cytiva

SP **Sepharose** High Performance

Ion exchange resin

Instructions for Use

SP Sepharose™ High Performance is a BioProcess™ chromatography resin with a well-deserved reputation as a highly successful cation ion exchange resin for purifying a wide range of biomolecules. It shares an impressive list of operational characteristics that includes:

- High-resolution, high-capacity separations with high recovery
- Reliable and reproducible
- High chemical stability for effective CIP/sanitization
- Available in different convenient prepacked formats, such as PreDictor™ 96-well filter plates, PreDictor Robocolumn™, HiTrap™, HiScreen™, and HiPrep™ columns
- Easy to scale up

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Read these instructions carefully before using the products.

Safety

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

1 BioProcess 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 process validation and submissions to regulatory authorities. BioProcess resins cover all purification steps from capture to polishing.

2 Resin characteristics

SP Sepharose High Performance is a strong cation exchanger based on cross-linked, beaded agarose with a particle size of ~ 34 µm. The ion exchange group is a sulphopropyl group, see the Figure below, which remains charged and maintains consistent ionic capacity over the entire operating pH range of 4 to 13. The ionic exchange groups are coupled to the base matrix through chemically stable ether bonds.

Fig 1. Partial structure of SP Sepharose High Performance

The high ionic capacity of SP Sepharose High Performance is illustrated by the titration curve in the Figure below. Ionic capacity is 0.15 to 0.20 mmol/mL resin. Dynamic capacity is in the range 50 to 100 mg/mL resin, for example, binding capacity for Ribonuclease is approx. 55 mg/mL resin (column diameter: 5 mm, bed height: 5 cm, sample concentration: 5 mg Ribonuclease/mL, and buffer: 100 mM sodium acetate, pH 6.0).

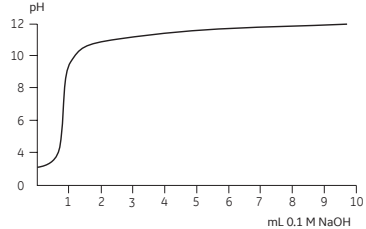

Fig 2. Titration curve. Approx 5 mL of SP Sepharose High Performance resin in 50 mL 1 M HCl.

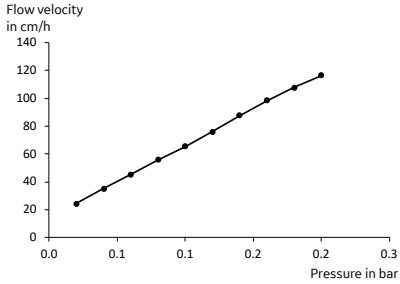

Fig 3. Pressure/Flow curve for SP Sepharose High Performance in a 10 L ReadyToProcess column with 25 cm diameter, 20 cm bed height using buffers with the same viscosity as water at 20°C.

SP Sepharose High Performance has excellent physical and chemical stabilities, see Table below. Strong oxidizing agents, however, must be avoided. This resin combines good kinetics with high physical stability to give excellent performance even at flow velocities up to 90 cm/h in columns with 20 cm bed height. The pressure/flow curve is shown in the Figure above.

Table 1. Characteristics of SP Sepharose High Performance

Matrix	Cross-linked agarose, spherical
Functional group	$CH_2CH_2CH_2SO_3^-$, sulphopropyl
Ionic capacity	0.15 to 0.20 mmol (H^+)/mL resin
Exclusion limit [M_r] Globular proteins [1]	~ 1×10^7
Particle size (d_{50v}) [2]	~ 34 µm
Recommended maximum operational flow velocity	90 cm/h[3]
Binding capacity	~ 70 mg BSA/mL resin

pH stability, operational [4]	4 to 13
pH stability, CIP [5]	3 to 14
pH ligand fully charged [6]	Entire pH range
Working temperature	4°C to 30°C
Chemical stability	Stable to commonly used aqueous buffers, 1.0 M NaOH[7], 1.0 M acetic acid[7], 8 M urea, 6 M guanidine hydrochloride, 30% isopropanol, 70% ethanol
Avoid	Oxidizing agents, cationic detergents
Storage	0.2 M sodium acetate in 20% ethanol, 4°C to 30°C

1 Exclusion limit measured on the base matrix.

2 Median particle size of the cumulative volume distribution.

3 In a 10L ReadyToProcess column with 25 cm diameter and 20 cm bed height using buffer with the same viscosity as water at 20°C.

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

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

6 pH range where ligand is fully charged; although the ligand is fully charged throughout the entire pH range, only use the resin within the stated stability ranges.

7 1.0 M NaOH and 1.0 M acetic acid 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. Design of the method in 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 SP Sepharose High Performance is recommended. Flow velocity can also be included in the scouting.

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 PreDictor plates is preferentially included in the method development. The PreDictor plates are 96-well filter plates prefilled with chromatography resins, which can be used for rapid screening of chromatographic conditions in small scale. The suggested workflow with PreDictor plates is shown in *Fig. 4, on page 8*, where a large design space can be explored prior to further experiments in packed column formats, such as prepacked HiScreen columns.

