Column packing instruction for BioPro IEX and BioPro IEX SmartSep for laboratory columns

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1. Introduction

BioPro IEX and BioPro IEX SmartSep are ion exchange chromatography (IEX) resins of YMC. They are based on hydrophilic spherical polymer beads and bonded with functional groups for strong anion exchange chromatography (quaternary ammonium group; Q) or cation exchange chromatography (sulfo group; S). These media are designed for demanding purifications, such as proteins, antibodies, peptides and oligonucleotides. Their capacity, resolution and recovery enable maximum productivity while their physical and chemical robustness guarantees efficiency for your process.

As these resins are available in a variety of particle sizes, they can be universally used for initial capture, intermediate purification and final polishing of target compounds. Good column packing is essential for IEX separation. A poorly packed column results in peak broadening and loss of resolution. In this regard, this instruction contributes to a successful purification.

2. Specification

Parameter	BioPro IEX SmartSep Q/S		BioPro IEX Q/S
Particle size (μm)	20	30	75
Recommended working pressure	≤ 2 MPa		≤ 0.3 MPa
Pressure limit	3 MPa		0.3 MPa
Optimum usage	Polishing and intermediate purification		Initial capture and intermediate purification

3. Column packing instructions

The following instructions exemplarily explain the packing procedure using biocompatible column hardware (glass column, acrylic column or similar). If you are using a different column type, please refer to the corresponding column manual to adjust the packing procedure accordingly.

In addition, check the tools used for filling in advance and prepare clean ones without deterioration or scratches.

3.1 Recommended packing parameters

Compression factor (CF)		20 µm	1.05 - 1.15
		30 µm	1.05 - 1.15
		75 µm	1.10 - 1.20
Slurry Concentration (C_{SLURRY})	Recommended	30% - 50%	
	Maximum	70%	
Slurry solvents *		1 M NaCl, 0.5 M Na_2SO_4	
Packing flow		More than twice as fast as flow rate using for purification	

* It is recommended to use the highest ionic strength solution in actual purification operation, such as a mobile phase at elution step. 20% Ethanol and 100% H_2O can also be used as a slurry solvent. However, packing procedures may have to be changed to get a good chromatographic performance.

- CF: The degree of bed compression is calculated by the ratio of the volume of uncompressed resin settled by gravity and the volume of compressed material.
- C_{SLURRY}: The proportion of the resin volume to total slurry volume.

3.2 Calculations for column packing

Volume of compressed resin: bed volume (V_{BED}) (r = radius of column, L = required bed length)	$V_{BED} = \pi \times r^2 \times L$
Volume of required material (V_M)	$V_M = V_{BED} \times CF$
Total slurry volume (V_{SLURRY})	$V_{SLURRY} = V_M / C_{SLURRY}$

3.3 Resin preparation: Removal of fines

Materials needed:

- Resin (in the original packaging)
- A graduated, container with a capacity of 2 times or more than the material volume needed to pack the column (V_M)
- Mixing tool (Do not use a sharp-edged paddle or a magnetic stirrer to avoid fine generation)
- Slurry solvent

Step	Operation
1	Suspend the resin by careful shaking and transfer it into the prepared container
2	Allow the slurry to settle for the sedimentation time specified for each particle below and measure the amount of the resin
3	Add or remove suspension, if required, to achieve the required material volume (V_M)
4	After sedimentation, decant the supernatant
5	Add the volume of slurry solvent equal to the amount of material, carefully homogenize with the mixing tool
6	Allow the resin to settle for the sedimentation time
7	Repeat 4 - 6 at least 3 times

Sedimentation time for each particle size

20 µm	300 minutes ~ overnight
30 µm	120 minutes ~ overnight
75 µm	60 minutes ~

3.4 Slurry preparation

Materials needed:

- Resin (from step 3.3)
- Container with a capacity larger than the slurry volume (V_{SLURRY})
- Mixing tool (Do not use a sharp-edged paddle or a magnetic stirrer to avoid fine generation)
- Filtration equipment (e.g. funnel & paper or glass filter)
- Slurry solvent (as specified in 3.1)

Step	Operation
1	The clear supernatant after step 3.3 is decanted
2	Carefully transfer the resin to your filtration equipment, rinse with slurry solvent if required
3	Filter the resin using three times the material volume (V_M) of slurry solvent
4	Transfer the washed slurry into a container with a capacity larger than the slurry volume (V_{SLURRY})
5	Add slurry solvent until V_{SLURRY} is achieved

3.5 Column preparation

Materials needed:

