
NanoGel-50SP

Ion Exchange Chromatography Resin

Product Instruction Manual

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NanoMicro has developed the new generation of high-performance ion exchange chromatography Resin NanoGel-50SP through the application of a precision microsphere manufacturing technology to meet the separation and purification requirements of macromolecules (e.g., mAbs and viral vaccines) in order to improve the efficiency and lower the cost of large-scale manufacturing of biopharmaceuticals. These series products use the polystyrene/divinylbenzene (PS-DVB) microspheres that have outstanding mechanical strength, excellent chemical stability, uniform particle size, and ultra-large open pore structure as matrix and lower the non-specific adsorption by the application of hydrophilic modification of surface and a unique surface functionalization technology. Therefore, they have the merits of high flow rate, high resolution, high loading capacity and low back pressure in IEC.

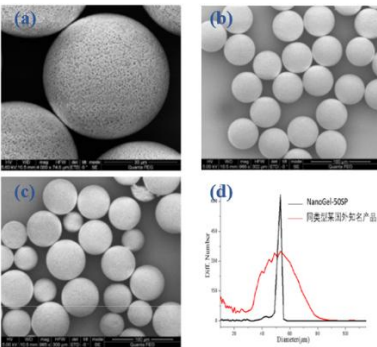


Figure 1. Comparison of the scanning electron microscope of NanoGel-50SP from Nanomicro Technology and a certain internationally renowned filler P-50XS, as well as the measurement results of Coulter particle size.

Note: the figure a, b is NanoGel - 50 sp, figure c is P - 50 xs, figure d is Kurt particle size measurement for comparison of two kinds of medium. Figure 1 is ion exchange chromatography medium NanoGel - 50 series and monodispersity of P - 50 xs contrast figure, can be seen from the diagram, NanoGel - 50 series chromatography medium microspheres particle size uniform, while P - 50 xs present a microsphere size distribution width and contain some reunion (agglomeration) ingredients.

Table 1. Classification and uses of ion exchange functional groups

Project	NanoGel-50SP
Separation principle	SP
Matrix	PS/DVB
Particle size	~50 μm
Ligand	-CH ₂ CH ₂ CH ₂ SO ₃ ⁻
Ligand density	~0.16meq·mL ⁻¹
Dynamic loading capacity	~100 mg/mL (hlgG)
Maximum pressure resistance	2.0 MPa
CIP	1 M NaOH
Recommended	300-1200 cm/h

flow rate	
pH stability	1~14
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. 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.

High flow rate, low back pressure

High flow rate is good for achieving shorter chromatographic purification cycle and higher production efficiency. NanoGel-50 product series for their perfect monodisperse microsphere shape have outstanding physical properties and the merits of low back pressure and high flow rate in addition to the high mechanical strength of conventional PS-DVB hard agarose gel. The pressure-flow rate profiles presented in the following figure indicate the good linear relationship between pressure-flow rate of NanoGel-50 ion exchange chromatography Resin even in high flow rate range.

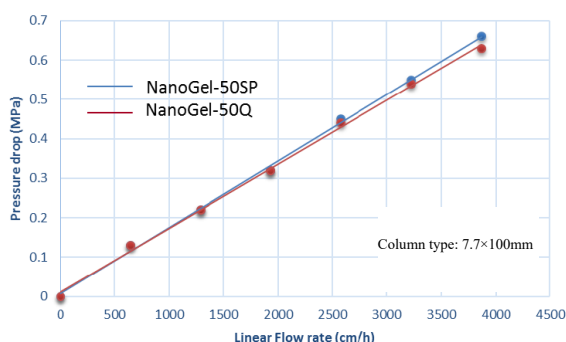


Figure 2. Pressure-flow rate profiles of NanoGel-50SP and NanoGel-50Q

High resolution

NanoGel series products show excellent resolution by virtue of the physical properties of monodisperse Resin and the highly-efficient mass transfer feature by ultra-large open pores. Example: Compared with a foreign well-known similar products that is famous for good resolution, NanoGel -50SP has better resolution in standard protein separation test (see Figure 3).

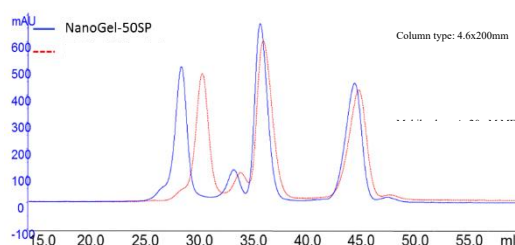


Figure 3. Performance in standard protein separation test - NanoGel-50SP vs. P-50XS

Dynamic loading capacity remains stable in a wide range of salt concentration

NanoGel series products have impressive salt-tolerance. Compared with similar products, they have more stable dynamic loading capacity under different salt concentration conditions (Figure 6). This feature enables them to adsorb proteins of interest or remove impurities in a wide range of conductivity process conditions. Therefore, they can improve the flexibility in purification process development and production efficiency.

