
NW Super 75

Size Exclusion Chromatography Resin

Product Instruction

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Size exclusion chromatography resin

NW Super 75 is a chromatography resin designed for industrial purification of biomolecules through size exclusion chromatography. This resin is created using a composite matrix that combines the exceptional size exclusion properties of dextran with the physical and chemical stability of cross-linked agarose. It can be widely used in the purification, separation or detection of virus, proteins, nucleic acids, polysaccharides, etc.

NW Super 75 has the following advantages:

- High chemical and physical stability
- High yield and high resolution at higher flow rates
- Easy scale-up for production

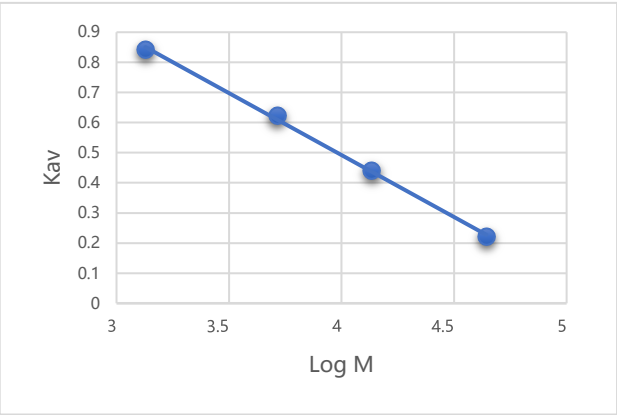


Figure 1. Selectivity curves for NW Super 75.

The separation and purification of NW Super 75 is based on the size exclusion chromatography, a simple and gentle chromatography technique used primarily for high-resolution separations. This separation is based on differences in biomolecular size, allowing samples to be separated into one or more components. It's important to note that natural proteins have spatial structures, so in some cases, the molecular weight of

a protein may not align with its actual structural size. This means that the separation behavior of certain macromolecular proteins with natural structures in gel filtration may be smaller than linear molecules with a slightly smaller molecular weight.

Table 1. Technical parameters of NW Super 75.

Matrix	Cross-linked agarose and dextran
Exclusion limit (Globular molecule)	3 KD-70 KD
Particle size	22-44μm
Recommended flow rate	30-60cm/h
Maximum flow rate	90cm/h
Maximum pressure	0.5MPa
pH stability	3-12 (Operational), 1-14 (CIP)
Chemical stability	Stable in commonly used aqueous buffers. 1M NaOH, 6M guanidine hydrochloride, 70% ethanol, 30% acetonitrile, etc.
Storage	20% ethanol, 4-30°C

Resin slurry preparation

1. Calculate the amount of the resin

Resin required based on the volume of the column:

Column volume = bottom area of the column × column height

Resin amount = Column volume × 1.15 (Packing factor)

Slurry amount = resin amount / resin slurry concentration

Note:

- The buffer or water used needs to be degassed (water for injection does not require degassing).
- Generally, the slurry concentration in the original package is about 75%. If the slurry concentration is not determined, it can be obtained by pouring the slurry into a measuring cylinder and allowing it to settle overnight (typically 12-24 hours) and calculating the natural settling volume of the resin.
- The resin of original package is stored in 20% ethanol. The packing buffer is replaced by negative pressure filtration with sand core funnel for three times (generally, buffer containing 0.15 M NaCl or ultra-pure water is used). Do not mix with excessive force.
- If autoclaving is required, it can be done in high pressure aqueous solution at 121°C for 20 min.
- Do not use a magnetic stirrer during gel stirring, as it may cause the resin particles to break.

2. Determinate the slurry concentration

The available resin is prepared into a 50% to 75% slurry, and the slurry concentration is prepared according to the packing volume and the empty column volume. It is easy to produce air bubbles when the slurry concentration is more than 75%, so it should be stirred well for later use.

Note:

- High temperature (after sterilization) may damage the column during packing.
- Magnetic stirring may cause damage to resin beads.

Column packing

Please refer to the column manual (columns from different companies are used in different ways, and improper operation may cause damage to columns and resin).

- a) The packing buffer is usually water or equilibrium buffer.
- b) Level the column.
- c) Vent the bottom membrane of the column, and retain 0.5-1cm packing buffer at the bottom of the column.
- d) Install a column packer. When the packing height exceeds 2/3 of that of column, a column loader is required.
- e) Pour the stirred slurry into the column slowly at one time. Be careful to avoid air bubbles. Stir it well again with a stirring rod after pouring.
- f) 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.
- g) Insert the column head into the column so that the bottom membrane comes into contact with the liquid surface, and remove air bubbles between the liquid surface and the head membrane.
- h) Connect the system or peristaltic pump and pack the column at the recommended linear flow rate of 500 cm/h. Keep the pressure within 3 bar; if the flow rate of the column packing pump is not enough, the maximum pump flow rate should be used.
- i) With the liquid flow, the resin interface gradually drops. Mark the position of the interface when the resin interface is stable (the interface no longer drops within 30 min).
- j) Stop the pump and remove the column loader (if necessary). Lower the column head to the resin interface (0.5 cm further down from the marked position).
- k) Continue pressing the gel for 20-30 min according to the flow rate before stopping the pump. If the resin interface is stable, it indicates the packing is completed.

