Millipore®

User Guide

Eshmuno® CPS Chromatography Resin

Contents

The life science business of Merck KGaA, Darmstadt, Germany operates as MilliporeSigma in the U.S. and Canada.

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Lab Scale Column Packing

Materials

- Eshmuno® CPS resin
- Graduated cylinder
- Packing buffer (150 mM NaCl)
- Tracer solution
- Lab scale chromatography column and extension tube
- Syringe
- Fitting to connect the syringe to the column outlet (bottom)

Compression and Resin Calculations

An accurate determination of the slurry volume and slurry concentration is important to achieve good packing results. An error will result in inaccurate packed bed compression, giving rise to high or low operating pressures and possibly poor HETP/A_s values.

Compression Factors

Column Size	Recommended Compression	
	Factor (CF)	Percent
Lab Scale	1.09 to 1.11	8 to 10%

Refer to Pilot Scale Column Packing for Pilot Scale Compression Factors.

Compression and Resin Calculation Formulas

Calculate packed bed volume (PBV):
PBV = π × column radius^2 × bed height

Calculate settled bed volume required at a given percent compression for a target packed column bed volume (SBV):

$$SBV = PBV \times CF$$
or
$$SBV = PBV/(100\% - \%\ compression)$$

Calculate the slurry volume required for a target bed height:

$$slurry\ volume = SBV/slurry\ concentration$$

Where: slurry concentration = gravity settled volume of resin/ total slurry volume

Calculate compression factor (CF):

$$CF = 100\%/(100\% - \%\ compression)$$

Example

Pack Eshmuno® CPS resin to a target bed height of 200 mm in a 10 mm i.d. column:

$$PBV = \pi \times (0.5\ cm)^2 \times 20\ cm = 15.7\ mL$$

At 8% compression, the settled bed volume is:

$$SBV = 15.7\ mL/(100\% - 8\%) = 17.1\ mL$$

Therefore, 17.1 mL of resin is needed to pack a stable bed at 20 cm bed height.

The resin is supplied in a storage solution (20% ethanol solution + 150 mM NaCl, 70% slurry concentration), the volume of slurry needed is:

$$slurry\ volume = 17.1\ mL/70\% = 24.4\ mL$$

Resin Slurry Preparation

NOTE DO NOT pack the resin in the storage solution.

1. Thoroughly mix the slurry into a homogeneous resin suspension and transfer the slurry into a graduated cylinder.
2. Let the resin settle under gravity for ≥ 4 hours then determine the slurry concentration.

NOTE Settling time depends on the slurry concentration, packing solution, and height of the container.

3. Remove the supernatant.
4. Add 2 M sodium chloride solution to obtain a 50% slurry concentration.
5. Mix the resin into a homogeneous slurry. Ensure there are no clumps of resin at the bottom of the container.
6. Repeat steps 2– 5 at least two additional times.
7. Determine the required slurry volume and adjust the total volume to 70% slurry concentration.

Packing Procedure

1. Mark the target bed height on the column tube.
2. Install and mount the column vertically. Connect an extension tube or place a funnel with a large enough capacity on top of the column.
3. Fill a syringe with 1 to 2 mL of packing buffer (150 mM NaCl) and connect to the bottom of the column.
4. Use the syringe to fill the bottom of the column with 1 to 2 cm of packing buffer to wet bottom bed support. Leave the syringe connected to the column.
5. Mix the slurry in the graduated cylinder into a homogeneous suspension.
6. Add the slurry to the column assembly. Avoid air entrapment by pouring the slurry down the column wall using a funnel or a glass rod.
7. Add a few milliliters of water or packing buffer to the cylinder. Mix this with any leftover resin in the cylinder and add this slurry to the column. Rinse any leftover resin from the column tube wall using water or packing buffer.
8. Apply suction by pulling the plunger of the syringe and draw liquid through the bottom of the column. As the liquid is pulled out, observe the formation of the bed. Keep the column vertical during this step to prevent uneven settling. Stop applying suction with the syringe when the liquid level is approximately 1 cm above the packed bed.
9. Disassemble the extension tube and/or remove the funnel from the top of the column.
10. Disconnect the syringe from the bottom of the column and connect to the packing system.
11. Insert the top flow adapter and lower it to the target bed height based on the mark. The final bed height should be kept within the compression marks.
12. Connect the column to the chromatography system.
13. Pump packing buffer (150 mM NaCl) in the upward direction. Start with a low flow rate of 1 mL/min. Increase the flow rate in stages of 5 mL/min to 800 cm/h for 10 - 20 min. This allows for removal of any air that may be present in the column, e.g. visible particularly on the column tube wall.
14. Stop the flow, reconnect the system, and pump packing buffer (150 mM NaCl) in the downward direction at up to 800 cm/h for 10 - 20 min.

