instructions

Sepharose High

Q Sepharose™ High Performance and SP Sepharose High Performance

Q Sepharose High Performance, and SP Sepharose High Performance, are strong ion exchangers. They are based on rigid, highly cross-linked, beaded agarose with a mean bead diameter of 34 µm. Some characteristics of Q Sepharose High Performance and SP Sepharose High Performance are listed in table 1.

With their high physical and chemical stability, very high batch-to-batch reproducibility, and Regulatory Support File back-up, Q Sepharose High Performance and SP Sepharose High performance are ideal for all stages of an industrial scale operation – from research and process development through scale-up and into production.

Preparing the gel

Pour the gel into a graduated glass filter or a graduated laboratory beaker. The settled gel should have a volume of 1.25 x packed gel bed volume. In table 2 examples of different volumes

2 Tween 20 is added to decrease the surface tension, which makes the gel slurry more even.

ⓘ 71-7128-00 Edition AE

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for different column sizes (XK 16/20 and XK 26/20) are shown.

Wash the gel with 5–10 gel volumes of distilled water on a glass filter.

Suspend the gel with distilled water to a volume of 475 ml and add 250 µl of Tween 20[2].

Table 2. Gel characteristics

Type of ion exchanger	Strong anion (Q), Strong cation (SP)
Total ionic capacity(mmol/ml gel)	0.15–0.20
Exclusion limit (globular proteins)	4 x 10^6 (mw)
Gel matrix	6% highly cross-linked agarose
Mean bead size	34 µm
Bead size range	24–44 µm
Max. back pressure	0.5 MPa (5 bar, 70 psi)
Recommended linear flow rate*	up to 150 cm/h
Chemical stability	all commonly used buffers 8 M urea 6 M guanidine hydrochloride 70 % ethanol
pH stability**	
Long term:	2–12 (Q), 4–13 (SP)
Short term:	2–14 (Q), 3–14 (SP)
Autoclavable	With Cl^- (Q) and Na^+ (SP) respectively as counter ions at 121 °C, pH 7, for 30 min. For SP 0.1 M sodium acetate is recommended
Stored in	20% ethanol (Q), 20% ethanol in 0.2 M sodium acetate (SP)

* At room temperature in aqueous buffer. Linear flow rate = Volumetric flow rate (cm^3/h)/column cross-sectional area (cm^2).

** The ranges given are estimates based on our knowledge and experience. Please note the following:

i) *pH stability, long term* refers to the pH interval where the gel is stable over a long period of time without adverse effects on its subsequent chromatographic performance.

II) *pH stability, short term* refers to the pH interval for regeneration, cleaning-in-place and sanitization procedures.

Packing methods

Two alternatives of column packing are described below. Method 1 uses a P-1 peristaltic pump (Pharmacia). Method 2 is a more sophisticated packing method using constant pressure and therefore requires a pump with a pressure sensor, for example pump P-6000 (Pharmacia). Method 2 is prepared for separations which require extremely high resolution.

The described packing methods cover the columns XK 16/20 and XK 26/20.

Materials

Instruments for packing method 1 using P-1 peristaltic pump
Peristaltic pump P-1 using 3.1 mm diameter PVC-tubing
Packing reservoir RK 16 or RK 26

Instruments for packing method 2 using P-6000 (or equivalent) pump
Pump P-6000 or equivalent[1]
Packing reservoir RK 16 or RK 26

Column
XK 16/20 or XK 26/20

Solvents and detergents
Distilled water
Tween® 20[2]
20% ethanol in distilled water
15% isopropanol in distilled water

Packing preparations (method 1 and method 2)

1. Mount the packing reservoir (RK 16 or RK 26) at the top of the column and rinse with distilled water.
2. Mount filter and bottom piece on the column.
3. Wet the bottom filter by injecting 20% ethanol through the effluent tubing.
4. Mount 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 media slurry into the column and packing reservoir and top up carefully with distilled water.

1 Pump P-6000 has a flow rate capacity of 3000 ml/h up to a pressure of 5.0 bar. A pump that could be used for this packing method should have a minimum flow rate capacity of 600 ml/h and a pressure stability of 5 bar. It must have a built-in pressure sensor, alternatively a possibility to connect an external one.

