AC)
- multimodal chromatography (MMC)
- size exclusion chromatography (SEC)

3.2 Resin properties

The products have a highly cross-linked and spherical agarose matrix with high physical and chemical stability which allows for the use of high flow rates. This permits rapid processing of large sample volumes. The products combine high capacity, high flow rate and low back pressure properties to reduce process cycle times which improves productivity and process economy in downstream operations.

Capto HIC resins can be grouped by ligand type. The type of ligand affects the interaction with sample components:

- Capto Butyl ImpRes has an alkyl chain ligand (butyl) with a purely hydrophobic character.
- Capto Phenyl ImpRes has an aryl ligand (phenyl) with a mixed-mode behavior in which aromatic and hydrophobic interactions and lack of charge play simultaneous roles.

The table below lists the main resin properties.

	Capto Phenyl ImpRes	Capto Butyl ImpRes
Matrix	Highly cross-linked agarose, spherical	
Particle size, d_{50v}[1]	~ 40 µm	
Ligand	Phenyl	Butyl
Dynamic binding capacity, Q_{B10}[2]	~ 19 mg BSA/mL resin	~ 37 mg BSA/mL resin
Recommended operating flow velocity[3]	≤ 220 cm/h	
Pressure/flow characteristics[4]	≤ 220 cm/h at ≤ 0.3 MPa	
pH stability		
Operational[5]	3 to 13	
CIP[6]	2 to 14	
Chemical stability	See *Table 3.1, on page 6*.	
Storage	20% ethanol, 4°C to 30°C	
Autoclavability	17 min at 121°C in 0.1 M KH_2PO_4, pH 8.0, 10 cycles	

1 Median particle size of the cumulative volume distribution.
2 Dynamic binding capacity at 10% breakthrough by frontal analysis at a mobile phase velocity of 150 cm/h in a Tricorn™ 5/100 column at 10 cm bed height (4 min residence time). Buffer conditions: 0.1 M Na_2SO_4, 1.2 M $(NH_4)_2SO_4$, pH 7.
3 In a 1 m diameter column, with a 20 cm bed height, at 20°C, using buffer with the same viscosity as water.
4 In a 1 m diameter column, with a 20 cm bed height, at 20°C, using buffer with the same viscosity as water. Flow velocity is dependent on the column used. The pressure given must not be taken as the maximum pressure of the resin.
5 pH range where resin can be operated without significant change in function.
6 pH range where resin can be subjected to cleaning-in-place without significant change in function.

Chemical stability in aqueous solutions

The products have tested stable for incubation periods of up to a week in the aqueous solutions marked with an X, unless specified otherwise.

Table 3.1: Chemical stability for Capto HIC ImpRes resins

Solution	Capto Phenyl ImpRes	Capto Butyl ImpRes
1 M sodium hydroxide	X	X
1 mM hydrochloric acid	X	X
10 mM hydrochloric acid	X	-
100 mM hydrochloric acid	X	-
1 M acetic acid	X	-
70% formic acid	X	-
30% acetonitrile	X	-
30% isopropanol	X	X
8 M urea	X	-
6 M guanidine hydrochloride	X	-
2 M ammonium sulfate	X	X
3 M ammonium sulfate	X	-
2% sodium dodecyl sulfate	X	-
20% ethanol[1]	X	X

[1] Long-term stability.

X = Stable.

- = Stability to be evaluated by customer.

4 Method optimization

Overview

This chapter gives a general description of how to use the mechanisms of HIC separation to optimize the method. Since method optimization is preferably performed with small column volumes in order to save sample and buffer, certain parameters such as the dynamic binding capacity can be optimized using shorter bed heights than those for the final scale.