NOTE Reduce the linear velocity of this step if the system pressure exceeds the pressure limit of the column, particularly when packing long bed heights and/or if using columns with pressure limit of ≤5 bar.

15. Check the quality of the packed bed.

Packed Column Evaluation

Check the quality of the packing by measuring the packed column efficiency.

Measuring the Packed Column Efficiency

Run the column at a flow rate of ~150 cm/h and inject 1 to 2% of the packed bed volume recommended tracer solution. Monitor the conductivity (1M NaCl or water as tracer) or the UV absorption (acetone as tracer) of the column effluent.

The parameters to describe column efficiency are the height equivalent to a theoretical plate (HETP) and asymmetry (A_s).

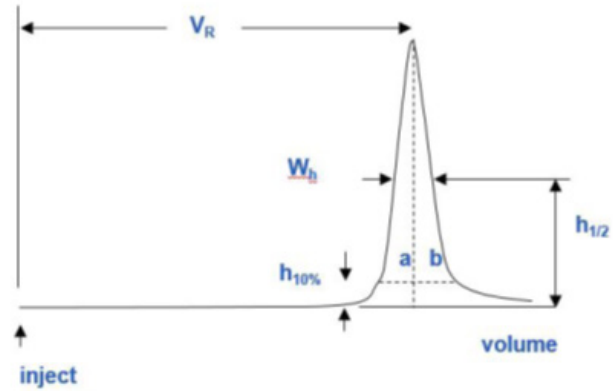
The values for HETP and A_s will depend on the specific test conditions (concentration and volume, flow rate and system tubing/pipework). It should be used only as a reference and the conditions maintained when directly comparing specific values.

Test the column at a linear flow rate of 50 to 200 cm/h using one of the sample buffers listed here:

Sample	Mobile Phase
1 M NaCl	200 mM NaCl
Water	200 mM NaCl
2% v/v acetone in running buffer	200 mM NaCl or running buffer

NOTE The conductivity based test systems in this table are recommended to minimize the charge interaction of buffer ions with the functional groups of the ion exchange resin. Using other test systems may result in test artifacts (tailing or fronting).

Calculating HETP and Asymmetry



$$HETP = L/N$$

$$N = 5.54(V_R/W_h)^2$$

Where:

V_R = Retention volume
W_h = Peak width at half peak height
L = Bed height
N = Number of theoretical plates
V_R and W_h must be in the same units.

$$A_s = b/a$$

Where:

a = 1st half peak width at 10% of peak height

b = 2nd half peak width at 10% of peak height

Guideline values for packed column quality of Eshmuno® CPS resin are N > 3000/m and asymmetry values between 0.7 and 1.6 at laboratory scale.

Pilot Scale and Large Scale Column Packing

Materials

- Eshmuno® CPS resin
- Graduated cylinder
- Packing buffer (150 mM NaCl)
- Tracer solution

Compression and Resin Calculations

An accurate determination of the slurry volume and slurry concentration is important to achieve good packing results. An error will result in inaccurate packed bed compression, giving rise to high or low operating pressures and possibly poor HETP/A_s values.

Compression Factors

Column Size	Recommended Compression	
	Factor (CF)	Percent
Pilot Scale	1.14 to 1.16	12 to 14%

See Compression and Resin Calculation Formulas and refer to Lab Scale Column Packing for Lab Scale Compression Factors.

Resin Slurry Preparation

Eshmuno® CPS resin is supplied as an approximately 70% suspension in 20% ethanol solution containing 150 mM NaCl.

