
NW Rose Plus HAM HP

Multimodal Chromatography Resin

Product Instruction

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Version: A0



NW Rose Plus HAM HP

Multimodal chromatography resin

NW Rose Plus HAM HP is a multimodal resin that go by a multimodal of ion exchange, hydrophobic interaction. It is designed for purification of monoclonal antibodies with unique selectivity. This resin is fully compatible with recommended procedures for process hygiene and maintenance, and meet the need for reliable, economic, and safe production of biological material in large-scale operation. NW Rose Plus HAM HP is based on highly cross-linked agarose matrix, which give good physical stability and chromatographic qualities, and enables processing of large volumes, making this media suitable for use at process scale. It is easy to work with, and tolerate working conditions of temperature, pH, and chemical agents typically used in biopharmaceutical production processes. Its packed columns can be sanitized and cleaned-in-place to minimize production losses through column maintenance.

NW Rose Plus HAM HP resin has the following characteristics:

- High dynamic binding capacity at high flow rate
- High physical and chemical stability
- Easy to scale-up from lab to large-scale manufacture
- Wide operational window of conductivity and pH

Table 1. The characteristics of NW Rose Plus HAM HP

Product name	NW Rose Plus HAM HP
Chromatography technique	Multimodal
Matrix	Highly cross-linked, rigid agarose beads
Particle Size	~40 μm
Ionic Capacity	0.8-0.12 mmol Cl ⁻ /ml medium
Maximum Pressure	0.3 MPa
Clean in Place	0.5 M NaOH
Recommended Flow Velocity	60-300 cm/h (XK50/30 Column, height 10 cm)
Maximum flow velocity	600 cm/h (0.1 MPa XK26/30 Column, height 15 cm, 25 °C)
pH stability	2-12 (operational), 2-14 (CIP)
Chemical stability	Stable in commonly used aqueous buffers, 6 M guanidine hydrochloride, 30% isopropyl alcohol, 70% ethanol, etc.
Operational temperature	4-40 °C
Storage	20% ethanol, 4-30 °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 HAM HP, packing factor is 1.1-1.2.

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

Lab-scale small column (I.D. ≤ 50 mm):

Subject the calculated amount of resin slurry in 20 % ethanol to suction filtration, add the loading solution (in 3 times the volume of the slurry) into the filter funnel, gently stir with a plastic spoon and then perform suction filtration again. Repeat the above-mentioned procedure twice so that the 20% ethanol in the medium slurry is completely replaced by the loading solution. Finally, add loading solution to make up the total volume of the slurry to 1.67~2 times the target column volume (Cs: 50%~60%).

Larger scale column (I.D. > 50 mm):

Measure and transfer the calculated amount of resin slurry in 20 % ethanol into a homogenate tank and allow the slurry to

settle for more than 2 hours, then remove the supernatant (which might be a bit turbid). Replenish loading solution in the same volume of the removed supernatant. Gently stir the slurry and then allow it to settle for more than 2 hours, then remove the supernatant. Repeat the above-mentioned procedure twice or thrice so that the ethanol is completely replaced by the loading solution. Finally, add loading solution to make up the total volume of the homogenate to 1.67~2 times the target column volume (Cs: 50%~60%).

Note: During the preparation of the homogenate for column-packing, avoid grinding or rubbing the resin; do not use magnetic stirrer or silicone tube peristaltic pump; if mechanical agitator is used, the paddle should not be too close to the column wall. Furthermore, when it is necessary to re-pack the column, homogenate should be prepared according to the above-mentioned filtration or settlement procedure.

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.

Flow packing of column:

- Thoroughly homogenize the above-mentioned resin slurry and transfer it into the column tube.
- Start the column-packing with the loading solution as mobile phase (the effluent may be a bit turbid at the beginning of the column-packing but will become clear after the flow of 1-2 CV of loading solution).
- Note: The starting flow rate for column packing should not be too high.
- When the column bed height stabilizes, increase the flow rate to maximum target value (Recommended value: 2 times the operation flow rate) or increase the column-packing pressure to maximum target value (Recommended value: 3 bar). Once the pressure becomes constant, allow the

- column-packing to stabilize for another 20 minutes.
- e. Simply adjust the piston to 2-3mm below the gel surface.

Axial compression packing of column (I.D. > 300 mm):

- Clean and degas the column.
- Connect the homogenate tank to the column, remove the surplus solution in the column, lower the piston to 5cm away from the lower frit.
- Open the feeding valve, lift the piston for draw-off. Draw-off rate: 200~300 cm/h. Determine the draw-off amount according to the homogenate concentration, and calculate the piston travel distance. Close the feeding valve, move the piston downwards for column compression at a compression rate of 60~100 cm/h.
- Keep compressing until the complete settlement of resin; stop the piston when the column bed height no longer increases, then record the column bed height. Calculate the final position of the piston with the compression ratio.

Note: Generally, the mass of resin is calculated using a percentage over 120% since the ingress of air during the slurry draw-off is inevitable.