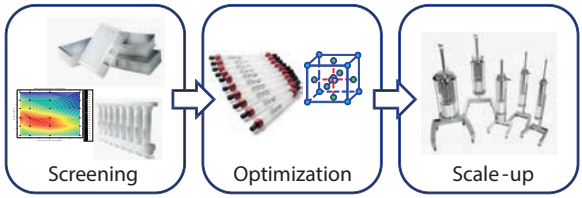

Fig 4. The recommended workflow is described in the figure. It starts with screening of conditions in high throughput formats, followed by optimization, preferably by applying Design of Experiments (DoE), in small columns and finally scale-up to large columns.

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	• pH • Conductivity • Column volume • Column bed height • Particle content • Temperature
2. Sample application	Manual or automatic application onto the column	• Flow rate • Sample pH • Sample conductivity • Upward/downward flow
3. Wash	Wash out unbound material with clean binding buffer	• Flow rate • Upward/downward flow • Buffer choice (normally same as column equilibration buffer)

Phases	Activity	Conditions to consider
4. Elution	Elute the material from the column either with salt or by change in pH	• Sample load • pH • Conductivity • Flow rate • Upward/downward flow

For more information about method development and optimization, consult the handbooks, *Ion exchange Chromatography: Principles and Methods (11000421).*

4 Scale-up

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 preferentially 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 in 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 approximated as the bed height (cm) divided by the flow velocity (cm/h) applied during sample loading.

Procedure

Step	Action
1	Select bed volume according to required binding capacity. Keep sample concentration and gradient slope constant.
2	Select column diameter to obtain the bed height (10 to 40 cm) from method optimization. ***Note:*** *The excellent rigidity of the high flow base matrix allows for flexibility in choice of bed heights.*
3	The larger equipment used when scaling up might cause some deviations from the method optimized at small scale. In such cases, check the buffer delivery and monitoring systems for time delays or volume changes. Different lengths and diameters of outlet tubing can cause zone spreading on larger systems. Check also the compatibility of the hardware and chromatography resin pressure limits with expected pressure during packing and operation.

5 Column packing

Packing HiScale™ and XK columns

The following instructions are for packing HiScale 16/20, HiScale 26/20, XK 16/20 and XK 26/20 with 10 cm bed height.

For more details about packing HiScale columns, see instructions *HiScale columns (16, 26, 50) and accessories (28967470)*.

For more details about packing XK columns, see *Instruction (28992023)*.

Recommended lab-scale and production-scale columns	Inner diameter (mm)	Bed volume	Bed height (cm)
AxiChrom™	50 to 200	up to 16.7 L	max. 50
AxiChrom	300 to 1600	up to 1005 L	max. 50
Tricorn 5/20	5	up to 0.5 mL	max. 2.6
Tricorn 5/50	5	0.2 to 1.1 mL	0.8 to 5.6
Tricorn 10/20	10	up to 2.1 mL	max 2.6
Tricorn 10/50	10	up to 4.4 mL	max. 5.6
Tricorn 10/100	10	3.6 to 8.4 mL	4.6 to 10.6
XK 16/20	16	up to 31 mL	max. 15.5
XK 16/40	16	17 to 70 mL	8 to 35
XK 26/20	26	up to 66 mL	max. 12.5
XK 26/40	26	45 to 186 mL	8.5 to 35

Materials needed

- SP Sepharose High Performance
- HiScale column or XK column
- HiScale packing tube
- Plastic spoon or spatula
- Glass filter G4
- Vacuum suction equipment
- Filter flask
- Measuring cylinder
- Distilled water

Equipment

- Chromatography system, such as ÄKTA™ system, or a stand-alone pump, depending on the flow rate required, can be used for packing.
- Pressure monitor

Equilibrate all materials to room temperature.

Preparation of the slurry

To measure the slurry concentration, let the resin settle in 20% ethanol at least overnight in a measuring cylinder or use the method for slurry concentration measurement described in *application note (28925932)*. This method can also be used for HiScale and XK columns.

Washing the resin

Attach a glass filter funnel onto a filtering 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 5 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.

Packing preparations

Step	Action
1	Attach the packing reservoir at the top of the column and rinse with distilled water.
2	Assemble filter and bottom piece on the column.
3	Wet the bottom filter by injecting 20% ethanol through the effluent tubing.
4	Attach the column and packing reservoir vertically on a laboratory stand. Rinse them with distilled water.
5	Apply distilled water 2 cm over the column end piece and put a tubing clamp on the effluent tubing.
6	Pour all the separation resin slurry into the column and packing reservoir and top up carefully with distilled water.

Packing procedure

Step	Action
1	Connect the pump[1] outlet to the inlet on the packing reservoir and open the column outlet.
2	Pack the column with distilled water at a constant flow (see *Table 3, on page 14*, Step 1) until the resin bed is stable.

