
NW Rose Plus Q HP & NW Rose Plus SP HP

Ion exchange chromatography (IEX) resins

NW Rose Plus Q HP and NW Rose Plus SP HP

Ion exchange chromatography resins

Ion exchange chromatography is a world wide used chromatography method in modern biopharma downstream purification process which plays a very important role in biomolecular separation.

NW Rose Plus HP series is a novel series of IEX chromatography resins base on highly crosslinked, rigid agarose beads and in order to meet the demands for micro biomolecular large-scale manufacture, the particle size, pore size, ligand density, bonding technology, etc. has been optimized.

NW Rose Plus HP series IEX resins has the following characteristics:

- High dynamic binding capacity.
- Low back pressure at high flow rate.
- High physical and chemical stability.
- Easy to scale-up from lab to large-scale manufacture.

A strong anion IEX chromatography resin, NW Rose Plus Q HP and a strong cation IEX chromatography resin, NW Rose Plus SP HP are available.

Table 1. The characteristics of NW Rose Plus Q HP and NW Rose Plus SP HP.

Product name	NW Rose Plus Q HP	NW Rose Plus SP HP
Chromatography technique	Strong anion IEX	Strong cation IEX
Matrix	Highly cross-linked, rigid agarose beads	
Particle Size	50 μm	
Ligand	Q	SP
Ionic Capacity	140-180 $\mu\text{mol} \cdot \text{mL}^{-1} (\text{Cl}^-)$	120-170 $\mu\text{mol} \cdot \text{mL}^{-1} (\text{H}^+)$
Maximum Pressure	0.5 MPa	
Clean in Place	0.5 M NaOH	
Recommended Flow Velocity	150 cm/h (50/30 Column, bed height 10 cm)	
Maximum flow velocity	600 cm/h, 16/20 Column, bed height 10 cm	
pH stability	3-12 (operational) 2-14 (CIP)	
Chemical stability	Stable in commonly used aqueous buffers, 0.1 M NaOH, 30% isopropyl alcohol, 6 M guanidine hydrochloride, 8 M urea, 70% ethanol, etc.	
Operational temperature	4-40 $^{\circ}\text{C}$	
Storage	20% ethanol, 4-30 $^{\circ}\text{C}$	

Prepare the resin slurry

i. Calculate the amount of Resin

Column volume = $S \times h$,

Resin volume = column volume $\times$ packing factor / slurry ratio,

For NW Rose Plus HP series resins, packing factor is 1.15.

Notes:

- The buffer or solution used needs to be degassed (degassing is not necessary for water for injection);
- The slurry ratio of origin package is about 75% generally. If the slurry ratio is not known, it can be determined by pouring the resin into a measuring cylinder for overnight (typically 12 to 24 hours) and calculating the ratio of gravity settling volume to origin volume.
- The origin package of resin is stored in 20% ethanol. The packing buffer is replaced by negative pressure filtration for three times with buffer containing 0.15-0.5 M NaCl, ultra-pure water or equilibrium buffer. Do not mix the resin with excessive force.
- It can be sterilized at 121 °C for 20 min if required.
- Do not use a magnetic stirrer, it may cause the resin particles to break.

ii. Prepare the slurry

Prepare the slurry concentration to 50-75%, stir well for later use.

Notes:

- High resin temperature (especially after sterilization) during column packing may damage the column.

Pack the column

Please refer to the column user manual (Chromatography columns produced by different vendors are used in different ways and improper operation may bring damage to columns and resin). Ultra-pure water and equilibrium buffer is used as packing buffer.

- Adjust the column level and lock it in place before pack;

- Vent the bottom membrane of the column and retain 1 cm packing buffer at the bottom of the column;
- Install a column loader if the packing height exceeds 2/3 of the column height;
- Pour the slurry into the column slowly at one time. Be careful to avoid air bubbles. Stir it well with a stirring rod after pouring;
- Connect the column head, add a pressure gauge between the column head and the system or peristaltic pump (required for industrial-scale columns), and vent the head membrane of the column;
- Insert the column head into the column until the bottom membrane reaching to the liquid surface and remove air bubbles;
- Connect the system or peristaltic pump and pack the column at an appropriate flow rate to ensure the back pressure under 2.5 bar;
- The resin interface gradually drops with mobile phase. Mark the position of the interface until the resin interface is stable (The interface is no longer drops within 20 min);
- Stop the pump and remove the column loader (if necessary). Lower the column head to the interface of the resin (0.5 cm further down from the marked position).
- Continue pressing the resin for 20-30 min. If the resin interface is stable, it indicates the column packing is completed.

Qualify the column

i. Evaluate of the column efficiency

For evaluation of the column efficiency, acetone or sodium chloride can be used as the indicator and mobile phase can be prepared according to the following table:

Table 2. Conditions for column efficiency evaluation.