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	1 M NaCl solution
Loading volume	1.0% CV	1.0% CV
Mobile phase	Water	0.4 M NaCl solution
Flow rate	60 cm/h	60 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 (A_s) 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.

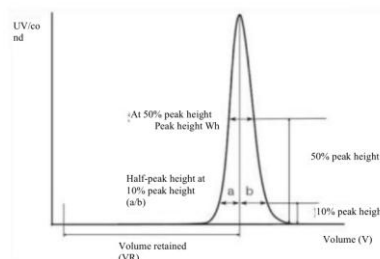


Figure 1. Column efficiency test.

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

Chromatography principle

The NW Rose Plus HAM HP resin is composed of a multimodal mode resin. The multimodal ligand interacts with the target molecule through a complex type of intermolecular force. Typically, this involves ionic interactions, and hydrophobic interactions may also be significant. The strength of these interaction forces depends on the process conditions.

Protein purification at high salt concentrations

Unlike traditional ion exchange chromatography, NW Rose Plus HAM HP, as a multimodal resin, is capable to purify proteins at higher salt concentrations. Details are as follows:

Condition:

Column: 7.7×100 mm, CV 4.65 mL

Buffer A: 25 mM Tris-HCl +0.3 M NaCl, 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 is dissolved in Buffer A and then filtered with a 0.45 μm membrane.

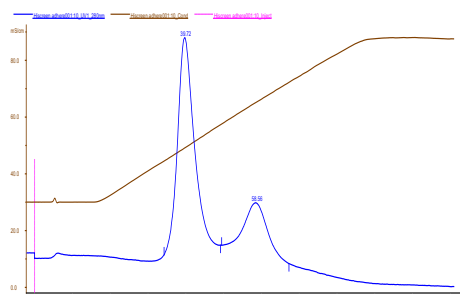


Figure 2. Purification chromatogram of NW Rose Plus HAM HP.

NW Rose Plus HAM HP can adsorb protein molecules under both low salt conditions, similar to traditional AEX resin, and high salt conditions, akin to the purification mode of HIC resin. This provides more options for the purification of proteins.

During the process design and exploration of biopharmaceutical manufacturing, the following separation modes are selected based on the characteristics of the samples and impurities:

1. When the target and impurity molecules have relatively similar properties, the adsorption mode is chosen. The protein to be purified is adsorbed onto the resin, and elution is achieved by increasing the salt concentration or lowering the pH.
2. When the sample has a high purity with low impurity content, the flow-through mode is recommended. The target protein flows through, while the impurities are

adsorbed onto the resin. This increases the binding capacity and improves the efficiency of purification.

Dynamic binding capacity test

Condition:

Column: 0.77×100 mm, CV: 4.65 mL

Buffer A: 50 mM Tris-HCl, pH 8.0

Sample: rIgG dissolved in buffer A

BSA dissolved in buffer A

Retention time: 5 min

Sample	Dynamic binding capacity
rIgG 10%DBC	75.42 mg/ml
BSA 10% DBC	50.23 mg/ml

Remove HCP from mAb by flow-through mode

Condition:

Column: 0.77×100 mm, CV: 4.65 mL

Buffer A: 20 mM NaAc, pH 5.5

Buffer B: 0.1 M HAc, pH 2.8

Sample: The collected solution after Protein A affinity purification

Retention time: 5 min

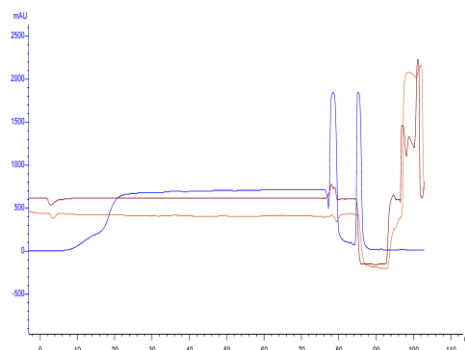


Figure 3. Remove HCP from mAb by flow-through mode with NW Rose Plus HAM HP.

Result:

Resin	NW Rose Plus HAM HP	A company's CA Resin
Sample concentration	5.54 mg/mL	4.3 mg/mL
Loading volume	75 mL	50 mL
HCP in sample	680.63 ppm	680.63 ppm

HCP residue	133.3 ppm	233.4 ppm
DBC _{10%}	89.35 mg/mL	46.23 mg/mL
Yield	88.11%	81.27%

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.
- The above two cleaning solutions can also be combined for cleaning, i.e., using a 30% isopropanol solution containing 1 M NaOH for CIP.

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 HAM HP 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 HAM HP 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 HAM HP resin in nature environment, incineration treatment is recommended to protect the environment.

Order information

Product	Package	Cat. No.
NW Rose Plus HAM HP	25 mL	60053-371500-2025
	100 mL	60053-371500-2100
	500 mL	60053-371500-2500
	1 L	60053-371500-1001
	5 L	60053-371500-1005
	10 L	60053-371500-1010

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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