- Column with a volume that exceeds the target bed volume (V_{BED})
- Packing reservoir (required if V_{SLURRY} exceeds the column volume)
- Suitable stand for a reliable fixation of the column in vertical position
- Small level gauge
- Chromatographic pump for solvent supply according to the required flow
- Capillaries

- Plugs and connectors
- Solvent reservoir with slurry solvent
- Solvent waste container

Step	Operation
1	Attach the packing reservoir to the column (if required)
2	Insert the lower piston into the column
3	Adjust the piston length to the target bed volume (V_{BED})
4	Fix the column into the stand and verify exact vertical installation with the air level
5	Connect the pump to the column bottom inlet
6	Pump 1 - 2 cm of solvent into the column from below (purges the air from the lower piston)
7	Quickly disconnect the pump & close the lower column outlet with a plug
8	Connect the pump to the upper piston, purge it with solvent

3.6 Column packing

Materials needed:

- All materials of step 3.5
- The prepared resin of step 3.4
- Mixing tool (Do not use a sharp-edged paddle or a magnetic stirrer to avoid fine generation)
- Erasable marker-pen suitable for the column body

General remark: In the following procedure, the slurry will be filled into the column. Please note that afterwards, sedimentation of the suspended material will begin immediately. This sedimentation may create zones with differing packing densities in the final column bed, which in turn may result in adverse chromatographic effects such as peak tailing. While it is hardly possible to fully avoid any sedimentation effects after introduction of the slurry, steps 3 - 9 should still be carried out as fast as reasonably possible in order to mitigate their effects.

Step	Operation
1	Verify that pistons & frits are still sufficiently wetted with solvent
2	Stir the resin gently using the mixing tool until a homogeneous mixture is formed
3	Pour the slurry into the column gently avoiding the introduction of air bubbles
4	Rinse the internal column wall with slurry solvent to release attached particles
5	Insert the upper piston into the solvent avoiding the introduction of air bubbles
6	Move the upper piston downwards until all air is removed from it (solvent appears on top)
7	Connect the pump to the column top inlet
8	Connect the column bottom outlet to the solvent waste container
9	Start the pump at one-fifth to one-half of the final packing flow
10	Slowly increase the flow rate to the packing flow (bed is increasingly compressed)
11	Wait until no further change in the bed length is noticeable

12	Mark the achieved bed length under flow using the marker-pen
13	Stop the flow and disconnect the pump
14	Close the column bottom outlet
15	Reduce filling level, dismount packing reservoir & reconnect the upper piston
16	Lower the upper piston until you reach the mark
17	Reopen the column outlet, reconnect the pump and start the flow again
18	Repeat steps 12 - 17 until no further compression of the bed is visible (If the desired bed length is not achieved after flow, push in the upper piston if necessary.)
19	Close the column outlet, disconnect the pump and apply a plug to the inlet as well

4. Column performance evaluation

When the column packing is completed, evaluate the column performance by injecting a sample to the column, and determine the column theoretical plate number (N/m) and peak asymmetry factor (As). Typical evaluation conditions are shown below. If there is a large difference between the value(s) obtained and typical performance listed below, adjust the packing parameter(s) (slurry concentration, CF etc.) and repeat the packing procedure.

Example of test conditions for the column packing evaluation

Detection	Conductivity	UV at 220 nm
Mobile phase	0.5 M NaCl	Low ionic strength buffer
		Strong anion exchanger (Q): 20 mM Tris-HCl buffer (pH 8)
		Strong cation exchanger (S): 20 mM Phosphate Buffer (pH 7)
Sample	1 M NaCl	Formamide (2 µL/mL)
Flow rate	Approximately 70 to 90 cm/hour	
Temperature	Ambient (25 ˚C)	
Injection volume	1 - 2 % of bed volume	

	Column performance		
Particle size (µm)	20	30	75
Theoretical plate number (N/m)	≥ 6,500	≥ 5,000	≥ 2,500
Asymmetry factor (As)	0.7 - 1.8		

These are just some guide values. Desired separation could be achieved even if the values obtained are out of the range shown above, depending on your usage and/or application.

Sample diffusion in the system flow path (extra-column volume) greatly affects column performance. If change of packing conditions does not affect the column performance, check the step 7 Troubleshooting.

5. Unpacking and storage of used product

The method of unpacking described below is an example. Please also refer to the instructions of your column.