4.1 Working principle of separation in HIC

Hydrophobic interaction chromatography separates proteins according to the differences in their surface hydrophobicity. The reversible interaction between the proteins and the hydrophobic ligand is affected by:

- hydrophobicity of the resin
- properties and composition of the sample
- presence and distribution of surface-exposed hydrophobic amino acid residues
- salt type and salt concentration used in the binding buffer

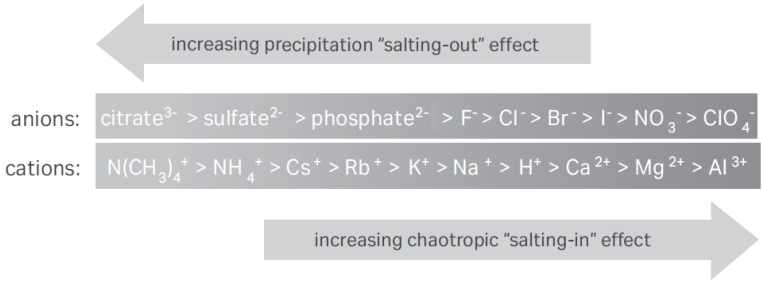
Equilibration

Add salt to the mobile phase to equilibrate the stationary phase to the desired start conditions.

Sample application and wash

Bind the target molecules to the HIC resin and wash out all unbound materials.

Binding is promoted by moderately high concentrations of anti-chaotropic salts such as ammonium sulfate and sodium sulfate. The Hofmeister series illustrated below arranges common anions and cations in order of their effects on the solubility of protein in aqueous solutions. Increasing the salting-out effect promotes hydrophobic interactions and increases the binding capacity of HIC resins for proteins. The opposite dominates when the chaotropic effect of the salts is increased.



Elution

Decrease the salt concentration of the buffer to elute the bound solutes. Use stepwise or gradient elution to separate the target biomolecules.

Regeneration

Remove the remaining bound biomolecules to prepare the stationary phase for the next run.

4.2 Factors affecting HIC

Important factors to consider when selecting a HIC resin and optimizing its chromatographic performance are:

- type of base matrix (for example, agarose, organic copolymers)
- ligand structure
- ligand concentration
- characteristics of target protein and other sample components
- salt type
- salt concentration
- temperature
- pH
- additives

The ligand structure and ligand concentration as well as the salt type and salt concentration added during the binding step determine the outcome. In general, the type of immobilized ligand determines the binding selectivity while its concentration determines the binding capacity.

Ligand choice must be determined empirically through screening experiments for each individual separation task.

For more detailed information, refer to the handbook *Hydrophobic Interaction and Reversed Phase Chromatography, Principles and Methods* (cytiva.com/handbooks).

4.3 Automated buffer preparation

ÄKTA™ chromatography systems with BufferPrep or BufferPro functionality provide different buffer recipes to screen chromatography resins over a range of pH values and elution conditions.

5 Packing columns

5.1 Packing preparations

Recommended columns

Column	Inner diameter	Column volume*	Bed height
Laboratory-scale			
Tricorn 5/100	5 mm	2 mL	10 cm
Tricorn 10/100	10 mm	8 mL	10 cm
HiScale™ 10/40	10 mm	8 to 20 mL†	max. 25 cm†
HiScale 16/20	16 mm	20 to 40 mL	max. 20 cm
HiScale 16/40	16 mm	20 to 70 mL	max. 35 cm
HiScale 26/20	26 mm	53 to 106 mL	max. 20 cm
HiScale 26/40	26 mm	53 to 186 mL	max. 35 cm
HiScale 50/20	50 mm	196 to 393 mL	max. 20 cm
HiScale 50/40	50 mm	196 to 687 mL	max. 35 cm
Production-scale			
AxiChrom™	50 to 200 mm	0.2 to 12.5 L	max. 40 cm
AxiChrom	300 to 1000 mm	7 to 314 L	max. 40 cm

* Column volume range calculated from 10 cm bed height to maximum bed height.

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

Definitions

The bed height of a gravity settled bed differs from the bed height of a bed settled at a given flow (consolidated). Therefore, the compression factor (CF) has to be separated from the packing factor (PF).