2 Tween 20 is added to decrease the surface tension, which makes the gel slurry more even.

Packing method 1 (peristaltic pump P-1)

1. Let the gel bed sediment without using the pump. It takes around 90 minutes.
2. When the level of the bed is stable, close the column outlet and remove the packing reservoir. Carefully fill the rest of the column with distilled water to form an upward meniscus at the top and insert the flow adaptor. The adaptor should be adjusted down to the bed surface.
3. Connect the flow adaptor to the pump and open the column outlet.
4. Pump 15% isopropanol through the column for 1 hour at maximum flow (3.1 mm PVC tubing).

5. Mark on the column the position of the bed surface, stop the pump, close the column outlet and adjust the adaptor to the bed surface and then push the adaptor a further 4-5 mm.

The column is now ready to use.

Packing method 2 (Pump P-6000 or equivalent)

1. Connect the pump outlet to the inlet on the packing reservoir and open the clamp on the effluent tubing.
2. Step 1: Pack the column with distilled water at a constant flow (see table 2) until the gel bed is stable.
3. Step 2: Adjust the flow rate to 2x the final one (see table 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. Remount the packing reservoir. Carefully fill the rest of the column with distilled water to form an upward meniscus at the top and insert the flow adaptor. The adaptor should be adjusted down to the bed surface.
5. Continue packing the column at 480 ± 20 kPa for 6 minutes.
6. Mark on the column the position of the bed surface, stop the pump, close the column outlet and adjust the adaptor to the bed surface and then push the adaptor a further 3 mm.

The column is now ready to use.

Table 2.

Column	Sedim.[3] gel/ml	Slurry ml	Height mm	Step 1 ml/min	Step 2 kPa	Final flow rate ml/min
XK 16/20	25	475	100	1.0	480 ± 20	[3] 12
XK 26/20	66	475	100	2.5	480 ± 20	[3] 30

[3] Sedimented gel volume = 1.25 x Packed gel volume

Performance testing of packed columns

To check the quality of the column packing it is recommended to do an efficiency test to determine the theoretical plate number and peak symmetry.

If the column is packed according to the instructions described above typical values should be:

Efficiency: N/m >10 000
Peak symmetry: A_f = 0.80–1.80

Chemicals
Distilled water
Sample acetone p.a. 20 mg/ml in distilled water

The columns are tested by pumping 100 µl (XK 16) or 200 µl (XK 26) through the column at a linear flow rate of 60 cm/h. The plate number is calculated as in Figure 1, using the formula:

$N/m = 5.54 (V_e / W_{1/2})^2 \times 1000/L$

and the a symmetry factor by the formula:
A_f = b/a (see figure 1)

Equilibration

Before starting a run, the ion exchanger has to be charged with counter ions and then equilibrated. This is done by pumping one column volume of a high ionic strength buffer followed by 5–10 column volumes of start buffer through the column until the conductivity and/or pH of the effluent is the same as for that of in-going solution.

The column is now equilibrated and ready for use.

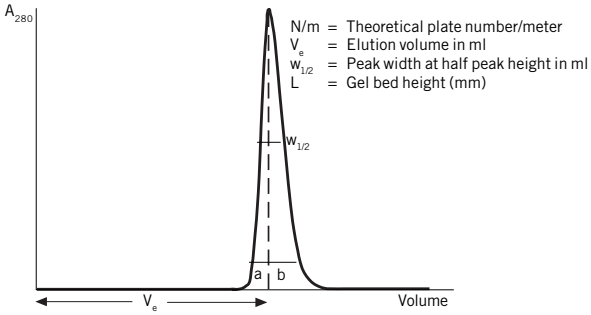

Fig.1. Example showing results obtained from the recommended column evaluation method.

