



Butyl Sepharose™ 4 Fast Flow Octyl Sepharose™ 4 Fast Flow

Hydrophobic interaction chromatography

Instructions for Use

Butyl Sepharose™ 4 Fast Flow and Octyl Sepharose 4 Fast Flow form part of the Cytiva resin range for hydrophobic interaction chromatography (HIC).

These instructions contain information about resin characteristics, column packing, and maintenance.

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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 Introduction

BioProcess™ resins

Butyl Sepharose 4 Fast Flow and Octyl Sepharose 4 Fast Flow belong to the BioProcess resins. BioProcess chromatography 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.

Resin description

The base matrix, Sepharose 4 Fast Flow, is a cross-linked, 4% agarose derivative with excellent kinetics, making the resins ideal for process-scale applications, particularly during initial capture and intermediate stages of a separation process when high flow rates are required.

The high physical and chemical stability of the matrix prevents bed compression and formation of fines, and allows for efficient maintenance procedures for increased resin lifetime. A typical pressure-flow curve for Butyl Sepharose 4 Fast Flow is shown below.

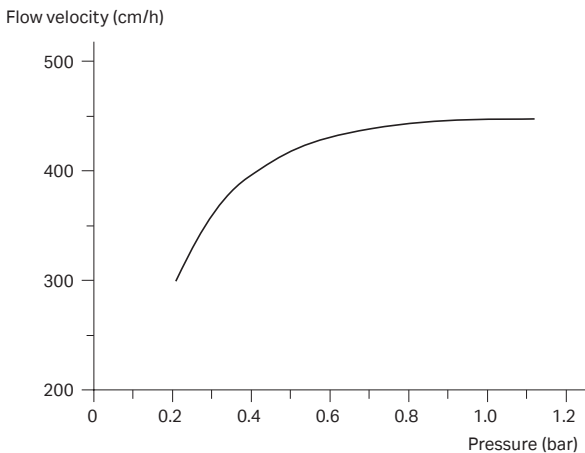


Fig 1. Typical pressure-flow curve for Butyl Sepharose 4 Fast Flow in an XK 50/30 column, bed height 15 cm; mobile phase 0.1 M NaCl.

Table 1. Characteristics of Butyl Sepharose 4 Fast Flow

Matrix	Cross-linked agarose, 4%, spherical
Type of ligand	Butyl: R-O-CH ₂ -CH(OH)-CH ₂ -O-(CH ₂) ₃ -CH ₃
Particle size, d_{50v}¹	~ 90 µm
Ligand concentration	~ 40 µmol butyl/mL resin
pH stability, operational²	3 to 13
pH stability, CIP³	2 to 14
Chemical stability⁴	Stable in commonly used aqueous buffers, 1 mM HCl, 1.0 M NaOH, 70% ethanol ⁵ , 30% isopropanol ⁵ , 6 M guanidine hydrochloride
Autoclavability	20 min at 121 °C in distilled water, pH 7, 5 cycles
Pressure-flow characteristics^{6,7}	150 to 250 cm/h at < 0.1 MPa in an XK 50/60 column with 5 cm diameter and 25 cm bed height (at 20 °C using buffers with the same viscosity as water).
Operating temperature	4 °C to 40 °C
Delivery conditions	20% ethanol

¹ Median particle size of the cumulative volume distribution.

² pH range where resin can be operated without significant change in function.

³ pH range where resin can be subjected to cleaning- or sanitization-in-place without significant change in function.

⁴ 1.0 M NaOH must only be used for cleaning purposes.

⁵ Consult local safety regulations to verify if safety precautions are required for the use of 70% ethanol or 30% isopropanol.

⁶ The pressure-flow characteristics describe the relationship between pressure and flow under the set circumstances. The pressure given is not the maximum pressure of the resin.

⁷ Pressure-flow test performed on the base matrix.