$L_{settled}$	Settled bed height/Gravity settled bed height Bed height measured after settling by gravity.	
L_{cons}	Consolidated bed height Bed height measured after settling the resin at a given flow velocity.	
L_{packed}	Packed bed height	
CF	Compression factor	$CF = L_{settled}/L_{packed}$
PF	Packing factor	$PF = L_{cons}/L_{packed}$
A_C	Cross-sectional area of the column	
V_C	Column volume	$V_C = L_{packed} \times A_C$
C_{slurry}	Concentration of the slurry	

Slurry preparation

Let the resin settle overnight in 20% ethanol in a measuring cylinder to determine the slurry concentration, or use the Slurry Concentration Kit (29096100).

5.2 Packing Tricorn columns

Materials and equipment

- Capto Phenyl ImpRes, or Capto Butyl ImpRes
- Tricorn column and an extra bottom piece
- Tricorn packing tube that is longer than the column tube
- appropriate Tricorn standard filter
- plastic spoon or spatula
- glass filter G3
- vacuum suction equipment
- filter flask
- measuring cylinder
- 0.15 M NaCl in ultrapure water (Capto Phenyl ImpRes), or 0.4 M NaCl in ultrapure water (Capto Butyl ImpRes)
- ÄKTA system, or stand-alone pump, depending on the flow rate required

Make sure that all equipment and materials are at room temperature.

To avoid column drainage during packing, a pressure relief valve can be attached to the outlet valve of the system. Setting a low back pressure of 0.02 MPa (0.2 bar, 2.9 psi) is sufficient.

Equilibration to packing solution

Step	Action
1	Attach a glass filter funnel to a filtering flask.
2	Suspend the resin by shaking the measuring cylinder and pour the slurry into the funnel.
3	Wash 5 times with 2 column volumes (CV) of packing solution. Gently stir with a spatula between additions.
4	Pour the washed resin from the funnel into a beaker.
5	Add packing solution to obtain a 55% slurry concentration.

Packing parameters for Tricorn columns

	Tricorn 5	Tricorn 10
Bed height (cm)	10	10
Slurry/packing solution	Capto Phenyl ImpRes: 0.15 M NaCl in ultrapure water Capto Butyl ImpRes: 0.40 M NaCl in ultrapure water	
Slurry concentration (%)	55	55
Packing flow velocity (cm/h)	2250	2250
Packing flow rate (mL/min)	7.4	29.4

Column preparation

For column assembly details, refer to *Tricorn Empty High Performance (28409488)*.

Step	Action
1	Wet a top filter and two bottom filters with 20% ethanol.
2	Insert the bottom filter into the filter holder.
3	Wet the O-ring on the filter holder by dipping the filter holder into water, buffer, or 20% ethanol.
4	Insert the filter holder into the column tube. Make sure that the keyed part of the filter holder fits into the slot in the threaded section of the column tube. Push the filter holder in position.
5	Thread the end cap onto the column tube.

Packing procedure

Step	Action
1	Attach a packing connector and a packing tube on top of the column tube. Fasten the column tube in a stand.
2	Fill the column with slurry suspended in packing solution and top up with packing solution.
3	Assemble a bottom unit to the top of the packing tube. Make sure that no air is trapped under the filter.
4	Connect the column top to the pump and start a packing flow. The packing flow velocity is shown in *Packing parameters for Tricorn columns, on page 12*.
5	Pack the column for 3 minutes.
6	Turn off the flow and attach a stop plug to the column bottom.
7	Disassemble the packing tube and remove excess resin, using a pipette.
8	Top up the column with packing solution.
9	Place the pre-wet top filter on top of the packing solution. Make sure that no air is trapped under the filter.
10	Screw the guiding ring inside the top adapter down to its end position so that it is level with the bottom of the adapter unit.
11	Wet the O-ring on the top adapter by dipping it into water, buffer or 20% ethanol.
12	Screw the guiding ring back 1.5 turns.
13	Screw the top adapter onto the column tube. The inner part of the guiding ring must fit into the slot in the column tube threads. Make sure that there are no air bubbles.
14	Connect the top adapter to the pump. Make a drop-to-drop connection to prevent air from entering the column.
15	Remove the stop plug from the column bottom.
16	Turn the adapter down until it is 1 to 2 mm above the resin bed to displace the air in the adapter tubing.
17	Start a packing flow. The packing flow velocity is shown in *Packing parameters for Tricorn columns, on page 12*. Let the flow continue for 20 minutes.

