Q Sefinose HP and SP Sefinose HP

Product information for SF013-QHP/SF014-SPHP:

Introductions:

Q Sefinose High Performance, and SP Sefinose High Performance are strong ion exchangers. They are based on rigid, highly crosslinked, beaded agarose with a mean bead diameter of 34 µm. Some characteristics of Q Sefinose High Performance and SP Sefinose 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 Sefinose High Performance and SP Sefinose High performance are ideal for all stages of an industrial scale operation – from research and process development through scale-up and into production.

Table 1

Type of ion exchanger	Q Sefinose™ HP	SP Sefinose™ HP
Cas #	SF013-QHP	SF014-SPHP
Charge	- $N^+(CH_3)_3$	- SO_3^-
Total ionic capacity	0.14-0.20 mmol Cl^-/ml	0.15-0.20 mmol H^+/ml
Binding capacity	70 mg BSA/ml resin	55 mg RNase /ml resin
Flow rate	30-150 cm/h	
Max. back pressure	0.5 MPa (5 bar, 70 psi)	
Mean bead size	34 µm	
Exclusion limit	Ig G 4 ×10^6	
Gel matrix	6% highly cross-linked agarose	
pH Stability	2-12 (long),1-14 (short)	4-13 (long),3-14 (short)
Chemical Stability	8 M urea , 6 M guanidine hydrochloride,70% ethanol, 1 M NaOH, 1 M sodium acetate	
Storage Buffer	20% ethanol (Q),0.2 M sodium acetate in 20% ethanol(SP)	
Storage Temperature	4°C to 30°C	

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. Wash the gel with 5–10 gel volumes of distilled water. Suspend the gel with distilled water to a volume of 475 ml and add 250 µl of Tween 20.

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.

A. Materials

Instruments for packing method 1 using P-1 peristaltic pump,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, Packing reservoir RK 16 or RK 26

Column

XK 16/20 or XK 26/20

Solvents and detergents

Distilled water,Tween-20, 20% ethanol in distilled water,15% isopropanol in distilled water

B.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.

Note :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.

Note :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 until the gel bed is stable.
3. Step 2: Adjust the flow rate to 2x the final one and decrease it step-wise until the pressure signal is 480 ± 20kPa. 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

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.

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 Sefinose High Performance

Buffer	Cation	Concentration	pKa (25 °C)
N-methylpiperazine	Cl^-	20 mM	4.8
piperazine	Cl^- $HCOO^-$	20 mM	5.7
L-histidine	Cl^-	20 mM	6.2
bis-Tris	Cl^-	20 mM	6.5
bis-Tris propane	Cl^-	20 mM	6.8
triethanolamine	Cl^- CH_3COO^-	20 mM	7.8
Tris	Cl^-	20 mM	8.2
N-methyldiethanolamine	SO_4^{2-} Cl^- CH_3COO^-	50 mM	8.5
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 Sefinose 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 Sefinose High Performance) or 20% ethanol, 0.2 M sodium acetate (SP Sefinose High Performance) at 4 °C.