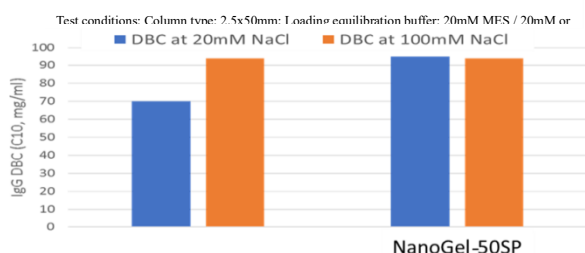
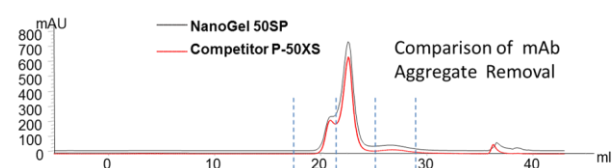


Figure 4. Dynamic loading capacity of NanoGel-50SP vs. P-50XS under different salt concentrations

For their high flow rate and high dynamic loading capacity features, NanoGel-50SP and NanoGel-50Q are very suitable for rapid high-performance chromatography applications. Moreover, their excellent resolution renders them better impurity removal capability than conventional ion exchange chromatography Resin in fine purification stage. For their ultra-large open pore structure, they are more advantageous when used for the chromatographic purification of macromolecules (including mAbs, viruses/vaccines, and macromolecular recombinant proteins, etc.). As shown in Figure7, NanoGel-50SP has excellent performance in the removal of polymers from monoclonal antibodies. Their polymer removal results and recoveries were comparable to those foreign well-known similar products.



	Samples	Purity (%)	Aggregate (%)	Yield of high purity (%)
Feed (mAb eluted from Protein A resin)	loading	98.36	1.64	
NanoGel 50SP	E-1	99.8	0.19	87.86
	E-2	99.02	0.93	
	E-3	97.89	2.11	
Competitor P-50XS	E-1	99.88	0.12	81.41
	E-2	99.18	0.82	
	E-3	97.52	2.4	

Figure5. Assessment of the application for removing polymers from monoclonal antibodies

Column Packing

NanoGel-50SP product is stored in 20% ethanol. 1.5 Kg of the product homogenate corresponds to 1L of medium volume. Recommended mobile phase for column-packing with the homogenate: 0.1 M NaCl or purified water. Recommended pack factor: 1.05-1.15.

NanoGel-50SP product is 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 pore size of column sieving plate: is10-23 μ m. Column-packing procedures:

1) Calculation of the required mass of the medium homogenate

Required mass of medium homogenate (Kg) = Target column volume (L) $\times$ 1.5 Kg/L $\times$ 1.05.

Example: The mass of homogenate required for packing a 25L column (40 cm I.D $\times$ 20 cm) is: 25 L $\times$ 1.5 Kg/L $\times$ 1.05=39.375 Kg. For guaranteed measurement accuracy of the medium 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. $\leq$ 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 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 medium 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 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 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 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.

Packing Efficiency Assessment

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 2. Packing efficiency test method for columns packed with NanoGel-50SP ion exchange chromatography Resin

Sample	2 M NaCl
Sample injection	1~5 % column volume
Eluent	0.5 M NaCl
Linear flow rate	100 cm/h
Detection	conductivity curve if 2M NaCl is used as sample
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, 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 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.

Wash sequentially with 5 CV of 0.5-1.0 M NaOH, 3 CV of water, 5 CV of 1-2 M NaCl, and finally with water until the conductivity stabilizes, then 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

If you have any problems with the NanoGel-50SP of ion exchange 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
	Inappropriate pH of eluent	Adjust pH of the eluent
	Residual highly hydrophobic impurities in the	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	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	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

Ordering Information

Format	Size	Catalog
NanoGel-50SP	30 mL	04062-050200-2030
	50 mL	04062-050200-2050
	100 mL	04062-050200-2100
	300 mL	04062-050200-2300
	500 mL	04062-050200-2500
	1 L	04062-050200-1001
	5 L	04062-050200-1005
	10 L	04062-050200-1010

	50 L	04062-050200-1050
	100 L	04062-050200-1100

We also offer 7.7 mm × 22 mm 、 16 mm × 25 mm 、 7.7 mm × 100 mm preppacked columns. More requirements about products or customized needs, please

NanoMicro Technologies, Inc.

National hotline: 400-828-1622

Email: info@nanomicro.com

(The company's products are only for scientific research
or industrial use)

Chinese website: www.nanomicro.com

English website: en.nanomicro.com

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Edition I

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