Millipore
Filtration, Separation
& Preparation

User Guide

Eshmuno® P Chromatography Resin

Contents

Notice

Operating Procedure

Materials

Ensure all buffers and sample feedstock are sterile (0.22 µm) filtered prior to use.

Before applying the sample to a newly packed column or one that is being reused after storage, ensure that it is equilibrated with the equilibration buffer. Optionally, run a blank cycle of the equilibration, elution and regeneration buffers, then reequilibrate the column before applying sample.

Solution	Specification
Equilibration Buffer	100 mM Acetate, pH 5.5
Elution Buffer	0.1 M glycine pH 2.7
Intermediate Wash Buffer	100 mM Acetate, 0.5 M NaCl, pH 5.5
Sanitization Solutions	0.5 M NaOH
	120 mM phosphoric acid, 167 mM sodium acetate, 2.2% benzyl alcohol (PAB)

1. Equilibrate the column with at least five column volumes (CV) of equilibration buffer or until the pH and conductivity at the column outlet have stabilized.
2. Load immunoglobglobin (Ig) feed containing anti-A/B antibodies on to the column, and collect the flow through which contains the target Ig.
3. Wash the column with equilibration buffer or intermediate wash buffer (preferred).
4. Apply up to five CV of elution buffer and collect fractions if desired.
5. Regenerate the column with five to ten CV of equilibration buffer.
6. Perform cleaning in place with 0.5 M NaOH or PAB for 30 to 60 minutes.
7. Apply up to five CV of 0.5 M NaCl.
8. Equilibrate the column with five to ten CV of equilibration buffer.

Sanitization

Sanitization reduces bioburden (i.e., microorganisms and spores) in the column. All columns should be sanitized on a regular basis.

Use PAB or 0.5 M NaOH for sanitization of Eshmuno® P resins. Eshmuno® P resins will maintain the ability to remove anti-A/B antibodies after 200 hours of exposure to PAB solution at room temperature. PAB or 0.5 M NaOH is not recommended for continuous long term storage.

Storage and Handling

Eshmuno® P resin is supplied in 20% ethanol. When using acrylic columns, check compatibility with benzyl alcohol. Store Eshmuno® P resins between 2 to 8 °C.

NOTE Do not freeze.
Do not store for prolonged periods without sanitizing solution.

If used under the recommended conditions, the product will be reusable over many cycles without significant loss of performance.

Lab Scale Column Packing

Introduction

The recommended compression factor (CF) for packing lab scale columns with an inner diameter ≤ 1.6 cm is 1.09 to 1.11.

User Supplied Materials

- Eshmuno® P anti-A or Eshmuno® P anti-B resin
- A lab scale chromatography column and extension tube
- Packing buffer: Deionized Water
- 25 mL Syringe
- Tracer solution to check symmetry (e.g. 1 M NaCl in running buffer)

Compression and Resin Calculations

Eshmuno® P resins should be packed to a CF of 1.09 to 1.11 in lab scale columns. CF is defined as:

$$CF = SV/CV$$

where: SV is the settled bed volume

CV is the packed bed volume.

The settled bed volume required at a given percent compression for a target packed column bed volume can be calculated as follows:

$$SV = CF \times CV$$

The slurry volume required for a target bed height is equal to:

$$(SV/\text{slurry concentration}) \times 100$$

Transfer the required slurry volume to a graduated cylinder before packing the column.

Example

To pack Eshmuno® P resin to a target bed height of 50 mm in a 10 mm inner diameter column (CV = 3.927 mL) using CF = 1.11, an aliquot of 8.72 mL resin suspension (aqueous solution of 20% v/v ethanol, 50% slurry concentration) is needed. This is equivalent to 4.36 mL of settled bed volume.

Resin Slurry Preparation

Eshmuno® P resins are rigid and do not swell in common buffers or solvents used in the biopharmaceutical processes. Storage buffer or equilibration buffer can be used for packing Eshmuno® P resins.

New Resin Slurry Preparation

Resin from a new container can be used for packing without exchanging the storage solution (aqueous solution of 20 % ethanol, 50 % slurry concentration). Mix the resin slurry thoroughly into a homogeneous suspension before transferring the required amount of slurry.

1. Mix the resin thoroughly into a homogeneous suspension and transfer the slurry to a graduated cylinder.
2. Let the resin settle under gravity for ≥ 4 hours and determine the slurry concentration.
3. Add additional storage buffer (aqueous solution of 20% ethanol) or packing buffer to make a 50% slurry concentration.
4. Refer to Compression and Resin Calculations to determine the required slurry volume.

Used Resin Slurry Preparation

Used resin slurry may be stored in buffers other than the original storage solution (aqueous solution of 20% ethanol) and may have a different slurry concentration.

1. Mix the resin slurry into a homogeneous suspension.
2. Take an aliquot of resin slurry. The aliquot volume should contain enough resin to achieve the target CV.

3. Pour the resin slurry into a funnel with a sintered frit.
4. Wash the resin five times with one to two CV of water.
5. Equilibrate the resin into storage buffer (aqueous solution of 20% ethanol) or packing buffer (deionized water) by washing five times with one to two CV of storage buffer or packing buffer.
6. Resuspend the resin in storage or packing buffer into a homogeneous suspension and transfer to a graduated cylinder.
7. Let the resin settle under gravity for ≥ 4 hours.
8. Adjust the total volume to 50% slurry concentration with storage or packing buffer.
9. Refer to Calculating Compression Percent and Required Resin to determine the required slurry volume.

