cytiva

Sepharose and Sepharose CL

Size exclusion chromatography

Instructions for Use

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71709800 AF

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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 Abstract

Sepharose™ is a spherical, agarose-based size exclusion chromatography resin. Sepharose is available with 2 different agarose contents, 4% and 6%, designated Sepharose 4B and Sepharose 6B respectively.

Sepharose CL resins are cross-linked derivatives of the above mentioned Sepharose resins. Sepharose CL is available in 2%, 4% and 6% agarose contents, designated Sepharose CL-2B, Sepharose CL-4B, and Sepharose CL-6B respectively. The cross-linked form of Sepharose is chemically and physically more resistant than Sepharose itself, offering the same selectivity with better flow characteristics. Cross-linked Sepharose resins are resistant to organic solvents and are thus the choice for separations in organic solvents.

Both Sepharose and Sepharose CL have broad fractionation ranges which makes them suitable for characterizing or cleaning-up samples containing components of diverse molecular weight.

The Sepharose resins mentioned above are part of the Cytiva BioProcess range of 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.

2 Resin characteristics

Sepharose	4B	6B	CL-2B	CL-4B	CL-6B
Matrix	agarose, 4%, spherical	agarose, 6%, spherical	cross-linked agarose, 2%, spherical	cross-linked agarose, 4%, spherical	cross-linked agarose, 6%, spherical
Fractionation range (M_r) Globular proteins	6×10^4 to 2×10^7	1×10^4 to 4×10^6	7×10^4 to 4×10^7	6×10^4 to 2×10^7	1×10^4 to 4×10^6
Particle size distribution (µm)	45 to 165[1]	45 to 165[1]	60 to 200[2]	45 to 165[1]	45 to 165[1]
Recommended maximum operating flow velocity (cm/h)[3]	11.5	14	15	26	30
pH stability, operational[4]	4 to 9	4 to 9	3 to 13	3 to 13	3 to 13
pH stability, CIP[5]	4 to 9	4 to 9	3 to 14	3 to 14	3 to 14
Chemical stability	Stable to commonly used aqueous buffers		Stable to commonly used aqueous buffers, ionic detergents, nonionic detergents and polar solvents		
Physical stability	Negligible volume variation due to changes in pH or ionic strength				
Sterilization	Chemical		Autoclavability 20 min at 120°C in phosphate buffer, pH 7		

1 ≥ 95% volume share within given range.

2 ≥ 90% volume share within given range.

3 In a column with 16 mm diameter and 50 cm bed height using buffers with the same viscosity as water at room temperature.

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.

3 Preparing the resin

Sepharose and Sepharose CL are supplied preswollen in 20% ethanol. Prepare a slurry with eluent buffer in a ratio of 75% settled resin to 25% buffer. Degas the resin slurry before packing.

The eluent buffer must not contain agents which significantly increase the viscosity. The column can be equilibrated with viscous buffers at reduced flow rates after packing is completed.

4 Packing Sepharose and Sepharose CL resins

The column packing method described below has two steps. The second step must be performed at the constant pressure given in *Table 1, on page 6*.

Resolution increases with increased bed height in size exclusion chromatography. It is therefore preferable to choose a bed height of at least 60 cm.

5 Materials

Instruments needed for packing

Pump: P-50, P-900 or similar pump

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

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

Packing connector

Table 1. Recommended packing flow velocities and pressures

	Step 1 Flow velocity (cm/h)	Step 2 Pressure (MPa, bar, psi)
Sepharose 4B	15	0.018, 0.18, 2.6
Sepharose 6B	30	0.025, 0.25, 3.6
Sepharose CL-2B	30	0.020, 0.20, 2.8
Sepharose CL-4B	30	0.025, 0.25, 3.6
Sepharose CL-6B	30	0.045, 0.45, 6.4

Step	Action
1	Equilibrate all material to the temperature at which the chromatography will be performed.
2	Prepare the resin as described above and degas the resin slurry.
3	Attach a packing reservoir, using the packing connector, at the top of a column and rinse with ultra pure water.
4	Insert an end piece or an adapter at the bottom of the column.

Step	Action
5	Assemble the column with the packing reservoir vertically on a laboratory stand. Eliminate air from the column dead spaces by flushing the end piece or adapter with eluent or ultra pure 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 ultra pure water.
7	Screw on the reservoir cap tightly and connect it to the pump.
8	Open the bottom outlet of the column and start the pump at the flow given for step 1 in *Table 1, on page 6*.
9	Pack the resin until the resin 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-assemble the column on the stand. Carefully fill the rest of the column with eluent to form an upward meniscus.