Table 2. Characteristics of Octyl Sepharose 4 Fast Flow

Matrix	Cross-linked agarose, 4%, spherical
Type of ligand	Octyl: R-O-CH ₂ -CH(OH)-CH ₂ -O-(CH ₂) ₇ -CH ₃
Particle size, d_{50v}¹	~ 90 µm
Ligand concentration	~ 5 µmol octyl/mL resin
pH stability, operational²	3 to 13
pH stability, CIP³	2 to 14
Chemical stability⁴	Stable in commonly used aqueous buffers, 1 mM HCl, 1.0 M NaOH, 70% ethanol ⁵ , 30% isopropanol ⁵ , 6 M guanidine hydrochloride
Autoclavability	20 min at 121 °C in distilled water, pH 7, 5 cycles
Pressure-flow characteristics^{6,7}	150 to 250 cm/h at < 0.1 MPa in an XK 50/60 column with 5 cm diameter and 25 cm bed height (at 20 °C using buffers with the same viscosity as water).
Operating temperature	4 °C to 40 °C
Delivery conditions	20% ethanol

¹ Median particle size of the cumulative volume distribution.

² pH range where resin can be operated without significant change in function.

³ pH range where resin can be subjected to cleaning- or sanitization-in-place without significant change in function.

⁴ 1.0 M NaOH must only be used for cleaning purposes.

⁵ Consult local safety regulations to verify if safety precautions are required for the use of 70% ethanol or 30% isopropanol.

⁶ The pressure-flow characteristics describe the relationship between pressure and flow under the set circumstances. The pressure given is not the maximum pressure of the resin.

⁷ Pressure-flow test performed on the base matrix.

Method design and optimization

The main purpose of optimizing a chromatographic step is to reach the predefined purity level with highest possible product recovery by choosing the most suitable combination of the critical chromatographic parameters. This means finding the conditions that give the highest possible productivity and process economy.

Recommendations for optimizing the critical operational parameters which affect the maximum utilization of a HIC step can be found in the *handbook (CY11248)*, available at cytiva.com.

Resin screening

The table below lists kits for screening and resin selection.

Screening kit	Contents
PreDicator™ HIC Screening High Hydrophobicity	Phenyl Sepharose 6 Fast Flow (low sub) Capto™ Butyl Phenyl Sepharose Fast Flow (high sub) Capto Phenyl (high sub)
PreDicator HIC Screening Low Hydrophobicity	Butyl-S Sepharose 6 Fast Flow Octyl Sepharose 4 Fast Flow Butyl Sepharose 4 Fast Flow Capto Octyl
HiTrap™ Capto HIC Selection Kit 5 × 1 mL columns	Capto Phenyl (high sub) Capto Phenyl ImpRes Capto Butyl Capto Butyl ImpRes Capto Octyl
HiTrap HIC Selection Kit 7 × 1 mL columns	Phenyl Sepharose High Performance Phenyl Sepharose 6 Fast Flow (low sub) Phenyl Sepharose 6 Fast Flow (high sub) Butyl Sepharose High Performance Butyl-S Sepharose 6 Fast Flow Butyl Sepharose 4 Fast Flow Octyl Sepharose 4 Fast Flow

2 Binding

The binding of proteins to hydrophobic resins is affected by:

- the structure of the ligand (for example carbon chain or an aromatic ligand)
- the ligand concentration
- the ionic strength of the buffer
- the salting-out effect (see The Hofmeister series below)
- the temperature

Salts that cause salting-out (for example ammonium sulfate) also promote binding to hydrophobic ligands. The column is equilibrated and the sample is applied in a solution of high ionic strength. A typical starting buffer is 1.7 M $(\text{NH}_4)_2\text{SO}_4$, which is just below the concentration employed for salting out proteins.

Hydrophobic interactions are weaker at lower temperatures. This must be taken into account if chromatography is performed in a cold room.