Step	Action
18	Mark the bed height and pause the pump.
19	Turn the adapter down to the mark, and approximately 1 mm further below the mark.
20	Start the packing flow again for a few seconds to make sure that no gap is formed between the bed and the adapter.
	Note: *If a gap is formed between the bed and the adapter during the last step, turn the adapter down to the bed without stopping the flow.*

The column is ready for efficiency testing.

5.3 Packing HiScale columns

Materials and equipment

- Capto Phenyl ImpRes, or Capto Butyl ImpRes
- HiScale column
- HiScale packing tube (depending on bed height)
- standard net ring filters
- plastic spoon or spatula
- glass filter G3
- vacuum suction equipment
- filter flask
- measuring cylinder
- 20% ethanol with 0.4 M NaCl
- ÄKTA system, or stand-alone pump, depending on the flow rate

Make sure that all equipment and materials are at room temperature.

Equilibration to packing solution

Step	Action
1	Attach a glass filter funnel to a filtering flask.
2	Suspend the resin by shaking the measuring cylinder and pour the slurry into the funnel.
3	Wash the resin 5 times with 2 CV of packing solution. Gently stir with a spatula between additions.
4	Pour the washed resin from the funnel into a beaker.
5	Add packing solution to obtain a slurry concentration of 50%.

Packing parameters for HiScale columns

Only the HiScale 10/40 and HiScale 50/20 columns require a conditioning flow for optimal packing. That is why the tables for the other columns state N/A (not applicable) for the conditioning flow values.

Note: *When two entries divided by a forward slash (/) are given, the first entry applies to Capto Phenyl ImpRes and the second entry applies to Capto Butyl ImpRes.*

	HiScale 10/40		
Bed height (cm)	10	20	25
Slurry/packing solution	20% ethanol with 0.4 M NaCl		
Slurry concentration (%)	50	50	50
Packing factor (PF)	1.05	1.04	1.04
Packing flow velocity (cm/h)	2674	1910	1528
Packing flow rate (mL/min)	35	25	20
Conditioning flow velocity (cm/h)	2674	1910	1528
Conditioning flow rate (mL/min)	35	25	20

	HiScale 16/20	HiScale 16/40	
Bed height (cm)	10	20	25
Slurry/packing solution	20% ethanol / 20% ethanol with 0.4 M NaCl		
Slurry concentration (%)	50 / 40	50 / 40	50
Packing factor (PF)	1.15	1.12	1.05 / 1.09
Packing flow velocity (cm/h)	550 / 600	500	400 / 350
Packing flow rate (mL/min)	18.4 / 20.1	18.7	13.4 / 11.7
Conditioning flow velocity (cm/h)	N/A	N/A	N/A
Conditioning flow rate (mL/min)	N/A	N/A	N/A

	HiScale 26/20	HiScale 26/40	
Bed height (cm)	10	20	35
Slurry/packing solution	20% ethanol / 20% ethanol with 0.4 M NaCl		
Slurry concentration (%)	50 / 40	50 / 40	50
Packing factor (PF)	1.15	1.12	1.05 / 1.09
Packing flow velocity (cm/h)	550 / 600	500	400 / 350
Packing flow rate (mL/min)	48.7 / 53.1	44.2	35.4 / 31.0
Conditioning flow velocity (cm/h)	N/A	N/A	N/A
Conditioning flow rate (mL/min)	N/A	N/A	N/A

	HiScale 50/20	HiScale 50/40	
Bed height (cm)	10	20	35
Slurry/packing solution	20% ethanol / 20% ethanol with 0.4 M NaCl		
Slurry concentration (%)	50 / 40	50/40	50
Packing factor (PF)	1.15	1.12	1.05 / 1.09
Packing flow velocity (cm/h)	550 / 600	500	400 / 350
Packing flow rate (mL/min)	180 / 196.3	163.6	130.9 / 114.5
Conditioning flow velocity (cm/h)	550 / 600	N/A	N/A
Conditioning flow rate (mL/min)	180 / 196.3	N/A	N/A

Column preparation

Step	Action
1	Assemble the column according to the column instructions *HiScale columns (10, 16, 26, 50) and accessories (28967470)*.
2	Fasten the column tube in 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. Perform this step when the nets are dry.
4	Remove any air that is trapped under the net by light suction from a syringe.
5	Connect the bottom adapter unit to the bottom of the column tube and tighten the O-ring by turning the end knob. *Result:* The column is prepared. Proceed with column filling.

