NanoSP-30L

Strong Cation Exchange Chromatography Resin

PRODUCT INSTRUCTION MANUAL

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Ion exchange is the most commonly used method for the separation and purification of biomacromolecules, which is based on the difference of molecular surface charge (type, number and distribution). The ion-exchange chromatography resin provided by NanoMicro is a polymer (polyacrylate or polystyrene-divinylbenzene copolymer) as the matrix, which is then bonded to the ion-exchange group after surface hydrophilic modification and has good biocompatibility and physical and chemical stability for bioactive macromolecules, which greatly improves the purification efficiency. NanoMicro provides the Nano series of ion exchange products, suitable for the purity of antibodies, proteins, peptides, nucleic acids and small molecule drugs.

Table 1. Specifications of NanoSP-30L

Product	NanoSP-30L
Separation principle	Strong cation exchange chromatography
Matrix	Monodisperse polystyrene/divinylbenzene (PS/DVB)
Particle size	~ 30 μm
Ligand	$—(CH_2)_3SO_3^-$
Ligand density	~0.19 meq/mL
Dynamic binding capacity	~60 mg/mL（Lys）
Maximum pressure	2.0 MPa
Cleaning-in-place	1 M NaOH
Recommended flow velocity	250-1000 cm/h
pH stability	2~12
Chemical stability	All common buffers, 1 M acetic acid, 1 M sodium hydroxide, 1 M hydrochloric acid, 100% ethanol, 100% Methanol, 100% n-propanol alcohol, 100% isopropyl alcohol, 100% DMSO, 100% DMF, 100% acetonitrile, 1% SDS, 6M guanidine hydrochloride, 8 M urea and other common organic solvents and salt solutions; Avoid exposure to strong oxidants. Do not be exposed to strong oxidants (such as hypochlorite), oxidizing acids (such as nitric acid), strong reducing agents (such as sulfites), acetone, or benzyl alcohol.
Operational temperature	4-30 °C
Storage	20% Ethanol, 4-25°C

Chromatographic column packing

Nano series products are stored in 20% ethanol, 1.5 Kg slurry corresponds to 1 L resin volume, it is recommended that the mobile phase of the column slurry be 0.1M NaCl or pure water, and the recommended pack factor is 1.05-1.1. Nano series products are high mechanical strength "hard gel", can be installed both low pressure column and high pressure column, the more suitable column sieve plate model is 3-5 μm. Packing column are as follows:

1) Determination of Slurry Percentage and Resin Volume for Column Packing

Mass of slurry required (Kg) = target column volume (L) × 1.5Kg /L ×1.05. For example, the mass of slurry required to fill a 25 L chromatographic column of 40 cm I.D×20 cm is 25 L×1.5 Kg/L ×1.05=39.375 Kg. In order to ensure the accuracy of the volume measurement of the resin, before weighing, it must be ensured that the slurry concentration is uniform (you can use a plastic spoon to slowly mix the slurry or use a mechanical mixing of less than 50 rpm, and the standing time of the slurry should not exceed 10 minutes).

2) Preparation of the Slurry 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 medium 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 medium suspension 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 (Slurry concentration: 50%~60%).

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

Measure and transfer the calculated amount of medium suspension in 20 % ethanol into a slurry 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 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 slurry to 1.67~2 times the target column volume (slurry concentration: 50%~60%).

Note: During the preparation of the slurry 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, slurry should be prepared according to the above-mentioned filtration or settlement procedure.

3) Packing

- Flow packing of column:

  3-1) Thoroughly slurry the above-mentioned medium 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 2.0 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 slurry 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 slurry 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 ingression of air during the slurry 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 60 cm/h. For the specific test parameters, refer to Table 2:

Table 2. Column efficiency test method for columns packed with chromatography

Sample	1 M NaCl
Sample injection	1~2 % column volume
Eluent	0.5 M NaCl
Linear flow rate	60 cm/h
Detection	Conductivity meter
Recommended qualification	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 PBS, pH=7.0), 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 medium’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 medium 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 medium 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. Recommended CIP Cleaning Procedure for Cation and Weak Cation Exchangers: sequentially use 5 CV 0.5~1.0 M NaOH, 3 CV water, 5 CV 1.0~2.0 M NaCl, then wash with water until conductivity is stable, and store in 20% ethanol. Recommended CIP Cleaning Procedure for Anion and Weak Anion Exchangers: sequentially use 5 CV 0.5~1.0 M NaOH, 3 CV water, 5 CV 1.0~2.0 M NaCl, 3 CV water, 5 CV buffer (pH 4~7, e.g., 20 mM PBS, pH 6.8), then wash with water until conductivity is stable, and store in 20% ethanol.

Storage

The used chromatography Resin or prepacked column should be sealed and stored in 20% ethanol after completely generation. 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

Problems that occasionally occur during NanoSP-30L chromatography and suggested solutions are listed below. We also have an experienced and dedicated application team to provide full technical support, from method development, scale-up design, to commercial production.

Problem	Possible Cause	Recommended Solution
Increase of 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 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 resin
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 shallower gradient or isocratic elution and to reduced 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 loading 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 #
NanoSP-30L	10 mL	04042-030100-2010
	30 mL	04042-030100-2030
	50 mL	04042-030100-2050
	100 mL	04042-030100-2100
	300 mL	04042-030100-2300
	500 mL	04042-030100-2500
	1 L	04042-030100-1001
	5 L	04042-030100-1005
	10 L	04042-030100-1010
	50 L	04042-030100-1050
	100 L	04042-030100-1100

Note: We can also provide you with a preparation column with an inner diameter of 10/21.2/30/50 mm and a length of 100/150/250 mm. Please contact us for further details.

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