3 Elution

Bound proteins are eluted by reducing the strength of the hydrophobic interaction. This can be done by:

- reducing the concentration of salting-out ions in the buffer with a decreasing salt gradient (linear or step)
- increasing the concentration of salting-in ions in the buffer with a positive gradient (linear or step)
- eluting with a polarity-reducing organic solvent (for example ethylene glycol)
- including detergent in the eluent

The Hofmeister series

← Increasing salting-out (precipitation) effect

Anions: $\text{PO}_4^{3-} < \text{SO}_4^{2-} < \text{CH}_3\text{COO}^- < \text{Cl}^- < \text{Br}^- < \text{NO}_3^- < \text{ClO}_4^- < \text{I}^- < \text{SCN}^-$

Cations: $\text{NH}_4^+ > \text{Rb}^+ > \text{K}^+ > \text{Na}^+ > \text{Cs}^+ > \text{Li}^+ > \text{Mg}^{2+} > \text{Ba}^{2+}$

Increasing salting-in (chaotropic) effect →

Increasing the salting-out (precipitation) effect strengthens hydrophobic interactions; whereas increasing the salting-in (chaotropic) effect weakens hydrophobic interactions.

A suggested starting gradient is a linear gradient from 0% to 100% B with:

Buffer A: 50 mM phosphate buffer, pH 7.0 + 1.7 M $(\text{NH}_4)_2\text{SO}_4$

Note: *When working with proteins that tend to aggregate, start with a lower $(\text{NH}_4)_2\text{SO}_4$ concentration to avoid the risk of precipitation.*

Buffer B: 50 mM phosphate buffer, pH 7.0

4 General column packing guidelines

Recommended columns

Column	Laboratory-scale		
	Inner diameter (mm)	Bed volume (mL)	Bed height (mm)*
Tricorn™			
Tricorn 5/50	5	0.2–1.1	8–56
Tricorn 5/100	5	1.2–2.1	58–106
Tricorn 5/150	5	2.1–3.1	108–156
Tricorn 10/50	10	0.0–4.4	0–56
Tricorn 10/100	10	3.6–8.4	46–106
Tricorn 10/150	10	7.6–12.6	96–156
XK			
XK 16/20	16	0–31	0–155
XK 26/20	26	0–66	0–125
XK 50/20	50	0–274	0–140
XK 50/30	50	0–559	0–280
HiScale™			
HiScale 10/40	10	8–31	100–400
HiScale 50/20	50	0–393	0–200
HiScale 50/40	50	274–785	140–400

* The minimum value corresponds to a packing performed with the adapters.

Column	Production-scale		
	Inner diameter (mm)	Bed volume (L)	Bed height (cm)
AxiChrom™			
	50–200	max. 15.7	max. 50
	300–1600	max. 1005	max. 50
BPG			
Variable bed, glass columns	100–450	–100	max. 58
Chromaflo™			
Variable and fixed bed columns	400–†	†	†

† For more details, refer to *Data file CY13377*.

General considerations

Purifying biological macromolecules by HIC is a typical high selectivity technique where the difference in retention for the molecules to be separated can be substantial at any specific ionic strength.

Therefore, relatively short columns can be used if the selectivity of the adsorbent is exploited in an optimal way. Typical bed heights range from 3 to 15 cm, which will minimize back pressure and allow high throughput.

Columns can be packed in different ways depending on the type of column and equipment used. Always read and follow the relevant column instruction manual carefully. Sepharose 4 Fast Flow based resins are easy to pack since their rigidity allows for the use of high flow values.

Two types of packing methods are described:

- pressure packing (for columns with adapters)
- Chromaflow packing

The following parameters refer to large-scale packing:

General packing procedures	
Preferred packing solution	10% to 20% ethanol
Resin slurry concentration	50%
Packing factor	1.15
Packing pressure	0.10 MPa (1.0 bar)
Packing flow velocity	200 to 300 cm/h

The packing quality of the column greatly affects the separation results. It is very important to pack and test the column according to the following recommendations.

Always start by determining the optimal packing flow velocity. Guidelines for determining optimal packing flow velocities for columns with adapters and fixed bed heights are given in the next section.