Column filling

Step	Action
1	Fill the column with approximately 1 cm of packing liquid using a pump or a syringe.
2	Disconnect the pump or the syringe and place a stop plug in the outlet.
3	Connect the packing tube to the top of the column tube.
4	Connect the top adapter to the pump and prime with a slow flow.
5	Turn the top adapter so the net faces up.
6	Fill the column with slurry suspended in packing solution.
7	If needed, top up the slurry with extra packing fluid so the top adapter when attached dips into the slurry to avoid air under the net.
8	Attach the top adapter on top of the packing column tube.
9	Tighten all O-rings firmly and remove the bottom stop plug.
10	Immediately proceed with resin packing.

Resin packing

Step	Action
1	Start a downflow with packing solution. The packing flow velocity is shown in *Packing parameters for HiScale columns, on page 16*.
2	Let the flow run until the bed consolidates.
3	Measure the consolidated bed height using the graduation marks on the column. The use of a light source can facilitate the measurement of the bed height.
4	Calculate the final bed height by dividing the consolidated bed height with the desired packing factor, $L_{packed} = L_{cons}/PF$.
5	Turn off the flow and attach a stop plug to the bottom of the column.
6	Disassemble the top adapter from the column packing tube.
7	While holding the packing tube over a beaker or a sink, disconnect the packing tube from the column.
8	Reassemble the top adapter to the column tube.

Mechanical compression and bed conditioning

Only the HiScale 10/40 and HiScale 50/20 columns require a conditioning flow for optimal packing.

Step	Action
1	Turn the adapter down until it is 1 to 2 cm above the resin bed to displace any air in the adapter tubing. Make sure that the bed surface is undisturbed.
2	Tighten the O-ring of the adapter.
3	Remove the bottom stop plug and carefully turn the bottom adapter down.
4	Keep turning the bottom adapter until the calculated final bed height is reached. Make sure that no pressure peaks exceed the pressure specifications for the resin.
5	Turn the adapter down to the marking for the calculated compression.
6	**For the HiScale 10/40 and HiScale 50/20**: Start a downflow with packing solution to condition the bed. The conditioning flow velocity is shown in *Packing parameters for HiScale columns, on page 16*.
7	**For the HiScale 10/40 and HiScale 50/20**: Let the flow run for 10 CV. *Result:* The bed is conditioned and the column is ready for efficiency testing.

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

6 Evaluation of column packing

6.1 Intervals

Test the column efficiency to evaluate the packing quality:

- after completion of a packing procedure
- at regular intervals during the working life of the column
- when a deterioration in separation performance is observed

6.2 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). These values can be determined by applying a sample such as 1% acetone solution to the column. Sodium chloride can also be used as a test sample. Use a concentration of 0.8 M NaCl in water with 0.4 M NaCl in water as eluent.

For more information about column efficiency testing, see *Column efficiency testing (CY13149)*.

Note: *The calculated plate number varies with the test conditions and must only be used as a reference value. For comparable results, test conditions and equipment must remain unchanged. Any changes in solute, solvent, eluent, sample volume, flow velocity, liquid pathway, or temperature influence the results.*

Sample volume and flow velocity

For optimal results, the sample volume must be at maximum % of the column volume and the liquid velocity must be 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 use.

6.3 Method for measuring HETP and A_S

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

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

L = bed height (cm)

N = number of theoretical plates

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

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

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:

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

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

As a guideline, a value of < 3 is very good.

The peak must be symmetrical, and the asymmetry factor as close to 1 as possible. (A typical acceptable range could be $0.8 < A_S < 1.8$).