Step	Action
3	Adjust the flow rate to 2x the final one (see Table below, Step 2) and decrease it step-wise until the pressure signal is 480 ± 20 kPa. Pack the column at the flow rate which gives 480 ± 20 kPa for 45 minutes.
4	Detach the packing reservoir.
5	Carefully fill the rest of the column with distilled water to form an upward meniscus at the top and insert the flow adapter. The adapter must be adjusted down to the bed surface.
6	Continue packing the column at 480 ± 20 kPa for 6 minutes.
7	Mark on the column the position of the bed surface, stop the pump, close the column outlet and adjust the adapter to the bed surface and then push the adapter a further 3 mm.

Table 3. Packing parameters

Column	Sedim.[1] resin (mL)	Slurry (mL)	Height (mm)	Step 1 (mL/min)	Step 2 (kPa)	Final flow rate (mL/min)
HiScale or XK 16/20	25	50	100	1.0	480 ± 20	~ 12
HiScale or XK 26/20	66	132	100	2.5	480 ± 20	~ 30

[1] Sedimented resin volume = 1.25 × Packed resin volume.

6 Evaluation of packed column

Introduction

The packing quality needs to be checked by column efficiency testing. The test must be done after the packing, and at regular intervals during the working life of the column, and also when the separation performance is 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., influence the results.*

For more information about column efficiency testing, consult the application note *Column efficiency testing* (Product code: 28937207).

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:

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

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 $0.8 < A_S < 1.5$.

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.

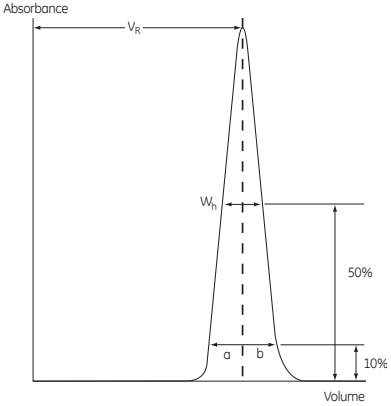

Fig 5. A typical test chromatogram showing the parameters used for HETP and A_s calculations.

7 Maintenance

For best performance from SP Sepharose High Performance and to maximize the lifetime of the resin, follow the procedures described below.

Equilibration

After packing, and before a chromatographic run, equilibrate with start buffer by washing with 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 desired 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 five bed volumes of start buffer or until the column effluent shows stable conductivity and pH values.

Cleaning-in-place (CIP)

Regular CIP prevents the build-up of contaminants in the packed bed and helps to maintain capacity, flow properties and general performance.

A specific CIP protocol should be designed for each process according to the type of contaminants present.

Precipitated, hydrophobically bound proteins or lipoproteins	Wash with 1.0 M NaOH solution with reversed flow direction. Contact time depends on the type of contaminant present.
Ionically bound proteins	Wash with 2 M NaCl with reversed flow direction. Contact time 1 to 2 h.

Lipids and very hydrophobic proteins	Wash with 2 to 4 column volumes of 0.5% nonionic detergent (e.g., 1 M acetic acid) with reversed flow direction. Contact time 1 to 2 h. Alternatively, wash with 2 to 4 column volumes of up to 70% ethanol or 30% isopropanol with reversed flow direction. Contact time 1 to 2 h.

CAUTION
70% ethanol can require the use of explosion-proof areas and equipment.

Sanitization

To reduce microbial contamination in the packed column, sanitization using 0.5 to 1.0 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.

Storage

Store in 20% ethanol, 0.2 M sodium acetate, at a temperature of 4°C to 30°C.

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

8 Ordering information

Product	Quantity	Product code
SP Sepharose High Performance	75 mL	17108701
	1 L	17108703

Product	Quantity	Product code
	5 L	17108704
	10 L	17108705
	60 L[1]	17108708

[1] Pack size available on request.

SP Sepharose High Performance is supplied in suspension in 20% ethanol, 0.2 M sodium acetate. For additional information, contact your local Cytiva representative.

Related products

Product	Quantity	Product code
PreDictor RoboColumn SP Sepharose HP	8 × 200 µL	28986104
PreDictor RoboColumn SP Sepharose HP	8 × 600 µL	28986193
HiTrap SP HP	5 x 1 mL	17115101
HiTrap SP HP	5 x 5 mL	17115201
HiScreen SP HP	1 x 4.7 mL	28950515
HiPrep SP HP 16/10	1 x 20 mL	29018183
Tricorn™ 5/100 column	1	28406410
Tricorn 10/100 column	1	28406415
HiScale 16/20	1	28964441
HiScale 16/40	1	28964424
HiScale 26/20	1	28964514
HiScale 26/40	1	28964513
HiScale 50/20	1	28964445
HiScale 50/40	1	28964444

Accessories

Product	Quantity	Product code
Tricorn Glass Tube 5/100	1	18115306

Product	Quantity	Product code
Tricorn Packing Connector 5-5	1	18115321
Tricorn Packing Equipment 10/100	1	18115325
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	Product code
Data File: Q Sepharose High Performance, SP Sepharose High Performance	18117288
Handbook: Ion Exchange Chromatography, Principles and Methods	11000421
Handbook: High throughput process development with PreDictor plates	28940358
Instructions: Tricorn Empty High Performance Columns	28409488
Instructions: HiScale columns (16, 26, 50) and accessories	28967470
Application note: Column efficiency testing	28937207

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18106026 AI V:6 11/2020