Determine optimal packing flow velocity

The optimal packing flow velocity is dependent on column size and type, resin volume, packing solution, and temperature.

The optimal packing flow velocity must therefore be determined empirically for each individual system.

To determine the optimal packing flow velocity, follow these instructions:

Step	Action
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- | | |
|---|---|
| 1 | Calculate the amount of resin needed for the slurry (this is especially important for columns with fixed bed heights). The quantity of resin required per liter packed volume is approximately 1.15 L sedimented resin. |
| 2 | Prepare the column exactly as for column packing. |
| 3 | Start packing the resin at a low flow velocity (30 cm/h). |
| 4 | Increase the pressure in increments and record the flow velocity when the pressure has stabilized. Do not exceed the maximum pressure of the column, or the maximum flow velocity for the resin. |
| 5 | The maximum flow velocity is reached when the pressure-flow curve levels off or the maximum pressure of the column is reached. Stop packing and do not exceed this flow velocity. Make sure to achieve a packing factor of 1.15. The optimal packing flow velocity/pressure is 70% to 100% of the maximum flow velocity/pressure. |
| 6 | Plot the pressure-flow curve as in Resin description, on page 3 and determine the optimal packing flow velocity. |

The operational flow velocity/pressure must be < 70% of the packing flow velocity/pressure.

5 Packing laboratory-scale columns

HiScale and XK columns

The following instructions are for packing HiScale and XK columns with a 10 cm bed height. The instructions can be modified for columns of different dimensions while maintaining the packing pressure at 0.1 MPa (1 bar).

Materials and equipment needed

Material / equipment needed	Specification
Resin	Butyl Sepharose 4 Fast Flow or Octyl Sepharose 4 Fast Flow
Column	HiScale column or XK column
Equipment	• Chromatography system, such as ÄKTA™ system, or a stand-alone pump such as Pump P-900, depending on the required flow rate • Pressure monitor • Vacuum suction equipment
Other materials	• Measuring cylinder • Filter flask • Distilled water • Glass filter G4 • Plastic spoon or spatula

Prepare the slurry

To prepare the slurry, follow the steps below. If you only want to measure the slurry concentration, follow the first three steps of the instructions below or use the method for slurry concentration measurement described in the Applications content *How to determine resin slurry concentration*. The method can also be used for HiScale and XK columns.

Step	Action
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- | | |
|---|---|
| 1 | Let the resin settle in 20% ethanol at least overnight in a measuring cylinder. |
| 2 | Measure the sedimented resin in the measuring cylinder. |
| 3 | Calculate the slurry concentration. |
| 4 | Attach a glass filter funnel onto a filter flask. |
| 5 | Shake the resin to suspend it into a homogenous slurry. |
| 6 | Pour the resin into the glass filter funnel. |
| 7 | Wash the slurry five times with 1 to 2 mL distilled water/mL resin. |

Note:

Gently stir with a spatula between each wash step.

- | | |
|---|--|
| 8 | Transfer the washed slurry from the glass filter funnel into a beaker. |
|---|--|

Step	Action
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- | | |
|---|--|
| 9 | Add enough distilled water to obtain a 50% slurry concentration. |
|---|--|

Wash the resin

Step	Action
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- | | |
|---|---|
| 1 | Wash five times with 1–2 mL distilled water/mL resin. |
| 2 | Gently stir with a spatula between additions. |
| 3 | Move the washed resin from the funnel into a beaker and add distilled water to obtain a 50% slurry concentration. |

Prepare the column for packing

Step	Action
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- | | |
|---|--|
| 1 | Attach the packing tube to the top of the column and rinse with distilled water. |
| 2 | Attach the filter and the bottom piece to the column. |
| 3 | Wet the bottom filter by injecting 20% ethanol through the effluent tubing. |
| 4 | Assemble the column and the packing tube vertically on a laboratory stand and rinse them with distilled water. |

