

User Guide

Eshmuno® CMX Chromatography Resin

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Lab Scale Column Packing

Materials

- Eshmun® CMX resin
- Graduated cylinder
- Packing buffer (150 mM NaCl)
- Tracer solution (2% acetone in 150mM NaCl)
- Lab scale chromatography column and extension tube
- 20% ethanol solution containing 150 mM NaCl

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	1.06 to 1.08	6 to 8%

Refer to [Pilot Scale Column Packing](#) for Pilot Scale Compression Factors.

Formulas

Calculate packed bed volume (PBV):
 $PBV = \pi \times \text{column radius}^2 \times \text{bed height}$

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

$$SBV = PBV \times CF$$

or

$$SBV = PBV / (100\% - \% \text{ compression})$$

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):

$$CF = 100\% / (100\% - \% \text{ compression})$$

Example

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

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

At 8% compression, the settled bed volume is:

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

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:

$$\text{slurry volume} = 17.1 \text{ mL} / 70\% = 24.4 \text{ mL}$$

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 20% ethanol solution containing 150mM NaCl or 0.1M NaOH 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.

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. Connect the bottom of the column to the chromatography system.
4. Pump liquid through the bottom to wet the bottom bed support and fill the column with 1 to 2 cm of packing buffer (150 mM NaCl).
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. Ensure the column outlet is closed. Connect the top flow adapter while venting air out of the inlet tube. Lower the top adapter as much as needed to remove the air, i.e. a few millimeters into the slurry.
9. Connect the column to the chromatography system.

10. Start pumping at a low flow rate (2 mL/min) and prime the column inlet line.
11. Open the bottom outlet and make a liquid to liquid connection to the column inlet. Ensure there are no leaks or air inside the column near the top adapter.
12. Immediately pump packing buffer in the downward direction at 500 cm/h until all the resin has settled onto the packed bed and all the liquid above the packed bed is clear.

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.

13. Stop the flow and close the bottom outlet of the column.
14. If an extension tube has been used, remove the top adapter and the extension tube and then reconnect the top adapter into the column as described above. If an extension tube has not been used, open the bottom outlet of the column and move to next step.
15. Lower the top adapter to the target bed height.
16. Apply downward flow at 500 cm/h for 1 CV.
17. 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

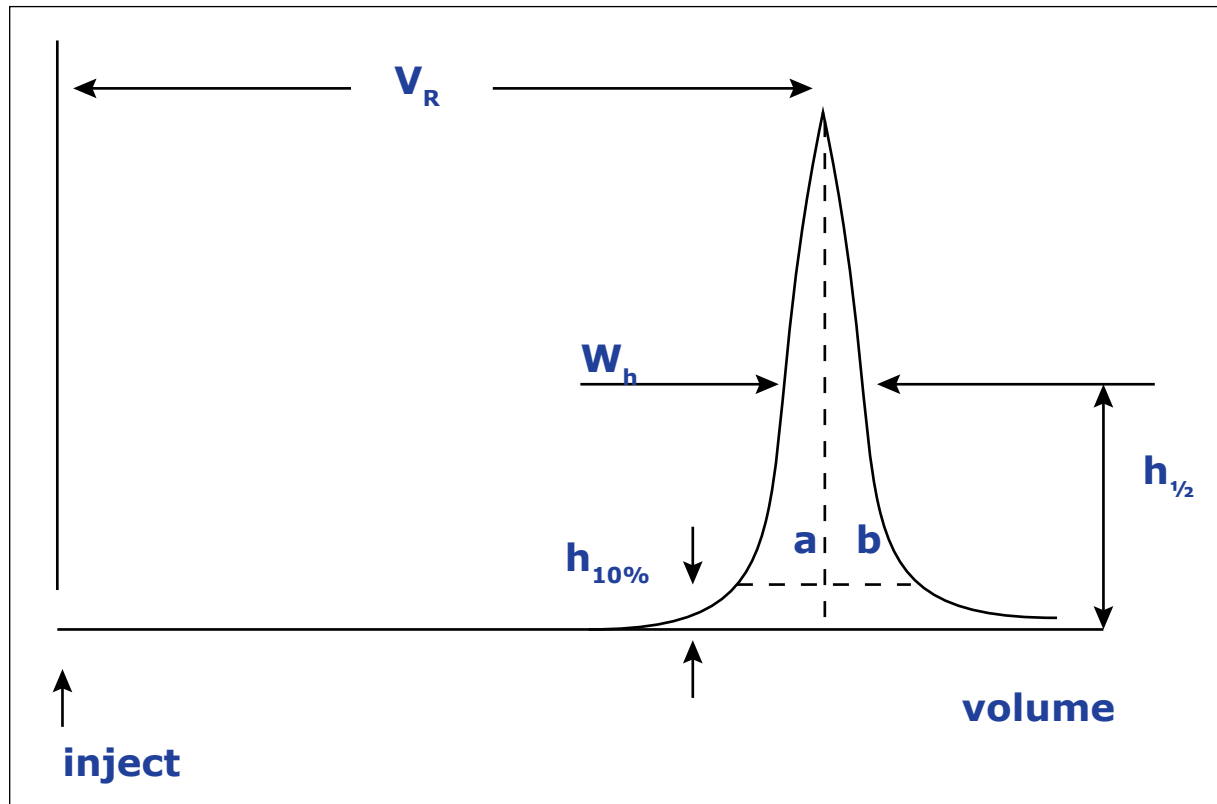
Run the column at a flow rate of ~ 100 cm/h using 150 mM NaCl as mobile phase and inject 1 to 2% of the packed bed volume recommended tracer solution. Monitor the UV absorption (acetone as tracer) of the column effluent.

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

The values for HETP and As 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.

NOTE Using other test systems may result in test artifacts (tailing or fronting).

Calculating HETP and Asymmetry



$$\text{HETP} = L/N$$

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

$$A_s = b/a$$

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.

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® CMX 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® CMX resin
- Graduated cylinder
- Packing buffer (150 mM NaCl)
- Tracer solution (2% acetone in 150 mM NaCl)
- 20% ethanol solution containing 150 mM NaCl

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	1.11 to 1.15	11 to 15%

See [Compression and Resin Calculation Formulas](#) and refer to [Lab Scale Column Packing](#) for Lab Scale Compression Factors.

Resin Slurry Preparation

Eshmun® CMX 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 0.1M NaOH solution.

NOTE Media compression can be higher with 0.1M NaOH. A compression between 11 and 13% can be targeted with 20% ethanol solution containing 150mM NaCl and a compression between 13 and 15% can be targeted with 0.1M NaOH solution.

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.

Eshmun® CMX 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 300-500 cm/h flow rate 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.

- 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.
- 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.

volume. These values should only be used as references and these conditions maintained constant when directly comparing specific values.

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® CMX resin at pilot scale are $N > 3000/m$ and asymmetry values from 0.7 to 1.8.

Standard Product Warranty

The applicable warranty for the products listed in this publication may be found at www.millipore.com/terms (within the "Terms and Conditions of Sale" applicable to your purchase transaction).

Packed Column Evaluation

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

- Run the column at a flow rate of ~100 cm/h and inject 1 to 2% of the packed bed volume of the tracer solution.
- Monitor the UV absorption of the column effluent (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

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and worldwide contact information
please visit:
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For additional information and
documentation please contact:
Merck KGaA, Darmstadt, Germany
Corporation with General Partners
Frankfurter Str. 250
64293 Darmstadt, Germany
Phone: + 49 6151-72 0

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