
NW Rose Plus Butyl HP & NW Rose Plus Phenyl HP

Hydrophobic Interaction Chromatography (HIC) Resins

Product Instruction

File code: NM-A-DF-0404

Version: A0



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Hydrophobic interaction chromatography (HIC) resins

Hydrophobic interaction chromatography (HIC) utilizes reversible interactions between proteins and HIC resins to separate proteins based on their different hydrophobicity.

The NW Rose Plus HP series HIC resin is made by highly cross-linked agarose beads coupled with different ligands, Butyl and Phenyl which

offer different selectivity. These products have the following characteristics

- Higher binding capacity, higher resolution lead to an improved production efficiency and reduced production costs.
- Higher flow rate to increase processing capacity per unit time.
- Excellent physical and chemical stability

Table 1. The characteristics of NW Rose Plus HP series HIC resins.

Product name	NW Rose Plus Butyl HP	NW Rose Plus Phenyl HP
Matrix	Highly cross-linked agarose beads	Highly cross-linked agarose beads
Ligand	Butyl	Phenyl
Particle size	40-50 μm	40-50 μm
Dynamic binding capacity (BSA)	30 $\text{mg} \cdot \text{mL}^{-1}$	40 $\text{mg} \cdot \text{mL}^{-1}$
Maximum pressure	0.3 MPa	
CIP	0.5 M NaOH	
Recommended flow rate	200-350 cm/h (XK50/30 column, ID=50 mm, column height=10 cm)	
Maximum flow rate	600 cm/h (0.1 MPa XK26/30 column, column height=15 cm, 25 $^{\circ}\text{C}$)	
pH stability	3~13 (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.	
Storage	20% ethanol, 4-30 $^{\circ}\text{C}$	
Operational temperature	4-40 $^{\circ}\text{C}$	

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 HIC resins, packing factor is 1.15.

Notes:

- The buffer or solution used needs to be degassed (Injection water does not need to be degassed);
- The slurry ratio of origin package is about 75% generally. If the slurry ratio is not unknown, 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 sterilization 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 vendor 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 reaches 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.

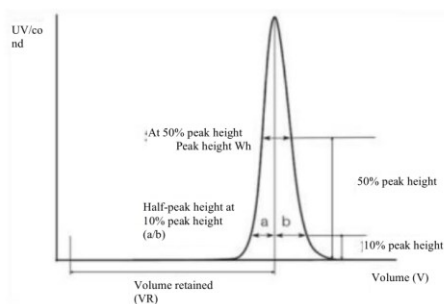


Figure 1. Column efficiency test.

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

1. When using HIC resins, $(NH_4)_2SO_4$ buffer is generally as the equilibrium buffer, and the $(NH_4)_2SO_4$ concentration is determined depending on different samples.
2. The sample also needs to be dissolved in $(NH_4)_2SO_4$ buffer.
3. Select a suitable HIC resin.
4. Conduct a preliminary experiment using a laboratory column, as shown in the following figure:

The experimental conditions are as follows:

Sample: Ribonuclease A, lysozyme, and chymotrypsin were dissolved in equilibrium buffer and use $0.45 \mu m$ filter before use.

Column: $0.77 \times 100 mm$

Equilibrium buffer: 20 mM PB +1.7 M $(NH_4)_2SO_4$ pH 7.0

Elution buffer: 20 mM PB pH 7.0

Flow rate: $1 mL \cdot min^{-1}$ (128 cm/h)

The use and optimization of salt in HIC:

1. The commonly used salt is ammonium sulfate, and other salts can also be used.
 $Na_2SO_4 > K_2SO_4 > (NH_4)_2SO_4 > Na_2HPO_4 > NaCl$
2. Temperature has a significant impact on the solubility of salt, and it is generally recommended to operate at 20-25 °C. If it is necessary to operate at low temperatures, the saturation and dissolution of salt must be considered.
3. HIC is generally performed around pH neutrality.

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:

1. 3-5 CV pure water to remove the tightly bound proteins.

2. 2-3 CV 1 M NaOH + 5-10 CV pure water to remove strong hydrophobic proteins and precipitated proteins.
3. 5-10 CV 70% ethanol or 30% isopropanol + 10 CV pure water to remove lipoproteins and lipid substances.

Sterilization

The NW Rose Plus HP series HIC resins 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 HIC resins are 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 HIC resins in nature environment, incineration treatment is recommended to protect the environment.

Order information

Product	Package	Cat. No.
NW Rose Plus Phenyl HP	25ml	60033-545101-2025
	100ml	60033-545101-2100
	500ml	60033-545101-2500
	1 L	60033-545101-1001
	5 L	60033-545101-1005
	10 L	60033-545101-1010
NW Rose Plus Butyl HP	25ml	60033-545901-2025
	100ml	60033-545901-2100
	500ml	60033-545901-2500
	1 L	60033-545901-1001
	5 L	60033-545901-1005
	10 L	60033-545901-1010

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

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