Step	Action
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- | | |
|---|---|
| 5 | Add distilled water, up to 2 cm over the column endpiece, and attach a tubing clamp to the effluent tubing. |
| 6 | Pour all the slurry into the column and packing tube. |
| 7 | Top up the column with distilled water. |

Pack the column

Step	Action
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- | | |
|---|---|
| 1 | Connect the pump outlet to the inlet on the packing tube and open the column outlet. |
| 2 | Pack the column with distilled water at a constant flow rate (see table below) until the resin bed is stable. |
| 3 | Adjust the flow rate to twice the final flow rate (see table below) and decrease the flow rate stepwise until the pressure signal is 0.08 ± 0.02 MPa. |
| 4 | Pack the column at the flow rate which gives 0.08 ± 0.02 MPa for 45 minutes. |
| 5 | Disconnect the packing tube. |
| 6 | Carefully fill the rest of the column with distilled water to form an upward meniscus at the top and insert the flow adapter. |

Note:

Lower the adapter down to the bed surface.

Step	Action
7	Continue packing the column at 0.08 ± 0.02 MPa for 6 minutes.
8	Mark the position of the bed surface on the column and stop the pump.
9	Close the column outlet and lower the adapter down to the bed surface.
10	Lower the adapter a further 3 mm into the resin bed.

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

Packing parameters

Parameter	Column inner diameter			
	10 mm ¹	16 mm	26 mm	50 mm
Sedimented resin (mL)²	9.2	23	61	230
Slurry (mL)	18.4	46	122	460
Bed height (mm)	100	100	100	100
Flow rate (mL/min)³	0.8	2.0	5.0	20
Pressure (MPa)⁴	0.08 ± 0.02	0.08 ± 0.02	0.08 ± 0.02	0.08 ± 0.02
Final flow rate (mL/min)	~ 4	~ 10	~ 25	~ 93

¹ Only applies to HiScale.

² Sedimented resin volume = $1.15 \times$ packed resin volume.

³ Recommended flow rate at first packing step.

⁴ Pressure limit at second packing step.

6 Packing methods for process-scale columns

Packing AxiChrom columns

AxiChrom columns are packed using mechanical axial compression packing. For more information regarding the AxiChrom columns and packing procedures, refer to:

- *Data file (CY10003)*
- *AxiChrom 50–200 columns, Operating Instructions (29379190)*
- *AxiChrom 300–1600 columns, Operating Instructions (29065430)*
- *AxiChrom 300–2000 columns, User Manual (29388247)*
- *AxiChrom Column and AxiChrom Column Controller, User Manual (29720910)*

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

Intelligent Packing in AxiChrom columns

When packing AxiChrom 50 to 200 columns with an ÄKTA system, Intelligent Packing control is managed by the UNICORN™ system control software. For AxiChrom 300 to 1600 columns, Intelligent Packing is performed by the AxiChrom column control unit, a separate unit that comprises a touchscreen-operated user interface, or from the UNICORN software on the ÄKTA process™ system.

In the Intelligent Packing wizard, packing methods are created by entering values for the following packing variables:

- column
- resin
- packing factor
- slurry concentration
- target bed height

Pack BPG columns

BPG columns are supplied with a movable adapter. They are packed by conventional pressure-packing by pumping the packing solution through the chromatographic bed at a constant flow rate (or back pressure).

Step	Action
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- | | |
|---|---|
| 1 | Pour some water (or packing solution) into the column. Make sure that no air is trapped under the bottom net. Leave approximately 2 cm of liquid in the column. |
|---|---|