Mix the sedimented slurry with a paddle, rod or stirrer. If mixing a settled bed, start the mixing on top of the bed OR shake bottled resin by hand.

NOTE DO NOT USE permanent/intensive agitation within the settled bed.

DO NOT USE magnetic stirrers to resuspend the resin within the column as the bar will crush the beads.

To unpack a small diameter column, remove the bottom adjuster if the column design allows it.

To unpack a larger diameter column, resuspend the resin within the column and pump it out.

Buffer Exchange

Prior to packing, ethanol in the storage solution should be removed and disposed of according to local regulations.

1. After allowing resin to settle in the shipping container, decant the storage solution (20% ethanol + 150 mM NaCl) once. Resuspend the resin using packing buffer.
2. Pour the desired amount of resin into the column or another appropriate container.
3. Perform at least two additional buffer exchanges. For each buffer exchange, let the resin settle under gravity for >4 hours and remove the supernatant using a pump or by decantation. These steps will remove all the ethanol prior packing, and clear the potential "fines" created during shipment, resulting from base bead abrasion.
4. Once the buffer exchanges have been performed, allow the resin to settle for four hours for an accurate measure of the settled bed height/volume (settling for less than four hours will result in an overestimation of the amount of resin available for packing.

Packing Procedure

Different column designs can have slightly different packing options. Consult the column manual for specifications.

Eshmuno® CPS resin can be packed with 10 µm and 20 µm bed support.

1. Add the appropriate volume of resin slurry to achieve the desired packed bed height at the recommended compression factor.
2. Reslurry the resin bed by mixing with a paddle to achieve a homogeneous suspension.
3. Rinse the walls of the column with packing buffer to ensure resin particles are not trapped between the top adapter seal and the column wall.
4. Secure the column top, engage the seal and lower the top adapter to the surface of the liquid slurry, allowing excess liquid to escape through the inlet line.
5. Make sure the column inlet line is full of liquid before connecting the column inlet to the pump.
6. Open the column outlet and pack the column with the packing buffer at a starting flow rate > 300 cm/h until the packed bed height is stable. Do not recirculate the packing buffer during this step.
7. Turn off the pump.

NOTE Use a packing flow rate at least 20% higher than the maximum process flow rate.

8. Lower the top adapter to the target packed bed height (this will generally be below the bed height achieved during packing). Exhaust the liquid through the top of the column. If the resistance of the bed is too high to lower the adjuster manually to the targeted bed height, reapply a flow at 300 cm/h in downflow mode, to recompress the bed. Once the bed is stable again, stop the flow and lower the adapter to the target bed height.
9. Condition the packed bed by applying flow to the column for 10 minutes in the upward flow direction at 2 bar gross pressure, followed by flow in the downward direction for another 10 minutes at 2 bar gross pressure.

Packed Column Evaluation

The quality of the packing can be checked by measuring the packed column efficiency.

1. Run the column at a flow rate of ~150 cm/h and inject 1 to 2% of the packed bed volume of one of the recommended tracer solutions listed below.
2. Monitor the conductivity or the UV absorption of the column effluent, respectively (conductivity: 1M NaCl or water as tracer; UV absorption: acetone as tracer).

The qualification parameters, e.g. asymmetry, depend on the specific test conditions: sample concentration and volume, flow rate and system hold-up volume. These values should only be used as references and these conditions maintained constant when directly comparing specific values.

Recommended test sample/buffer systems as tracer solution

Sample	Mobile Phase
1 M NaCl	200 mM NaCl
Water	200 mM NaCl
2% v/v acetone in running buffer	200 mM NaCl or running buffer

The conductivity-based test systems in this table are recommended to minimize the charge interaction of buffer ions with the functional groups of the ion exchange resin. Using other test systems may result in test artifacts (tailing or fronting).

Calculating HETP and Asymmetry

See Compression and Resin Calculation Formulas for information on calculating these values.

Guideline values for packed column quality of Eshmuno® CPS resin at pilot scale are N > 3000/m and asymmetry values from 0.7 to 1.8.

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