Sample	1.0% (v/v) acetone	2 M NaCl solution
Loading volume	1.0% CV	1.0% CV
Mobile phase	Water	0.4 M NaCl solution
Flow rate	30 cm/h	30 cm/h
Detection	UV 280 nm	Conductivity

ii. Calculate the column efficiency

Calculate the theoretical plate height (HETP), theoretical plate number (N) and asymmetry factor (As) according to the UV or conductivity curve with the following equation:

$$HETP = L/N$$

$$N = 5.54 \times \frac{V_R^2}{W_h^2}$$

Where,

V_R is retained volume;

W_h is peak width at half-height;

L is the column height;

N is the number of theoretical plates.

The units of V_R and W_h should be the same.

$$A_s = \frac{b}{a}$$

Where,

a is the first half peak width at 10% peak height;

b is the second half peak width at 10% peak height.

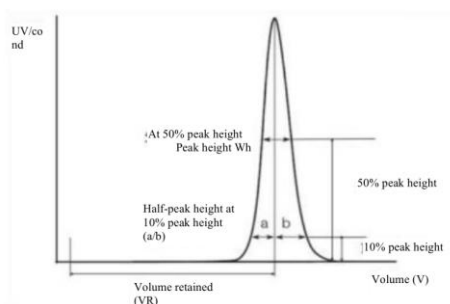


Figure 1. Column efficiency test.

iii. Result evaluation

It is judged to be qualified if the value of HETP is less than three times of the average particle diameter of the resin beads and the asymmetry factor is between 0.8 and 1.8. It is necessary to analyze the reasons and repack the column, if the HETP and A_s is unsatisfactory.

Chromatography method

NW Rose Plus HP series IEX resin is usually used in mediate and polishing purification step after capture step.

- IEX chromatography resin can separate molecular according to the charge difference of biomolecules, and the isoelectric point or charged state of the target sample should be known before use.
- Select the buffer according to the stability and other properties of the target molecule, and determine the pH value. Generally, the CEX is 1 pH lower than the isoelectric point, and the AEX is 1 pH higher than the isoelectric point.
- Conduct a preliminary experiment using a 10-20cm high column.
- Optimize experimental conditions based on the analysis of pre-experimental results.

Purification example of NW Rose Plus Q HP

Experimental condition:

Resin: NW Rose Plus Q HP;

Column: 7.7×100 mm (CV: 4.65 ml);

Buffer A: 25 mM Tris-HCl, pH 8.5;

Buffer B: 25 mM Tris-HCl + 1.0 M NaCl, pH 8.5;

Sample: 8 mg/ml BSA, 5 mg/ml starch glycosidase dissolved in buffer A.

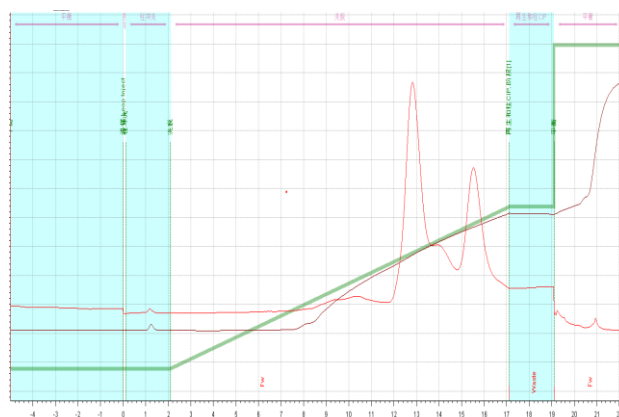


Figure 2. Purification diagram of NW Rose Plus Q HP

Purification example of NW Rose Plus SP HP

Experimental condition:

Resin: NW Rose Plus SP HP;

Column: 7.7×100 mm (CV: 4.65 ml);

Buffer A: 25 mM PB, pH 6.8;
 Buffer B: 25 mM PB + 1.0 M NaCl, pH 6.8;
 Sample: 2.5 mg/ml lysozyme, 4 mg/ml cytochrome C dissolved in buffer A.

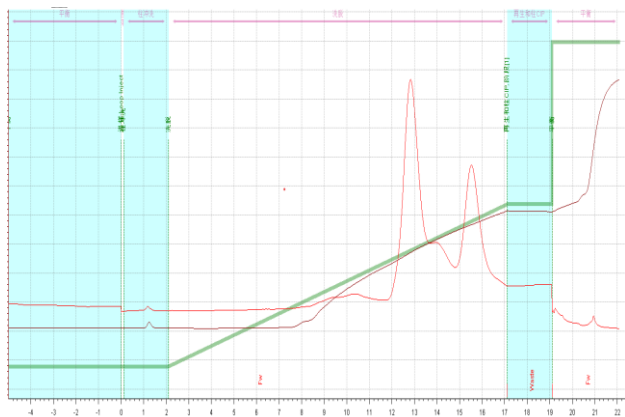


Figure 2. Purification diagram of NW Rose Plus SP HP

NW Rose Plus Q HP、NW Rose Plus SP HP show a high dynamic binding capacity in purification of antibodies.

