
NW Rose Plus Plasmid

Plasmid Affinity Chromatography Resin

Product Instruction

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Plasmid affinity chromatography resin

Cell therapy and gene therapy are new therapeutic approaches following monoclonal antibody drug therapy. Plasmid plays an important role as the carrier of cell therapy and gene therapy.

NW Rose Plus Plasmid, a chromatography resin specifically designed for purifying superhelical plasmids, is crafted by immobilizing 2-mercaptopyridine, a sulfur-rich compound, onto high-flow agarose beads. The thiophilic affinity chromatography principle purifies superhelix plasmids by leveraging the interaction between electron donors and acceptors, strengthened by high salt and weakened by low salt.

Table 1. The characteristics of NW Rose Plus Plasmid.

Product name	NW Rose Plus Plasmid
Matrix	Highly cross-linked agarose bead
Ligand	2-mercaptopyridine
Particle Size	50-60 μm
DBC	$\sim 4 \text{ mg} \cdot \text{mL}^{-1}$
Maximum Pressure	0.3 MPa
Recommended Flow rate	200 cm/h (50/30 column ID 50 mm, h=10 cm)
Maximum flow rate	500 cm/h (50/30 column ID 50 mm, h=10 cm)
pH stability	2-12 (working), 2-14 (CIP, short term)
Chemical stability	Common aqueous chromatography solution, including 0.1 M NaOH, 8 M urea, 6 M guanidine hydrochloride, 70% ethanol, 30% isopropanol, 1% SDS, etc.
Storage	20% ethanol, 4-30 $^{\circ}\text{C}$

Prepare the resin slurry

i. Calculate the amount of Resin

$$\text{Column volume} = S \times h,$$

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

For NW Rose Plus Plasmid, packing factor is 1.15.

Notes:

- The buffer or solution used needs to be degassed.
- The slurry ratio of origin package is about 75% generally. If the slurry ratio is 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 $^{\circ}\text{C}$ 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 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.

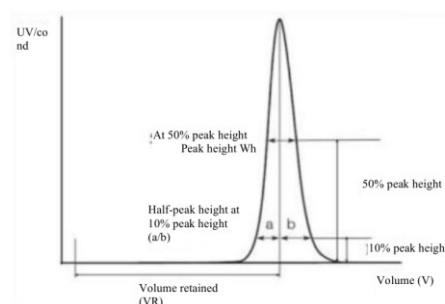


Figure 1. Column efficiency test.

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_e is unsatisfactory.

Chromatography method

NW Rose Plus Plasmid is a thiophilic affinity resin. Its optimized ligand density and superhelical DNA have appropriate affinity. Under high salt, the affinity between superhelical plasmid and ligand is higher than that of open-loop and notched plasmid DNA.

Sample preparation

The key to plasmid purification is sample preparation. According to the above process flow of plasmid purification: after the completion of E. coli fermentation, use centrifuge or 0.2 μ m mesoporous fiber for solid-liquid separation, collect bacteria; wash the bacteria with TE buffer; lyse E. coli with NaOH, neutralize and use centrifugation or mesoporous fiber membrane for solid-liquid separation, remove impurities, replace the buffer; use supernatant as sample of NW Rose Plus Plasmid after purification by NW Rose 6FF.

Buffer preparation

Equilibrium buffer:

2.0M $(\text{NH}_4)_2\text{SO}_4$ + 10mM EDTA + 100mM Tris-HCl, pH 7.5

Elution buffer:

1.7M $(\text{NH}_4)_2\text{SO}_4$ + 0.3M NaCl + 10mM EDTA + 100mM Tris-HCl, pH 7.5

Due to the high concentration of ammonium sulfate, the buffer is prepared and filtered through a 0.45 μ m membrane in order to protect the column.

Plasmid capture steps

- i. Equilibrium: equilibrate 2 CV of column with equilibrium buffer at a flow rate of 100cm/h.
- ii. Sample loading: remove samples collected by the first step of the NW Rose 6FF by the RNA step at a flow rate of 100 cm/h.
- iii. Re-equilibrium buffer: re-equilibrate 4-5 CV using equilibrium buffer at a flow rate of 100 cm/h until the UV260 signal is stable and the conductivity is stable; in this step, open-loop plasmid

DNA is washed out of the column and the UV260 peaks are collected for analysis.

- iv. Elution: elute 3-5 CV with elution buffer at a flow rate of 100 cm/h, and collect UV260 peaks, for analysis and later use. If the sample collected in the first RNA removal step is not processed, steps A-D can be repeated; the sample loading can be repeated 2-5 times depending on the sample conditions. Flowing out of the peak when the sample is loaded indicates the need of regeneration of chromatographic column.
- v. Regeneration: rinse with 3-4 CV with purified water (if water for injection is used in cGMP environment).
- vi. CIP: rinse with 2-3 CV with 0.5 M NaOH at 50 cm/h. (If the cGMP environment requires sterilization and removal of endotoxin, add 2 CV, stop the flow when the pH of the outflowing liquid becomes alkaline, keep it at room temperature for 15-30 min and then continue to rinse to remove endotoxins for best results.)

Equilibrium buffer:

equilibrate 3-5 CV, and equilibrate 5 CV after sample loading.

Sample loading amount:

after the RNA removal step, integrate the plasmid peak area to calculate the plasmid DNA content roughly, and calculate the loading amount of Plasma capture column (2-3 mg/ml packing resin). Too much sample loading results in low resolution of superhelix and open loop.

Sterilization

The resin can be treated with 1 M NaOH for more than 0.5 to 1 hour to achieve sterilization and pyrogen removal.

Storage

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

Order information

Product	Package	Cat. No.
NW Rose Plus Plasmid	25 mL	60043-402401-2025
	100 mL	60043-402401-2100
	500 mL	60043-402401-2500
	1 L	60043-402401-1001
	5 L	60043-402401-1005
	10 L	60043-402401-1010

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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