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

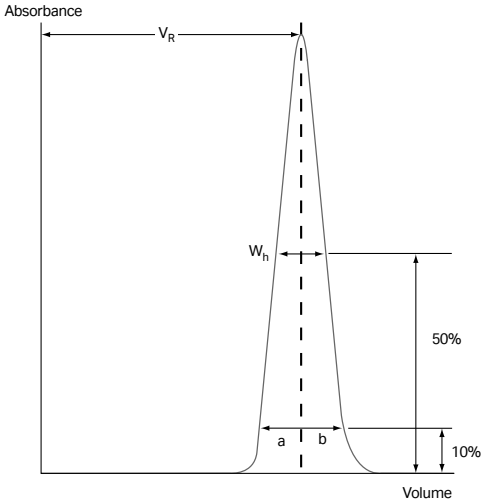
Peak asymmetry factor calculation:

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

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.



7 Cleaning-In-Place (CIP)

Overview

CIP removes very tightly bound, precipitated, or denatured substances from the resin. The accumulated contaminants can affect the chromatographic properties of the packed column, reduce the capacity, or contaminate subsequent runs.

CIP must be performed regularly to prevent the enrichment of the contaminants and to maintain the capacity, flow properties, and general performance of the packed columns.

It is recommended to perform a CIP:

- before first-time use or after long-term storage
- after every cycle with real feed
- when an increase in the back pressure is noticed
- if a reduced column performance is observed
- to prevent potential cross-contamination, when the same column is used for purification of different proteins

7.1 CIP protocol

Capto HIC resins can be regenerated by washing with distilled water, but they are also alkali-tolerant. The properties of the sample determine the CIP protocol, so the protocols below might require optimization. Cleaning agent concentration, contact time, and frequency are the main parameters to alter during CIP optimization. The CIP protocols below remove common contaminants.

Regular cleaning

Regular cleaning is performed with 0.5 to 1.0 M NaOH. NaOH dissolves proteins and saponifies fats.

Step	Action
1	Wash with 3 CV water or elution buffer.
2	Wash with at least 3 CV 0.5 to 1.0 M NaOH at half the flow rate used during purification or 65 to 130 cm/h, preferably in reversed flow direction. The contact time must be at least 15 minutes, preferably 30 minutes to improve the bactericidal effect.
3	Wash with at least 3 CV water or elution buffer. To neutralize the liquid more rapidly, a phosphate buffer of neutral pH can be used for washing.

Removal of strongly bound substances

Strongly bound substances are removed by washing with up to 70% ethanol or 30% isopropanol. For extreme cases, use 30% isopropanol in 1 M NaOH.

Step	Action
1	Wash with 3 CV water or elution buffer.
2	Wash with at least 3 CV up to 70% ethanol or 30% isopropanol (or 30% isopropanol in 1 M NaOH) at half the flow rate used during purification or 65 to 130 cm/h, preferably in reversed flow direction. The contact time must be at least 15 minutes, preferably 30 minutes to improve the bactericidal effect.
3	Wash with at least 3 CV water or elution buffer. To neutralize the liquid more rapidly if 30% isopropanol in 1 M NaOH was used, a phosphate buffer of neutral pH can be used for washing.

8 Scale-up

Overview

After method optimization at laboratory scale, the process can be scaled up. Scale up is typically performed by keeping bed height and linear liquid velocity constant while increasing bed diameter and volumetric flow rate. Because optimization is preferably performed with small column volumes to save sample and buffer, parameters like dynamic binding capacity can be optimized using shorter bed heights than those 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 like clearance of critical impurities, can change when column bed height is modified and must be validated using final bed height. Bed heights of 10 to 25 cm are generally considered appropriate. The residence time is approximated as the bed height (cm) divided by the linear liquid velocity (cm/h) applied during sample loading.

Select the bed volume according to the required binding capacity. Keep sample concentration and gradient slope constant. Larger equipment used when scaling up can cause deviations from the method optimized at a 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.

9 Storage

Store the product in a 20% ethanol solution at 4°C to 30°C.