Step	Action
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- | | |
|---|--|
| 2 | Mix the packing buffer with the resin to form a 50% to 70% slurry. (Sedimented bed volume/slurry volume = 0.5 to 0.7). Pour the slurry into the column. Insert the adapter and lower it to the surface of the slurry, making sure that no air is trapped under the adapter. Secure the adapter in place. |
| 3 | Seal the adapter O-ring and lower the adapter a little further into the slurry to fill the adapter inlet with packing solution. |
| 4 | Connect a pump and a pressure gauge, then start packing at the predetermined packing flow rate (or pressure). Keep the flow rate (or pressure) constant during packing and check the pressure at the column inlet. Never exceed the pressure limit for the column (0.08 ± 0.02 MPa (0.8 ± 0.2 bar)). |
| 5 | When the bed has stabilized, mark the bed height on the column tube, close the bottom valve and stop the pump. The bed starts rising in the column. Loosen the O-ring and lower the adapter to 0.5 to 1 cm from the bed surface. |
| 6 | Seal the O-ring, start the pump and continue packing. Repeat steps 5 and 6 until there is a maximum of 1 cm between bed surface and adapter when the bed has stabilized. |

Step	Action
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- | | |
|---|---|
| 7 | Close the bottom valve, stop the pump, disconnect the column inlet and push the adapter down to approximately 3 mm below the mark on the column tube without loosening the adapter O-ring. The packing solution will flush the adapter inlet. Remove any trapped air by pumping liquid from the bottom (after the inlet tubing and the bottom valve have been properly filled). |
|---|---|

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

Pack Chromaflow columns

Refer to the *Data file (CY13377)*, prepare the column for packing as described in the *Operating instructions (28962232)*, and pack the column appropriately for the resin type.

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

The column is now ready for equilibration and testing.

7 Evaluation of column packing

Introduction

The quality of the packed bed and the column performance must be evaluated initially and monitored throughout the lifetime. The method for measuring the efficiency of a packed column is in terms of the height equivalent to a theoretical plate (HETP) and the asymmetry factor (A_s).

Frequency

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 decrease in separation performance is observed

Column efficiency testing

Packed column efficiency is best expressed in terms of the height equivalent to a theoretical plate (HETP) and the asymmetry factor (A_s). These 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 used remain the same so that the results are comparable.

Test results are affected by changes in:

- solute
- solvent
- eluent
- sample volume
- flow velocity
- liquid pathway
- temperature
- chromatography system

For more information about column efficiency testing, refer to the application note *Column efficiency testing* available on cytiva.com.

Sample volume and flow velocity

For optimal column efficiency testing results, the sample volume must be approximately 1% of the CV and the flow 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.

Method for measuring HETP and A_s

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

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

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 unit

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, an acceptance value of < 3 for (h) can be used.

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

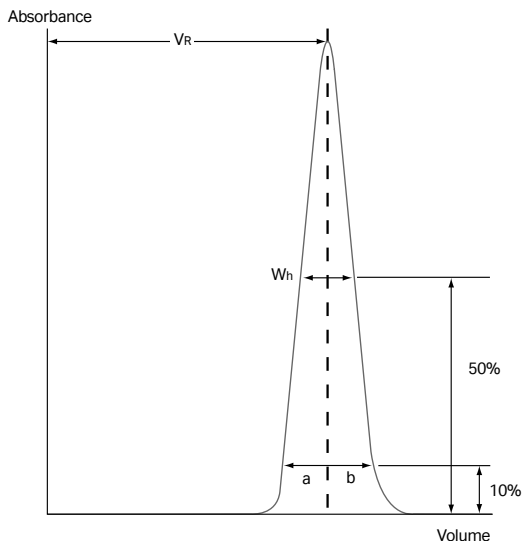
A change in the shape of the peak is usually the first indication of bed deterioration due to excessive use.

The peak asymmetry factor is calculated as follows:

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

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

The figure below shows a UV trace for acetone in a typical test chromatogram from which the HETP and A_5 values are calculated.



8 Maintenance

Regeneration

For best performance from the resins, bound substances must be washed from the column after each chromatographic cycle.

Wash with 2 bed volumes of water, followed by 2 to 3 bed volumes of starting buffer.

To prevent a buildup of contaminants or impurities on the column over time, it is possible that more rigorous cleaning protocols have to be applied on a regular basis.

Cleaning-in-place (CIP)

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 packed bed and helps to maintain 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 listed below.

