
NM90Agarose Series

Multimodal Chromatography Resins

Product Instruction Manual

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Multimodal chromatography resin is the most commonly used method for separation and purification of macromolecules by their differences in surface charge (type, quantity and distribution of charge). NanoMicro NM90Agarose series Multimodal chromatography resins have selectivity profiles different from those of conventional IEC resin because the multimodal chromatography resins are highly cross-linked agarose microspheres bonded with ionic groups of diverse functions.

The technical parameters of NM90Agarose series are as shown in Table 1.

Table 1. Technical parameters of NM90Agarose

Product	NM90Agarose HCM	NM90Agarose HAM
Separation principle	Multimodal weak cation exchange	Multimodal strong anion exchange
Matrix	Agarose	Agarose
Particle size	90 μm	90 μm
Ligand	Phenyl group/carboxyl group	Phenyl group/quaternary ammonium salt
Dynamic binding capacity	~ 50 mg·mL ⁻¹ (Lys)	~ 30 mg·mL ⁻¹ (BSA)
Maximum pressure resistance	0.3 MPa	0.3 MPa
Clean-in-place (CIP)	1 M NaOH	1 M NaOH
Recommended flow rate	150-250 cm/h	150-250 cm/h
pH stability	2~12	2~12
Chemical stability	All commonly used buffers, 1 M acetic acid, 1 M sodium hydroxide, 1 M hydrochloric acid, 70% ethanol, 30% isopropanol, 30% acetonitrile, 1% SDS, 6M guanidine hydrochloride, 8M urea and other commonly used organic solvents; contact with strong oxidizers should be avoided.	All commonly used buffers, 1 M acetic acid, 1 M sodium hydroxide, 1 M hydrochloric acid, 70% ethanol, 30% isopropanol, 30% acetonitrile, 1% SDS, 6M guanidine hydrochloride, 8M urea and other commonly used organic solvents; contact with strong oxidizers should be avoided.
Temperature	4-30 °C	4-30 °C

High flow rate, low back pressure

Chromatographic column packing

NM90Agarose products are stored in 20% ethanol. 1.5 Kg of the product homogenate corresponds to 1L of resin volume. Recommended mobile phase for column-packing with the homogenate: 0.1 M NaCl or purified water. Recommended pack factor: 1.15-1.2.

NM90Agarose products are made of a “hard agarose gel” of high mechanical strength. They can be packed into either low-pressure columns or mid-/high-pressure columns. Recommended column frit pore size: 10-23 μm. Column-packing procedures:

1) Calculation of the required mass of the resin homogenate

Required mass of resin homogenate (Kg) = Target column volume (L) ×1.5 Kg/L ×1.15.

Example: The mass of homogenate required for packing a 25L column (40 cm I.D×20 cm) is: 25 L×1.5 Kg/L ×1.15=43.125 Kg. For guaranteed measurement accuracy of the resin volume, the concentration homogeneity of the homogenate must be verified before weighing (the homogenate can be stirred slowly with a plastic spoon or a mechanical agitator at <50rpm to achieve the desired homogeneity and then allowed to stand for NMT 10 minutes).

2) Preparation of the homogenate for column-packing

Prior to the start of the packing, prepare sufficient amount of 0.1 M NaCl (or purified water) for use as loading solution for column-packing. The loading solution referred to hereinafter is 0.1 M NaCl (or purified water).

Lab-scale small column (I.D.≤50 mm):

Subject the calculated amount of resin suspension in 20 % ethanol to suction filtration, add the loading solution (in 3 times the volume of the suspension) 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 resin suspension 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 (Homogenate concentration: 50%~60%).

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

Measure and transfer the calculated amount of resin suspension in 20 % ethanol into a homogenate tank and allow the suspension 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 homogenate 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 (Homogenate concentration: 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.

3) Packing

● Flow packing of column:

3-1) Thoroughly homogenize the above-mentioned resin suspension and transfer it into the column tube.

3-2) 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.

3-3) 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: NMT 1.0 MPa). Once the pressure becomes constant, allow the column-packing to stabilize for another 20 minutes.

3-4) Simply adjust the piston to 2-3mm below the gel surface.

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

3-1) Clean and degas the column.

3-2) Connect the homogenate tank to the column, remove the surplus solution in the column, lower the piston to 5cm away from the lower frit.

3-3) 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 filler; 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 a compression ratio of 1.05.

Note: Generally the mass of filler is calculated using a percentage >120% since the ingress of air during the homogenate draw-off is inevitable.

Column Efficiency Evaluation

After the chromatographic column is packed, equilibrate the column with 2 CV 0.5 M NaCl solution and conduct column efficiency test at a flow rate of 100 cm/h. For the specific test parameters, refer to Table 3:

Table 3. Column efficiency test method for columns packed with NM90Agarose IEC resin

Sample	2 M NaCl
Sample injection	1~5 % column volume
Eluent	0.5 M NaCl
Linear flow rate	100cm/h
Detection	2 M NaCl loading: Conductivity meter
Recommended qualification specifications	As: 0.8-1.5; Plates (N/m): >3000