10 Ordering information

10.1 Products

For additional information, refer to *cytiva.com*.

Product	Pack size	Product code
Capto Phenyl ImpRes	25 mL	17548401
	100 mL	17548402
	1 L	17548403
	5 L	17548404
Capto Butyl ImpRes	25 mL	17371901
	100 mL	17371902
	1 L	17371903
	5 L	17371904

10.2 Related products

Screening kits

Format	Pack size	Product code
HiTrap™ column		
HiTrap Capto HIC Selection Kit:	5 × 1 mL	29321087
Capto Phenyl (high sub), Capto Phenyl ImpRes, Capto Butyl, Capto Butyl ImpRes, and Capto Octyl		
PreDictor™ plate		
PreDictor Capto HIC Screening Kit:	6 µL, 4 × 96-well plates	29711438
Capto Phenyl (high sub), Capto Butyl, Capto Octyl, Capto Phenyl ImpRes, Capto Butyl ImpRes, and Capto Butyl-S	20 µL, 4 × 96-well plates	29711439

Capto Phenyl ImpRes

Format	Pack size	Product code
HiTrap column	5 × 1 mL	17548411
	5 × 5 mL	17548412
HiScreen™ column	1 × 4.7 mL	17548410
PreDictor plate	6 µL, 4 × 96-well filter plates	29711440
	20 µL, 4 × 96-well filter plates	29711441
PreDictor RoboColumn unit	200 µL, 8 columns	29701638
	600 µL, 8 columns	17548441
Process Characterization Kit	3 × 25 mL (3 different ligand densities)	17545170
ReadyToProcess™ column	1 L (80/200)	29101697
	1.9 L (126/150)	29609021
	2.5 L (126/200)	29101698
	5 L (178/200)	29642661
	7.4 L (251/150)	29696391
	10 L (251/200)	29101700
	20 L (359/200)	29101702
	32 L (450/200)	29256253
	57 L (600/200)	29649594

Capto Butyl ImpRes

Format	Pack size	Product code
HiTrap column	5 × 1 mL	17371911
	5 × 5 mL	17371912
HiScreen column	1 × 4.7 mL	17371910
PreDictor plate	6 µL, 4 × 96-well filter plates	29711442
	20 µL, 4 × 96-well filter plates	29711443
PreDictor RoboColumn unit	200 µL, 8 columns	29701637
	600 µL, 8 columns	17371941
Process Characterization Kit	3 × 25 mL (3 different ligand densities)	17371970
ReadyToProcess column	1 L (80/200)	29713752
	2.5 L (126/200)	29655954
	5 L (178/200)	29647159
	10 L (251/200)	29138138
	20 L (359/200)	29229399
	32 L (450/200)	29256254
	57 L (600/200)	29474655

10.3 Related documentation

All items in the table below are available on *cytiva.com*.

Related documentation	Reference
Application notes	
Column efficiency testing	CY13149
High-throughput screening of HIC media in PreDictor plates for capturing recombinant Green Fluorescent Protein from *E. coli*	CY13374
Increasing productivity in hydrophobic interaction chromatography (HIC) using Capto resins	CY14112
Optimization of a hydrophobic interaction chromatography step for recombinant protein purification	CY27574
How to pack Capto HIC resins using verified packing methods	CY30548
Packing HiScale, XK, and Tricorn chromatography columns with Capto and MabSelect™ resins	CY14043
Data files	
Capto Phenyl ImpRes and Capto Butyl ImpRes	CY13700
HiScreen prepacked columns	CY13473
PreDictor 96-well filter plates and Assist Software	CY13663
Handbooks	
Hydrophobic Interaction and Reversed Phase Chromatography, Principles and Methods	CY11248
Instructions	
Tricorn Empty High Performance Columns	28409488
HiScale columns (10, 16, 26, 50) and accessories	28967470
Selection guides	
Hydrophobic Interaction Chromatography (HIC)	CY14016

Additional reading

Visit the *HIC resource center* on *cytiva.com* for additional reading and application content like:

- *Developing a HIC polishing step for removal of mAb aggregates*

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