

Sefinose™ and Sefinose™ CL

Product information for SF001-4B/SF002-6B/SF003-L4B/SF004-L6B:

Introduction

Sefinose™ is a bead-formed agarose-based gel filtration matrix. Sefinose is available with 2 different agarose contents: 4 and 6%, designated sefinose 4B and Sefinose 6B respectively. Sefinose CL gel is cross-linked derivatives of Sefinose 4B and Sefinose 6B. The cross-linked form of Sefinose is chemically and physically more resistant than Sefinose itself, offering the same selectivity but with better flow characteristics. Cross-linked Sefinose gel is resistant to organic solvents and hence is the choice if separation takes place in organic solvents. Both Sefinose and Sefinose CL have broad fractionation ranges which make them suitable for characterizing or cleaning-up samples containing components of diverse molecular weight.

Table 1

Resin	Sefinose™4B	Sefinose™6B	Sefinose™ CL- 4B	Sefinose™CL-6B
Cat#	SF001-4B	SF002-6B	SF003- L4B	SF004-L6B
Ingredient	4% Agarose	6% Agarose	4% Agarose	6% Agarose
Average size	45 – 165 μm	45 – 165 μm	45 – 165 μm	45 – 165 μm
Separation range	$6 \times 10^4 - 2 \times 10^7$	$1 \times 10^4 - 4 \times 10^6$	$6 \times 10^4 - 2 \times 10^7$	$1 \times 10^4 - 4 \times 10^6$
pH Stability (operation)	4 – 9	4 – 9	3 – 13	3 - 13
pH Stability (purgation)	4 – 9	4 – 9	2 - 14	2 - 14
Pressure/ Flow rate	(70 – 140 cm/h, High 10cm, diameter 5cm)	(100 – 200cm/h, High 10cm, diameter 5cm)	(80 – 150 cm/h, High 10cm, diameter 5cm)	(100 – 200 cm/h, High 10cm, diameter 5cm)

Preparing Gel

Sefinose and Sefinose CL are supplied pre-swollen in 20% ethanol. Prepare a slurry with eluent buffer in a ratio of 75% settled gel: 25% buffer. Degas the gel slurry before packing. The eluent buffer should not contain agents which significantly increase the viscosity. The column may be equilibrated with viscous buffers at reduced flow rate after packing is completed.

Packing Sefinose and Sefinose CL Gels

The column packing method described below has two steps. The second step should be performed at the constant pressure given in Table 2. Resolution increases with increased bed height in gel filtration. It is therefore preferable to choose a bed height of at least 60 cm.

Materials:

Instruments needed for packing

Pump: P-50, P-500 or similar pump

Recommended columns: XK columns series with one or two adaptors.

Packing reservoir (empty XK glasstube of same dimension as column)

Packing connector.

Table 2 Recommended packing flow rates and pressures

Gel	Step 1 Flow rate (cm/h)	Step 2 Flow rate (cm/h)	Step 2 Pressure (MPa,bar,psi)
Sefinose 4B	15	-	0.018, 0.18, 2.6
Sefinose 6B	30	-	0.025, 0.25, 3.6
Sefinose CL-4B	30	-	0.025, 0.25, 3.6
Sefinose CL-6B	30	-	0.045, 0.45, 6.4

1. Equilibrate all material to the temperature at which the chromatography will be performed.

2. Prepare the gel as described above and de-gas the gel slurry.
3. Mount a packing reservoir, using the packing connector, at the top of a column and rinse with distilled water.
4. Insert an end piece or an adaptor at the bottom of the column.
5. Mount the column with the packing reservoir vertically on a laboratory stand. Eliminate air from the column dead spaces by flushing the end piece or adaptor with eluent or distilled water. Make sure no air has been trapped under the net.
Close the column outlet with approximately one centimeter of eluent remaining in the column.
6. Pour the slurry in a single operation. Pouring the slurry down a glass rod held against the wall of the column will minimize the introduction of air bubbles. Fill the reservoir to the top with eluent or distilled water.
7. Screw on the reservoir cap tightly and connect it to the pump.
8. Open the bottom outlet of column and start the pump at the flow given for step1 in Table2.
9. Pack the gel until the gel bed has reached a constant height.
10. Stop the pump, close the column outlet and remove the packing connector and reservoir. This is most easily done by first removing the column from the stand and then unscrewing the reservoir over a sink.
11. Re-mount the column on the stand. Carefully fill the rest of the column with eluent to form an upward meniscus.
12. Insert the adaptor at an angle into the column, ensuring that no air is trapped under the net.
13. Slide the plunger slowly down the column so that the air above the net and in the capillary tubings is displaced by eluent. Valves on the inlet side of the column should be turned in all directions during this procedure to ensure that air is removed.
14. Lock the adaptor in position on the gel surface. Open the column outlet and apply a constant pressure given for step 2 in Table 2, until the gel bed has reached a constant height.
15. 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 3-4 mm.
Now the column is now ready to use.

Separation Conditions

It is recommended to use a buffer with an ionic strength of 0.15 or greater to avoid any unwanted ionic interactions between the solute molecule and the matrix.

For recommended flow rates for respective Sefinose gel, please refer to Table 1. The lower the flow rate, the better resolution.

Eluent and Sample Preparation

To avoid clogging of column filters, it is recommended to filter or centrifuge the sample to get rid of particulate matter.

Column Equilibration

Before applying the sample, equilibrate the column with at least two column volumes of the eluent to be used in the separation, or until the baseline is stable. Longer equilibration may be needed with detergent solutions. Equilibration is not needed between runs with the same eluent.

Sample Application

Recommended sample volumes is 2-5% of the total bed volume. The sample can be applied via sample applicators SA-5, SA-50 or by using sample loops with valves LV-4 or SRV-4.

Regeneration

Regeneration is normally performed by washing with 2-3 column volumes of buffer, followed by re-equilibration in the new buffer (if changing conditions). In some applications, substances such as denatured proteins or lipids do not elute in the regeneration procedure. These can be removed using the cleaning procedure described below.



Cleaning

Some observations which indicate that column cleaning is necessary;

- ✓ increased back-pressure
- ✓ colour change at the top of the column
- ✓ reduced resolution
- ✓ a space between the upper adaptor and the gel surface

If increased back-pressure is observed, check for stoppages in valves, tubing etc. before starting the column cleaning procedure. Remove precipitated proteins, non-specifically bound proteins and lipoproteins by washing the column with one column volume of 0.5 M NaOH at the recommended liner flow given in Table 1.

Remove strongly non-specifically bound proteins, lipoproteins and lipids by washing the column with two column volumes of non-ionic detergent solution, e.g. 0.1% Triton™ X-100, followed by at least 2-3 column volumes of eluent buffer.

The cleaning procedure given above can also be performed with the gel on a Bucher funnel.

Sanitization

Sanitization reduces microbial contamination of the gel to a minimum. Wash the column with 0.5 M NaOH at the recommended flow rate, given in Table 1. Re-equilibrate the column with 3-5 bed volumes of sterile buffer.

Storage

Store the gel, packed or unpacked, at 4-8°C in the presence of a bacteostatic agent, e.g. 20% ethanol.