

Care and Use Instructions Ion Exchange Media BioPro IEX SmartSep Q / BioPro IEX SmartSep S

Thank you for purchasing the BioPro IEX SmartSep Q/S. To ensure optimal performance and durability of the media, please read these instructions carefully before using.

1. Specification

Item	Strong-anion exchanger BioPro IEX SmartSep Q			Strong-cation exchanger BioPro IEX SmartSep S		
	Particle size (μm)	10	20	30	10	20
Matrix	Hydrophilic polymer beads					
Functional group	-R-N ⁺ (CH ₃) ₃			-R-SO ₃ ⁻		
pH range	2-12			2-12		
Temp. range (°C)	4-60			4-60		
Pressure range	Regular use: 3 MPa or less than 3 MPa	Regular use: 2 MPa or less than 2 MPa	Regular use: 3 MPa or less than 3 MPa	Regular use: 2 MPa or less than 2 MPa	Upper limit: 4 MPa	Upper limit: 3 MPa
	Upper limit: 4 MPa	Upper limit: 3 MPa	Upper limit: 4 MPa	Upper limit: 3 MPa		
Shipping solvent	20% ethanol aqueous solution					
Application	Highly efficient polishing step and intermediate purification step					

2. Packing instructions

NOTE) Also refer to the Instruction manual of the column using and follow the packing procedures described in it, when necessary.

For details packing instructions, please also refer to the Column packing instruction for BioPro IEX for laboratory columns and Column packing instruction for BioPro IEX.

2-1 Resin preparation (Removal of fines)

Recommended slurry solvent : Deionized water, buffer solution or 20% ethanol solution

Amount of media required : approximately 1.05 X to 1.10 X column volume

1. Suspend the settled resin in the shipping container(s) and transfer the slurry into a clean container with a capacity of 5 times or larger than the amount of resin.
2. Allow the slurry to settle for a certain time. Then measure the amount of the resin and decant the supernatant.
3. Add four times the resin volume of the slurry solvent stated above to the container.
4. Stir the solution with a rod or paddle to make homogeneous slurry. Do not use a sharp-edged paddle or a magnetic stirrer to avoid fine generation.
5. Allow the 30 μm resin to settle for 120 minutes. The smaller the particle size is, the more time for sedimentation is required.
6. Once resin has settled, decant the supernatant.
7. Repeat Step 3-6 until the supernatant at the Step 6 becomes clear.

2-2 Packing slurry preparation and column packing

Recommended packing solvent : A solution with high ionic strength (a component of mobile phase which has the highest ionic strength. For example, 1 M NaCl or 0.5 M Na₂SO₄)

1. Filter the slurry solution obtained after 3-1. If 20% ethanol is used as the slurry solvent at 3-1, rinse the resin by filtration with deionized water (This process can be skipped if deionized water or buffer is used at 3-1).
2. Rinse the resin on the filter with approximately three times the resin volume of the packing solvent stated above.
3. Transfer the resin to a clean container with a capacity of 5 times or larger than the amount of resin.
4. Add the packing solvent to adjust the slurry concentration to 30-50%. The slurry concentration can be calculated with a following equation. Slurry concentration (%) = Resin volume (L) / Total slurry volume (L) X 100
5. Stir the solution with a rod or paddle to make homogeneous slurry.
6. Once homogeneous slurry is formed, gently pour it into the column. Prevent air coming into the column during transfer.
7. Connect a pump to the column and start pumping the packing solvent at approximately two times of the actual flow rate in use. Maintain the flow rate until the packed bed becomes stable. Then disconnect the pump. The desired column volume can be

obtained by flowing the packing solvent through the column and compressing the resin.

8. When using an axial compression column, the column performance may be improved by pushing in after pumping.

2-3 Column performance evaluation

When the column packing is completed, evaluate the column performance evaluation by injecting a sample to the column, and determine the column theoretical plate number (N/m) and peak asymmetry factor (As). Typical evaluation condition is 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, flow rate of the slurry solvent etc.) and repeat the packing procedure.