Materials needed:

- The packed column after purification
- Solvent reservoir with 20% ethanol aqueous solution or another storage solvent
- Solvent waste container
- Container with a capacity larger than a slurry volume for resin storage
- Resin container

Step	Operation
1	Flush the column with 20% ethanol aqueous solution or another storage solvent to replace solution in the column with storage solvent completely.
2	Open the column bottom inlet and release the pressure in the column to atmosphere pressure.
3	Remove the bottom piston.
4	Pump 20% ethanol aqueous solution or another storage solvent into the column from the column top inlet. Gently push out the resin to a resin container (for storage).
5	Remove the upper piston.
6	Rinse the residual resin in the column with water or 20% ethanol aqueous solution.
7	Store the resin in 20% ethanol aqueous solution or another storage solvent at 4-35˚C.

6. Examples of actual packing and inspection

Practical packing example for BioPro IEX SmartSep S30 is shown. Column with 15 mm I.D. was packed by the following packing conditions. The inspection was carried out in accordance with the method described in Step 4 (conductivity method). Correlation of pressure and flow was determined for the resin with the column packed in this study. The operating pressure was proportional to linear flow rate in the tested flow rate range.

Resin	BioPro IEX SmartSep S30	
Column	Column diameter	15 mm I.D.
	Bed height (L)	300 mm
	Packing reservoir	500 ×15 mm I.D.
Packing conditions	Slurry concentration (C_{SLURRY})	30%
	Slurry solvent	1.0 M NaCl
	Packing flow	576 cm/h
	Compression factor (CF)	1.10
Test conditions	Mobile phase	0.5 M NaCl
	Flow rate	90 cm/h
	Sample	1.0 M NaCl
	Injection volume	1% of bed volume
	Temperature	Ambient

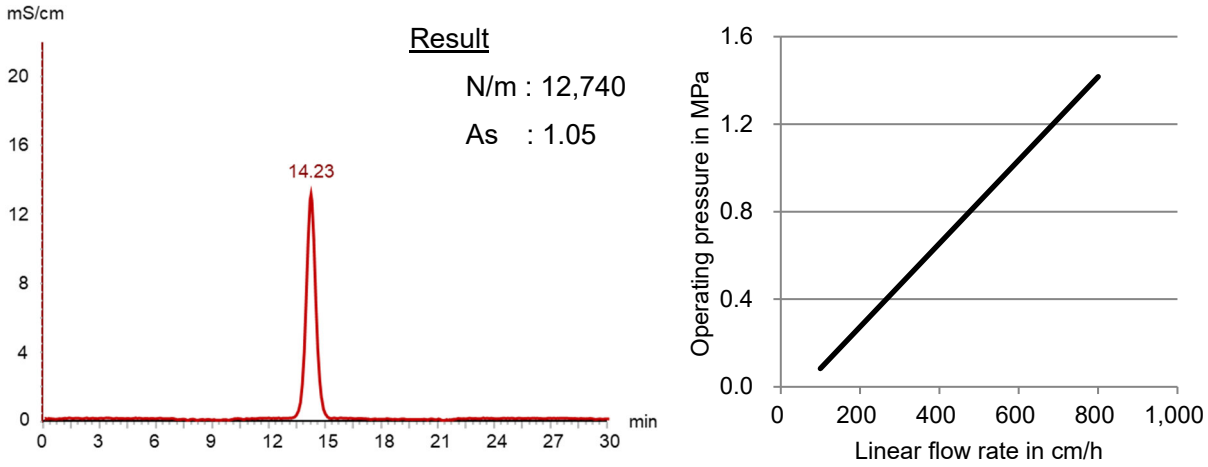

Figure. Packing example of BioPro IEX SmartSep S30

7. Troubleshooting

Trouble detail	Cause	Remedy
Peak tailing	Gap between piston and bed surface	Lower the upper piston until there is no gap.
	Bed is not compressed enough	Lower the upper piston to compress the packing bed layer. Repack the column with greater CF or a lower slurry concentration.
	Air or contaminants in column frits	Wet the frit thoroughly with packing solvent and install. Remove frit contamination. If not resolved, replacement of the frit is recommended.
	Sample diffusion in the system flow path (extra-column volume) is significant	Check if the inner diameter of connecting tubes and equipment performance are appropriate.
Peak fronting	Excessive column bed compression	Repack the column with smaller CF or a higher slurry concentration.
Split peaks	Channel(s) in packed bed	Repack the column.
	Resin is crushed or partially blocked frit (high column back pressure)	Repack the column after decanting the resin or replacing the frit.
Low plate count	Poor column packing	Review that the filling flow rate and slurry concentration are appropriate.
	Sample diffusion in the system flow path (extra-column volume) is significant	Check if the inner diameter of connecting tubes and equipment performance are appropriate.

8. Supplementary Information

Packing ECO and ECOPLUS Glass Columns
(https://youtu.be/V9wUbMll8Jg?si=x2CJWeqBP3bftzpo)