Column qualification

1. Column efficiency measurement

Acetone or NaCl can be used as the indicator, and the indicator solution and mobile phase can be prepared according to the following table:

Table 2. Column efficiency test conditions.

Probe	1.0% (v/v) acetone aqueous solution	2 M NaCl (soluble in water)
Probe volume	1.0% column volume	1.0% column volume
Mobile phase	Water	0.4 M NaCl solution
Flow rate	30 cm/h	30 cm/h
Detector	UV 280 nm	Conductivity

2. Calculation of column efficiency

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

$$HETP = L/N$$

$$N = 5.54(VR/Wh)^2$$

Where:

VR = volume retained

Wh = half peak width

L = column height

N = number of theoretical plates

The units of VR and Wh should be consistent;

$$As = b/a$$

Where:

a = first half peak width at 10% peak height;

b = second half peak width at 10% peak height.

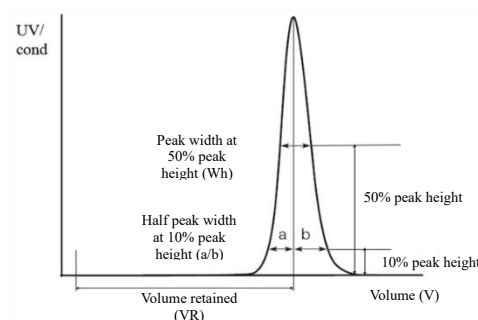


Figure 2. Column efficiency test

3. Result evaluation

The column efficiency is judged to be qualified if the value of HETP calculated by the above formula is less than three times the average particle diameter of the resin and the asymmetry factor is 0.8~1.8. For unsatisfactory column efficiency, it is necessary to analyze the reasons and re-pack the column.

For different applications, different column efficiencies are required.

High column efficiency is required when high resolution is required for purification and separation; HETP is at 2-4 times the average particle size of the medium. The HETP value of NW Super 75 is generally >8000.

Chromatography method

Separation principle of NW Super 75 is based on size exclusion which separates biomolecules according to their size (generally according to their molecular weight) by passing them through a column.

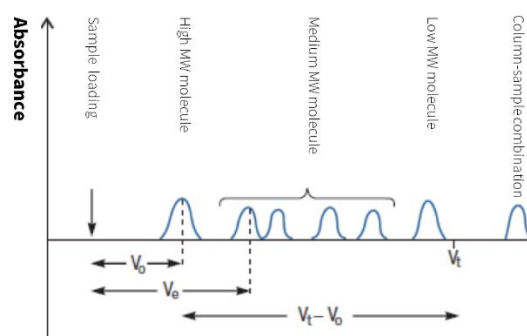


Figure 3. The principle of size exclusion chromatography.

Recommended column

The column used is determined according to the scale of purification and separation:

Laboratory scale

For laboratory scale purification, it is generally determined based on the required amounts of samples. The sample loading amount of size exclusion chromatography medium is generally 1-5% of the column bed volume; The separation result depends on the loading volume. It is good when the volume is lower than 1% CV and decreases when it is over 5% CV. It is recommended to choose a 60-100 cm height column.

Production scale

Columns for production scale should also be selected according to the sample volume. The height of the column should be the same as the column height in the laboratory experiment. The diameter of column is scaled up in proportion to the sample loading amount in the laboratory.

The column height should preferably be consistent during production scale-up. The diameter of the column: Generally, for columns with a diameter of <300 mm, one column is selected for separation. For columns with a diameter greater than 300 mm, two or more columns are generally selected in series.

Recommended buffers

The selection of buffers for the separation and purification process is generally based on the following principles:

- Biomolecule-stabilized buffer pH, salt concentration. The use of unstable buffer may lead to sample aggregation and precipitation in the separation and purification process, which will affect the column pressure and sample yield.
- To reduce non-specific adsorption (improve yield), 0.15 M salt (such as NaCl) is generally added to the buffer.
- Organic reagents can be added, but at high concentrations, the resin may shrink the beads. Therefore, it is necessary to pay attention to the tolerance of resin before adding.

- Appropriate addition of some surfactants may improve virus purity during virus purification. Pay attention to the effect of viscosity when using organic reagents (e.g. glycerol), because the buffer with high viscosity may affect the separation and purification effect. The buffer of the sample also affects the separation and purification effect.

General steps for purification with NW Super 75

- Equilibration: equilibrate the column with 1-2 CV of equilibrium buffer. The sample can be loaded when UV, conductivity and pH curves are stabilized.
- Loading: generally, a 1-5% CV is recommended.
- Regeneration: 1-1.5 CV equilibrium buffer is generally used.
- Samples can be continuously loaded according to test requirements.
- At the end of the test, 1-1.5 CV of preservation solution (degassed 20% ethanol) is used for preservation. Cleaning should be done in accordance with the CIP procedure, if required.