How to use

- 1) Equilibration** Equilibrate the column with more than 5 CV of equilibration buffer (e.g. Buffer A, 20 mM PB, pH7.0), until the conductivity and pH of the effluent remain constant (consistent with the equilibration buffer). The specific buffer system should be screened and optimized according to the stability and isoelectric point of the target protein and the type of ion exchange resin.
- 2) Loading** Dissolve the solid sample in the equilibration buffer; Low concentration sample solution can be concentrated in advance; and high concentration sample solution can be diluted with equilibrium buffer. Carry out the centrifugation or membrane filtration for the sample to prevent clogging of the column. Calculate the loading volume based on the resin's loading capacity and the target protein content in the sample and make sure that the loading buffer is consistent with the equilibration buffer before loading.
- 3) Elution** After sample loading, continue to wash the sample with equilibrium buffer until the baseline is stable. Elute the sample adsorbed on the chromatographic resin sequentially by changing the salt concentration or the pH of the mobile phase.
- 4) Clean in Place (CIP)** Regular CIP prevents the accumulation of precipitated protein contaminants in the resin bed and helps to maintain the loading capacity, separation efficiency, flow characteristics, etc. of the chromatography resin. Prepare a specific CIP method for each process based on the type of contaminant. The frequency of CIP depends on the nature of the starting feed liquid and process conditions. The recommended CIP method is as follows.

Wash sequentially with 5 CV of 1-2 M NaCl, 5 CV of 0.5-1 M NaOH, 3 CV of water and 5 CV of 1 M acetic acid.

Storage

The used chromatography resin or prepacked column after complete regeneration should be sealed and stored in 20% ethanol. The recommended storage temperature is 4 ~ 25 °C.

For unused chromatography resin or prepacked column, it is recommended to replace the 20% ethanol once every 3 months to prevent ethanol volatilization and growth of microorganisms.

Trouble Shooting Guide

If you have any problems with the NM90Agarose multimodal chromatography products, refer to the table below for solution or contact us.

Problem	Possible cause	Recommended solution
Increase in column pressure	Too high flow rate	Reduce the flow rate.
	Valve between pump and collector not opened	Open the valve.
	On-line filter clogged of instrument	Remove impurities and wash or replace the filter, and filter the sample and buffer through 0.45 µm or 0.2 µm membrane prior to use
	Sample precipitation on the column	Perform CIP (refer to regeneration conditions)
	Dirty sample or substances with a strong adsorption remaining on the resin	Perform CIP (refer to regeneration conditions)
	Column bed compressed	Repack the column.
	Long service time of the column	Replace the column or the chromatography filler
Insufficient sample adsorption	Too high ion strength in the sample solution	Reduce the ion strength in the sample solution by dilution or desalination, etc.
	Inappropriate pH of sample	Adjust pH to increase binding strength.
Failure to elute sample during the elution process	Too weak elution strength of the eluent	Replace the eluent with another one with stronger elution strength
	Inappropriate pH of eluent	Adjust pH of the eluent
	Residual highly hydrophobic impurities in the column	Perform CIP (refer to regeneration conditions)
Reduced resolution	Inappropriate elution conditions, e.g. too steep gradient or too high flow rate	Change the elution conditions to a slower gradient or isocratic elution and to reduce the flow rate
	Column packed improperly	Repack the column
	Dead volume left at the top or behind the column	Pack more resin or reduce the void behind the column
	Overload	Clean and re-equilibrate the column to reduce the load volume
Decreased binding of the sample after repeated injections	Impurities in the sample bound to the resin, which interfere with the normal binding	Perform CIP (refer to regeneration conditions)
Cracks in the column bed in use	Insufficient elimination of swelling	Mix well and fully equilibrate with 0.5 M NaCl
	Bubbles in the solution	Depressurize and filter to remove the air
	Outside air entered the system	Add more buffer to remove the air completely
	The column was not properly packed	Repack
Baseline drift	Chromatography column not well-equilibrated	Increase the equilibration time.
	Different absorption coefficients for eluents A and B at the same UV wavelength	Detect at different wavelengths or run a blank gradient.
Presence of unknown impurity peaks	Incomplete elution of the previous sample	Regenerate the column
	Impure eluent	Run a blank control gradient or use a high-purity chromatography grade reagent
	Trace ionic impurities bound to the column and concentrated during equilibration and loading, with peaks appearing during elution	Clean the column
Too long equilibration time	Direct re-equilibration after CIP	Equilibrate with 2 CV of buffer B (containing 1 M NaCl) and with buffer A

Order Information

Product	Size	Catalog #
NM90Agarose HCM	30 mL	04091-090001-2030
	50 mL	04091-090001-2050
	100 mL	04091-090001-2100
	300 mL	04091-090001-2300
	500 mL	04091-090001-2500
	1 L	04091-090001-1001
	5 L	04091-090001-1005
	10 L	04091-090001-1010
	50 L	04091-090001-1050
	100 L	04091-090001-1100
NM90Agarose HAM	30 mL	04092-090001-2030
	50 mL	04092-090001-2050
	100 mL	04092-090001-2100
	300 mL	04092-090001-2300
	500 mL	04092-090001-2500
	1 L	04092-090001-1001
	5 L	04092-090001-1005
	10 L	04092-090001-1010
	50 L	04092-090001-1050
	100 L	04092-090001-1100

Note: Chromatographic preppacked columns of 7.7 mm × 22 mm, 16 mm × 25 mm, 7.7 mm × 100 mm are available. Other sizes and custom configurations are also available. Please contact us for further details.

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