Buffer Exchange by Repeated Settling and Decanting

1. Let the resin settle for ≥ 4 hours in a graduated cylinder to accurately determine the settled volume.
2. Remove the supernatant and add storage or packing buffer.
3. Mix the resin into a homogeneous suspension and let the resin settle for ≥ 4 hours.
4. Adjust the total volume to 50% slurry concentration.
5. Repeat these steps two to three more times.
6. Refer to Compression and Resin Calculations to 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 adequate 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 (deionized water).
5. Mix the slurry in the graduated cylinder into a homogeneous suspension. Ensure there are no clumps of resin at the bottom of the container.
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. Rinse the graduated cylinder with a few mL of packing buffer. Add this slurry to the column. Use packing buffer to rinse any remaining resin from the column wall.
8. Ensure the column outlet is closed and connect the top flow adapter while venting air out of the inlet tube. Only 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 flow with the pump 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 (deionized water) 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. This step may take up to 20 to 30 minutes.

NOTE Reduce the linear velocity of this step if the system pressure exceeds the pressure rating of the column when packing long bed heights or columns rated < 5 bar.

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

NOTE Reduce the linear velocity of this step if the system pressure exceeds the pressure rating of the column when packing long bed heights or columns rated to < 5 bar.

18. Check the quality of the packed bed

Packed Column Evaluation

Before use, the quality of the packing should be checked by measuring the packed column efficiency. This may be repeated during column operation prior to use after storage or if deterioration in separation performance is observed.

The commonly used method for assessing column efficiency is in terms of the height equivalent to a theoretical plate (HETP) and asymmetry factor.

The values for HETP and asymmetry depend on the specific test conditions such as sample concentration and volume, flow rate and system tubing/pipework. These conditions should be maintained and used only as a reference when comparing specific values.

Test the column at a linear flow rate of 50 to 100 cm/h using a one of the sample buffers listed here:

Sample	Mobile Phase
1 M NaCl	water
1 M NaCl	50 mM NaCl
water	200 mM NaCl
2% v/v acetone in running buffer	50 mM NaCl or running buffer

The recommended asymmetry is 0.7 to 1.8.

Pilot Scale Column Packing

Introduction

The recommended compression factor (CF) for packing Eshmuno® P resin in pilot scale columns is 1.11 to 1.17.

Materials

- Eshmuno® P anti-A or Eshmuno® P anti-B resin
- Graduated cylinder
- Recommended packing buffer: Purified water or equilibration buffer
- Recommended tracer solution: 1 M NaCl

Slurry Preparation

Eshmuno® P resin is usually supplied as a nominal 70% resin suspension in 20% aqueous ethanol.

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.

Unpacking

Small diameter columns

Remove the bottom adjuster if the column design allows it

Large diameter columns

Resuspend the resin within the column and pump it out.

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

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% EtOH) 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 buffer exchanges in the column to remove the supernatant by syphoning or using a pump. If another container is used, perform the buffer exchange by decanting the supernatant into waste. These steps will remove the ethanol prior to packing, and clear the potential fines created during shipment from base bead abrasion. Between each buffer exchange, allow the resin to settle at least four hours.
4. Once the buffer exchange has 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. The mean particle size for Eshmuno® P resin is 50 µm so a 10 µm bed support is recommended.

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 water 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. Turn off the pump.

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

7. 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.
8. Condition of the packed bed by running the column for 1 CV in the upward flow direction at the highest processing flow rate or at 2 bar net pressure drop, followed by running the column for 1 CV in the downward flow direction at the highest process flow rate or 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 100 to 200 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	water
1 M NaCl	50 mM NaCl
water	200 mM NaCl
2% v/v acetone in running buffer	50 mM NaCl or running buffer

The recommended acceptance range for asymmetry is 0.7 to 1.8

Patent Sublicense for Use of Product

The life science business of Merck KGaA, Darmstadt, Germany, through its operating corporate entities, including but not limited to EMD Millipore Corporation and Millipore SAS, has the right to grant a nonexclusive sublicense to customers purchasing Eshmuno® P Anti-A and/or Eshmuno® P Anti-B resins for certain patents and patent applications owned and controlled by Laboratoire français du Fractionnement et des Biotechnologies S.A. (LFB).

Customers purchasing Eshmuno® P Anti-A and/or Eshmuno® P Anti-B resins are required to specify in a written form if they desire to seek or decline the foregoing sublicense from the life science business of Merck KGaA, Darmstadt, Germany. Please contact your local sales representative or technical service for more information.

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

For more information and
documentation please contact:

Merck KGaA, Darmstadt, Germany
Corporation with General Partners
Frankfurter Str. 250
64293 Darmstadt, Germany
Phone: + 49 6151-72 0

For requests from USA and Canada
please contact:

EMD Millipore Corporation
A subsidiary of Merck KGaA, Darmstadt, Germany
290 Concord Road
Billerica, MA 01821, USA
Phone: 1-800-645-5476

www.emdmillipore.com