10% break through DBC of Standard protein	NW Rose Plus Q HP	NW Rose Plus SP HP
IgG, mg/ml	77.5	68.3
BSA, mg/ml	56.2	-
Lysozyme, mg/ml	-	65.2

Cleaning and sanitization

As the cycle of resin increases, the accumulation of pollutants on the column also continues to increase. Regular in place cleaning can prevent the accumulation of pollutants and maintain a stable working state. Determine the frequency of in place cleaning based on the degree of contamination of the resin (if the contamination is severe, it is recommended to perform in place cleaning after each cycle to ensure repeatability of the results). The recommended cleaning

conditions for different types of impurities and pollutants are as follows:

- Use 2 M NaCl to remove the tightly bound proteins or 1 M NaAc, pH=3, pH 3 to remove strongly bound proteins.
- For the removal of strongly hydrophobic proteins and precipitated proteins: clean the column with 2-3 CV 1 M NaOH, and then immediately rinse with 5-10 CV pure water.
- Removal of lipoproteins and lipid substances: clean the column with 5-10 CV of 70% ethanol or 30% isopropanol, and then rinse with 5-10 CV of pure water.

Notes:

Degassing treatment should be carried out before using 70% ethanol or 30% isopropanol; During the in-place cleaning process, the flow rate can be selected from 30 to 60 cm/h; When the blockage is severe, reverse cleaning can be used.

Sterilization

The NW Rose Plus HP series IEX chromatography resin can be treated with 1 M NaOH for more than 0.5 to 1 hour to achieve sterilization and pyrogen removal.

Storage

The NW Rose Plus HP series IEX chromatography resin is sealed and stored in 20% ethanol. To prevent ethanol evaporation and microbial growth, it is recommended to replace it with fresh 20% ethanol every 3 months.

Destruction and recycling

Due to the difficulty in degrading the NW Rose Plus HP series IEX chromatography resin in nature environment, incineration treatment is recommended to protect the environment.

Troubleshoot

Q 1	After the resin is stirred to slurry, there is some small white foam float on the interface?
	This phenomenon is generally caused by the use of a magnetic stirrer or a peristaltic pump in the resin mixing or resin transfer or if the resin has not been used for a long time, the settling of the resin is too solid and the stirring is too intense.
Q 2	Protein purity is low after IEX chromatography?
	The resolution of the IEX chromatography mainly depends on the following factor: • The higher column efficiency, the higher resolution; • The smaller diameter of resin beads, the higher resolution; • Different elution condition effects the resolution, linear elution brings the higher resolution; • According to the charge difference between the target and impurity, find the appropriate pH and select the corresponding resin.
Q 3	Low yield.
	• Detect the concentration (or activity) of the sample in the flow through collection to confirm whether the sample is binding to the resin or partially bound (Sample overload is also a reason for low yield); • Test the regeneration solution to see if the target molecular is tightly bound (Buffer and resin types can be changed); • Is there any sample present in the CIP (sample precipitates in the column, change the chromatographic conditions).
Q 4	Target molecular is not bound.
	• Check whether it is overloaded or not and detect sample concentration activity during flow through; • The environment of the sample may not be charged or the charge may be the same as that of the resin.
Q 5	How to desalt after IEX purification?
	SEC technology can be used for desalination, such as NW DEX G-25C, or adjust pH for elution.
Q 6	Resin discoloration and agglomeration.
	This phenomenon is generally caused after use for a period of time, which is contaminated by pigments, lipids, and nucleic acid substances in the sample; Try different CIP methods to solve it. The NW Rose series resin can be used 300-600 times for general samples, and 100-200 times for complex samples.
Q 7	How to determine the lifetime of resin?
	Laboratory use: Generally, the lifetime of the resin is simply judged based on column pressure, flow rate, color, etc. Industrial production: it is necessary to strictly follow the process validation method to determine the medium life.

Order information

Product	Package	Cat. No.
NW Rose Plus Q HP	25 mL	60023-547000-2025
	100 mL	60023-547000-2100
	500 mL	60023-547000-2500
	1 L	60023-547000-1001
	5 L	60023-547000-1005
	10 L	60023-547000-1010
NW Rose Plus SP HP	25 mL	60023-546800-2025
	100 mL	60023-546800-2100
	500 mL	60023-546800-2500
	1 L	60023-546800-1001
	5 L	60023-546800-1005
	10 L	60023-546800-1010

We also offer 7.7 mm × 22 mm, 16 mm × 25 mm, 7.7 mm × 100 mm preppacked columns. More requirements about products or customized needs, please contact us.

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