Precipitated proteins	Wash the column with 4 bed volumes of 0.5 to 1.0 M NaOH at 40 cm/h with reversed flow direction, followed by 2 to 3 bed volumes of water.
Strongly bound hydrophobic proteins, lipoproteins, and lipids	Wash the column with 4 to 10 bed volumes of up to 70% ethanol or 30% isopropanol¹ with reversed flow direction, followed by 3 to 4 bed volumes of water. Apply gradients to avoid air bubble formation when using high concentrations of organic solvents. Alternatively, wash the column with 1 to 2 bed volumes of 0.5% nonionic detergent at 40 cm/h followed by 5 bed volumes of 70% ethanol to remove the detergent, and 3 to 4 bed volumes of water.

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

Sanitization

For inactivation of microbial contaminants, equilibrate the column with 0.5 to 1.0 M NaOH at a flow velocity of approximately 40 cm/h, contact time 30 to 60 minutes. For more details, refer to Application note *Use of sodium hydroxide for cleaning and sanitization of chromatography resins and systems (CY13951)*.

Wash the column thoroughly with running buffer after sanitization.

Sterilization

To sterilize the resins, dismantle the column and autoclave the resin for 20 minutes at 121°C.

Storage

Store the resins in 20% ethanol at 4°C to 30°C, to avoid microbiological growth.

9 Reference information

Ordering information

Product	Pack size	Product code
Butyl Sepharose 4 Fast Flow	25 mL	17098010
	200 mL	17098001
	500 mL	17098002
	5 L	17098004
	10 L	17098005
Octyl Sepharose 4 Fast Flow	200 mL	17094602
	1 L	17094603
	5 L	17094604
HiTrap Capto HIC Selection Kit	5 × 1 mL	29321087
HiTrap HIC Selection Kit	7 × 1 mL	28411007
HiTrap Butyl FF	5 × 1 mL	17135701
	5 × 5 mL	17519701
HiTrap Octyl FF	5 × 1 mL	17135901
	5 × 5 mL	17519601
HiPrep™ 16/10 Butyl FF	20 mL	28936547
HiPrep 16/10 Octyl FF	20 mL	28936548
PreDictor Capto HIC Screening Kit, 6 µL	4 × 96-well plates	29711438
PreDictor Capto HIC Screening Kit, 20 µL	4 × 96-well plates	29711439
PreDictor Screening Low Hydrophobicity, 6 µL	4 × 96-well plates	28992395

Product	Pack size	Product code
PreDictor Screening Low Hydrophobicity, 50 μ L	4 $\times$ 96-well plates	28992398
Tricorn 5/50	1	28406409
Tricorn 5/100	1	28406410
Tricorn 5/150	1	28406411
Tricorn 10/50	1	28406414
Tricorn 10/100	1	28406415
Tricorn 10/150	1	28406416
XK 16/20	1	18877301
XK 26/20	1	18100072
XK 50/20	1	28988952
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

Related literature

Description	Reference
Application notes	
Predictable scale-up through column design and robust packing methodology	CY13211
Use of sodium hydroxide for cleaning and sanitization of chromatography resins and systems	CY13951
Data files	
Butyl Sepharose 4 Fast Flow	CY14290
HiTrap HIC Selection Kit	CY13382
HiScreen preppacked columns	CY13473
BPG columns	CY978
Chromaflow columns	CY13377
AxiChrom columns	CY10003
Media Wand Media Handling Unit	CY13577
Handbook	
Hydrophobic interaction and reversed phase chromatography - Principles and methods	CY11248
Instructions	
AxiChrom 50–200 columns, Operating Instructions	29379190
AxiChrom 300–1600 columns, Operating Instructions	29065430
AxiChrom 300–2000 columns, User Manual	29388247
AxiChrom Column and AxiChrom Column Controller, User Manual	29720910
BPG columns, Operating Instructions	29193223
Chromaflow column 400–1000, Operating Instructions	28962232
Selection guide	
Hydrophobic Interaction Chromatography (HIC)	CY14016

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