Step	Action
12	Insert the adapter 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 must be turned in all directions during this procedure to make sure that air is removed.
14	Lock the adapter in position on the resin surface. Open the column outlet and apply a constant pressure given for step 2 in *Table 1, on page 6*, until the resin 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 adapter to the bed surface and then push the adapter a further 3 to 4 mm.

The column is now ready to use.

6 Performance testing of packed columns

To check the quality of the column packing, an efficiency test should be performed to determine the theoretical plate number and peak asymmetry factor.

Eluent: Ultra pure water

Sample: 2% (v/v) acetone in ultra pure water

The column is tested by pumping 200 µL of acetone (20mg/mL) through the column at the recommended flow velocity given in *Table 1, on page 6*. The plate number is calculated as in Figure 1, using the formula:

$N/m = 5.54 (V_R/W_h)^2 \times 1000/L$

and the peak asymmetry factor (A_s) by the formula:

$A_s = b/a$ (see Figure 1)

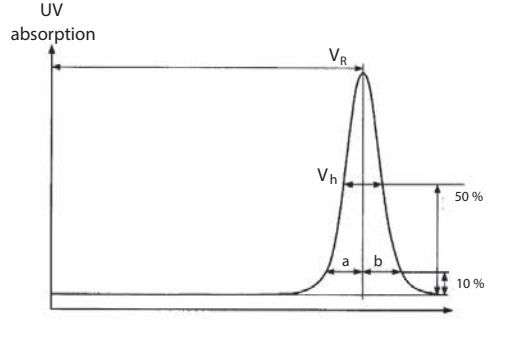

Fig 1. Example showing results obtained from the column evaluation method described above

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

Resin	Number of theoretical plates per meter	Peak asymmetry factor
Sepharose 4B	> 3000	0.7 to 1.3
Sepharose 6B	> 3000	0.7 to 1.3
Sepharose CL-2B	> 3000	0.7 to 1.3

Resin	Number of theoretical plates per meter	Peak asymmetry factor
Sepharose CL-4B	> 3000	0.7 to 1.3
Sepharose CL-6B	> 3000	0.7 to 1.3

7 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 velocities for respective Sepharose resin, refer to *Table 1, on page 6*. The lower the flow velocity, the better the resolution.

8 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.

9 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 might be needed with detergent solutions. Equilibration is not needed between runs with the same eluent.

10 Sample application

Recommended sample volumes is 2% to 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.

11 Regeneration

Regeneration is normally performed by washing with 2 to 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.

12 Cleaning

Some observations which indicate that column cleaning is necessary:

- increased back-pressure
- color change at the top of the column
- reduced resolution
- a space between the upper adapter and the resin surface

If increased back-pressure is observed, check for stoppages in valves, tubing etc. before starting the column cleaning procedure.

Remove precipitated proteins, nonspecifically bound proteins and lipoproteins by washing the column with one column volume of

0.5 M NaOH[1] at the recommended flow velocity given in *Table 1, on page 6*.

Remove strongly nonspecifically bound proteins, lipoproteins and lipids by washing the column with two column volumes of a nonionic detergent solution, followed by at least 2 to 3 column volumes of eluent buffer.

The cleaning procedures given above can also be performed with the resin on a Buchner funnel.

13 Sanitization

Sanitization reduces microbial contamination of the resin to a minimum.

Wash the column with 0.5 M NaOH[1] at the recommended flow velocity, given in *Table 1, on page 6*.

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

14 Storage

Store the resin, packed or unpacked, at 4°C to 30°C in the presence of a bacteriostatic agent, for example 20% ethanol.

1 Note that the stability of Sepharose 4B and Sepharose 6B cannot be guaranteed in this solution.

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15 Ordering Information

Product	Quantity	Product code
Sepharose 6B	1 L	17011001
Sepharose 6B	10 L	17011005
Sepharose 4B	1 L	17012001
Sepharose 4B	10 L	17012005
Sepharose CL-6B	1 L	17016001
Sepharose CL-6B	10 L	17016005
Sepharose CL-4B	1 L	17015001
Sepharose CL-4B	10 L	17015005
Sepharose CL-2B	1 L	17014001
Sepharose CL-2B	10 L	17014005

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71709800 AF V:5 06/2020