Suggested instruments:
Pump P-500 or P-6000
Injector V-7 equipped with 100 µl (XK 16) or 200 µl (XK 26) sample loops.
Recorder REC-481, 12 mm/min
Detector UV-M II, 280 nm, 5 mm path length

Binding

- The most common procedure is to let the molecules of interest bind to the ion exchanger and allow the others to pass through. However, in some cases it may be more useful to bind "contaminants" and let the molecules of interest remain in the flow through.
- For adsorption, it is critical to choose a buffer with an appropriate pH. Please refer to Table 3 and 4. The ionic strength of the buffer should be kept low so as not to interfere with sample binding. Recommended operating pH is within 0.5 pH units of the buffer's pKa and at least one pH unit below the isoelectric point (pI) of the molecule of interest.

Table 3. Suggested buffers for use with Q Sepharose High Performance

Buffer	Cation	Concentration	pKa (25 °C)
N-methylpiperazine	Cl^-	20 mM	4.8
piperazine	Cl^-	20 mM	5.7
	$HCOO^-$		
L-histidine	Cl^-	20 mM	6.2
bis-Tris	Cl^-	20 mM	6.5
bis-Tris propane	Cl^-	20 mM	6.8
triethanolamine	Cl^-	20 mM	7.8
	CH_3COO^-		
Tris	Cl^-	20 mM	8.2
N-methyldiethanolamine	SO_4^{2-}	50 mM	8.5
	Cl^-		
	CH_3COO^-		
diethanolamine	Cl^-	20 mM at pH 8.4 50 mM at pH 8.8	8.9
1,3-diaminopropane	Cl^-	20 mM	8.6
ethanolamine	Cl^-	20 mM	9.5
piperazine	Cl^-	20 mM	9.7
1,3-diaminopropane	Cl^-	20 mM	10.5

Table 4. Suggested buffers for use with SP Sepharose High Performance

Buffer	Counter ion	Concentration	pKa (25 °C)
Citrate	Na^+, Li^+	20 mM	3.1
Acetate	Na^+, Li^+	50 mM	4.8
Malonate	Na^+, Li^+	50 mM	5.7
Phosphate	Na^+	50 mM	7.2
BICINE	Na^+	50 mM	8.4

Elution

Desorption may be done using either an increasing salt gradient (linear or step) or an increasing pH gradient (linear or step).

Regeneration

Depending on the nature of the sample, regeneration is normally performed by washing with a high ionic strength buffer (e.g. 1–2 M NaCl) and/or increasing pH, followed by re-equilibration in start buffer.

In some applications, substances such as denaturated proteins or lipids do not elute in the regeneration procedure. These can be removed by cleaning-in-place procedures.

Cleaning-in-place (CIP)

Remove ionically bound proteins by washing the column with 0.5 bed volumes of a 2 M NaCl solution, contact time 10–15 minutes, reversed flow direction.

Remove precipitated proteins, hydrophobically bound proteins and lipoproteins by washing the column with 1 M NaOH solution at a linear flow rate of approximately 40 cm/h, contact time 1–2 hours, reversed flow direction.

Remove strongly hydrophobically bound proteins, lipoproteins and lipids by washing the column with four bed volumes of 70% ethanol or 30% isopropanol at 10 cm/h, reversed flow direction. Apply increasing gradients to avoid air bubble formation when using high concentrations of organic solvents.
Alternatively, wash the column with two bed volumes of 0.1–0.5% detergent in a basic or acidic solution. After treatment with detergent always remove residual detergent by washing with five bed volumes of 70% ethanol.

After the washing procedure the column should be equilibrated with at least 3 bed volumes of start buffer before use.

Sanitization

Sanitization reduces microbial contamination of the gel bed to a minimum.

Wash the column with 0.5–1 M NaOH at a flow rate of approximately 40 cm/h, contact time 30–60 minutes, reversed flow direction.

Re-equilibrate the column with 3–5 bed volumes of sterile start buffer.

Storage

It is recommended that the gel is stored for longer periods of time in 20% ethanol (Q Sepharose High Performance) or 20% ethanol, 0.2 M sodium acetate (SP Sepharose High Performance) at 4 °C.

Ordering information

Product	Pac size	Code No.
Q Sepharose High Performance	75 ml	17-1014-01
SP Sepharose High Performance	75 ml	17-1087-01
Ion Exchange Chromatography Principles and Methods		18-1114-21

• p11

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Produced by Wikströms, Sweden 1020978, 08.2002
Printed matter. Licence 341 051

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