Storage of the resin and packed column

Long-term storage:

It is recommended to remove the resin from the column and store it at room temperature in 20% ethanol (0.01 M NaOH) under sealed conditions.

Short-term storage:

It can be stored in the column at room temperature in 20% ethanol or 0.01 M NaOH.

Chromatography application:

1. High-resolution purification:

- NW Super series resin is generally used to separate 3KD-70KD proteins.
- Generally, a longer column length of more than 60 cm is chosen.
- The loading volume of sample size is 1-5% CV.
- Flow rate has a significant impact on resolution, and generally a lower flow velocity is used.

- b) Adding 150 mM NaCl is beneficial to reducing non-specific adsorption.

Sample:

Blue dextran 2000 (3mg/ml), MW 2000KDa.

Ovalbumin (50mg/ml), MW 45 KDa.

Ribonuclease A (100mg/ml), MW 13.7KDa.

VB12 (6mg/ml), MW 1.4KDa.

Loading volume: 100 μ L

Flow rate: 0.5 mL/min

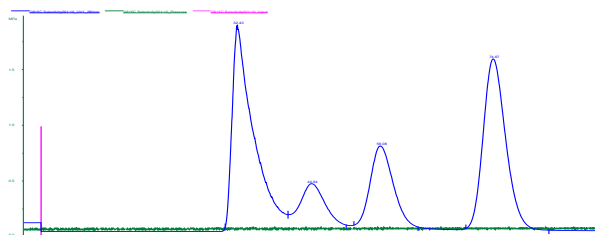


Figure 4. Purification chromatogram of NW Super 75.

Peak 1: Blue dextran 2000.

Peak 2: Ovalbumin.

Peak 3: Ribonuclease A.

Peak 4: VB12.

Clean in place

A decrease of column efficiency maybe observed when the NW Super 75 resin is used after a period of use. The following process can be used for cleaning and regeneration.

- Wash with 2 CV of pure water
- Rinse with 1 CV of 1 M NaCl
- Rinse with 1 CV of 0.2 M NaOH
- Wash with 4 CV of pure water

Sterilization

NW Super 75 resin in aqueous solution can be sterilized by treatment with 0.5 M NaOH for 30 min or 121°C for 30 min at a neutral pH.

Long-term storage

The resin is stored in 20% ethanol (or 0.01M NaOH) in a cool, dry, airtight place. It is recommended that freshly prepared 20% ethanol (or 0.01M NaOH) be replaced once every 3 months and stored at 4-30°C.

Destruction and recovery

Since the resin is hard to degrade in nature, incineration is recommended for environmental purposes.

Trouble shooting

If you have any problems when using NW Super 75 resin, please refer to the table below for solution or contact us.

Q1	After the resin is stirred to slurry, will there be some tiny white bubbles floating?
	This phenomenon is generally due to the use of a period of time, or the use of a magnetic stirrer during resin mixing or peristaltic pump during resin transfer, causing the beads to break. It may be because the resin has not been used for a long time, resulting in excessive resin settlement and excessive stirring. Or it is because the dehydration time of the resin is long when the buffer is replaced by suction filtration, resulting in the dehydration of the resin internal pores, and after adding new liquid, the resin hole is not completely saturated with liquid. The foam will disappear after stirring slowly and settling for 20-30 min.
Q2	Is it possible to continue using the resin beads if they are broken (with fragments)?
	If the fragment content is less than 1-3%, observe with a microscope. It can continue to be used for applications such as desalination. If the fragment content cannot be determined, a new resin is required.
Q3	Why do my samples always appear after 1 CV?
	Because the sample is adsorbed on the resin, and the behavior of the sample does not follow the principle of size exclusion. For this phenomenon, it is necessary to check whether the test purpose is achieved, and if the expectation is met, it can be separated according to this condition. Otherwise, the salt concentration of elution buffer should be increased. In a typical purification, the buffer concentration is in the range of 20-50 mM.
Q4	Is it possible to repeat the loading and how many times can it be repeated?
	It is required to detect whether the purification purpose is achieved and whether the resin is contaminated. The samples are generally clear, free of particles and precipitation, and the samples after separation are equally clear. Such samples can be repeated 10-15 times. If the sample is an emulsion, the column pressure may increase during the purification process. In this case, the column pressure should be observed, and the column will compress if it exceeds 1 bar.
Q5	Why is the diluted sample concentration low?
	The size exclusion is a dilution technique, which is a disadvantage. It can be partially solved by increasing the concentration or volume of the loaded sample. Pay attention to the viscosity of the sample when concentrating it.

Order Information

Product	Pack Size	Cat. No.
NW Super 75	150 mL	60013-025000-2150
	500 mL	60013-025000-2500
	1 L	60013-025000-1001
	5 L	60013-025000-1005
	10 L	60013-025000-1010

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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