Example of test conditions for the column packing evaluation

Condition 1		Condition 2		Column performance			
Mobile phase	0.5 M NaCl	Low ionic strength buffer	Strong anion exchanger (Q): 20 mM Tris-HCl buffer (pH 8)	Particle size	10 μm	20 μm	30 μm
		Strong cation exchanger (S): 20 mM Phosphate Buffer (pH 7)		Theoretical plate number (N/m)	≥ 14,000	≥ 6,500	≥ 5,000
Sample	1 M NaCl	Formamide (2 μL/mL)		Asymmetry factor (As)	0.7~1.8		
Detection	Conductivity	UV at 220 nm					
Flow rate	Approximately 70 to 90 cm/hour						
Temperature	Room temperature (25°C)						
Injection volume	1% of bed volume						

* 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 the packing conditions do not change the column performance, check if the inner diameter of connecting tubes and equipment performance are appropriate.

3. Equilibration and elution

- Equilibrate with about 5–10 column volumes of initial mobile phase before using a column for chromatographic separations.
- Generally, samples are adsorbed onto the top of the column with 20–50 mM of buffer as the initial mobile phase, and then eluted with a salt-concentration gradient method (for example, adjusting sodium chloride concentration between 0 and 0.5 M) or pH gradient method. It is recommended to flush the column with buffer containing about 1 M of sodium chloride after each run in order to remove residual impurities in the column staying with the final mobile phase.
- Water-soluble organic solvent (maximum of 30%), can be added to the mobile phase. Before adding such solvent, make sure that salt in the buffer will not precipitate.

4. Cleaning and regeneration

A change of retention time or peak shape and/or pressure increase might result from the adsorption of fat-soluble substances or precipitated impurities in a sample. In such cases, follow these steps for column cleaning and regeneration. If these procedures will not solve the problem, we recommend using new media.

4-1 Common cleaning methods

- Cleaning in place (CIP) - The following CIP is effective when there is a change in column performance or before long-term storage (it is recommended to wash the column without connecting to a detector).

First, 3 to 5 CV (CV : Column Volume) of 1 to 2 M NaCl is flowed through the column. Then, 3 to 5 CV of 0.1 to 0.5 M NaOH is flowed through the column. The cleaning efficiency of the column can be improved by increasing the concentration of NaOH (up to 1 M) and/or the exposure time by slowing the flow rate. In order to neutralize the column, 3 to 5 CV of 1 to 2 M NaCl is flowed through the column. After neutralization, equilibrate the column thoroughly with the same mobile phase used for the next step.

The state of contamination or type of washing solution can cause a pressure increase. In such cases, reduce the flow rate.

- Batch method

Soak and agitate the media in washing solution about 3–5 times of the media volume. After leaving it stationary, remove the supernatant fluid by decantation. Repeat the process 2–3 times. As a washing solution, the solvents used for CIP can be used.

4-2 Cleaning with surfactants and other additives

- Other additives such as urea (≤ 8 M) or guanidine hydrochloride (≤ 6 M), which are commonly used as protein denaturants are useful. Avoid solvents containing oxidant for the mobile phase.
- Nonionic surfactants, cationic surfactants or anionic surfactants are useful, but avoid anionic surfactants for BioPro IEX SmartSep

Q and avoid cationic surfactants for BioPro IEX SmartSep S.

- The state of contamination or type of washing solution (high viscosity, etc.) can cause a pressure increase. In such cases, reduce the flow rate.

5. Storage

Store the bulk media in 20% ethanol aqueous solution. Store the bulk media in the original container at temperatures from 4 to 35°C. Keep the container closed tightly.

NOTE) Product quality is maintained even if a product bottle is not stored or transported in the upright position, or the resin is adhering on wall or neck of the bottle.

However, it is recommended to store the product (bottle) in the upright position, and immerse all the resin in 20% ethanol solution (storage solvent in the bottle), from a standpoint of prevention of resin drying.