NanoGel-50SP and NanoGel-50Q

-Monodisperse Bioprocess Ion-Exchange Resins of High Performance

Product Description

NanoGel-50SP and NanoGel-50Q are, respectively, strong cation (CEX) and strong anion exchange (AEX) chromatography resins made with NanoMicro's precision monodisperse microsphere technology. Their supports are rigid poly(styrene-divinylbenzene) (PS-DVB) beads of uniformed particle size and large open pore structure (Figure 1). Through polyhydroxyl surface modification and special surface extender chemistry, these NanoGel ion exchange resins are enabled with low non-specific binding property. They are intended for general use in large-scale bioprocess purification of biomolecules, including large biologics such as viral particles and vaccines. The basic characteristics of NanoGel-50SP and NanoGel-50Q are summarized in Table 1. Figure 1 displays the monodisperse nature of these precision spherical polymer beads.

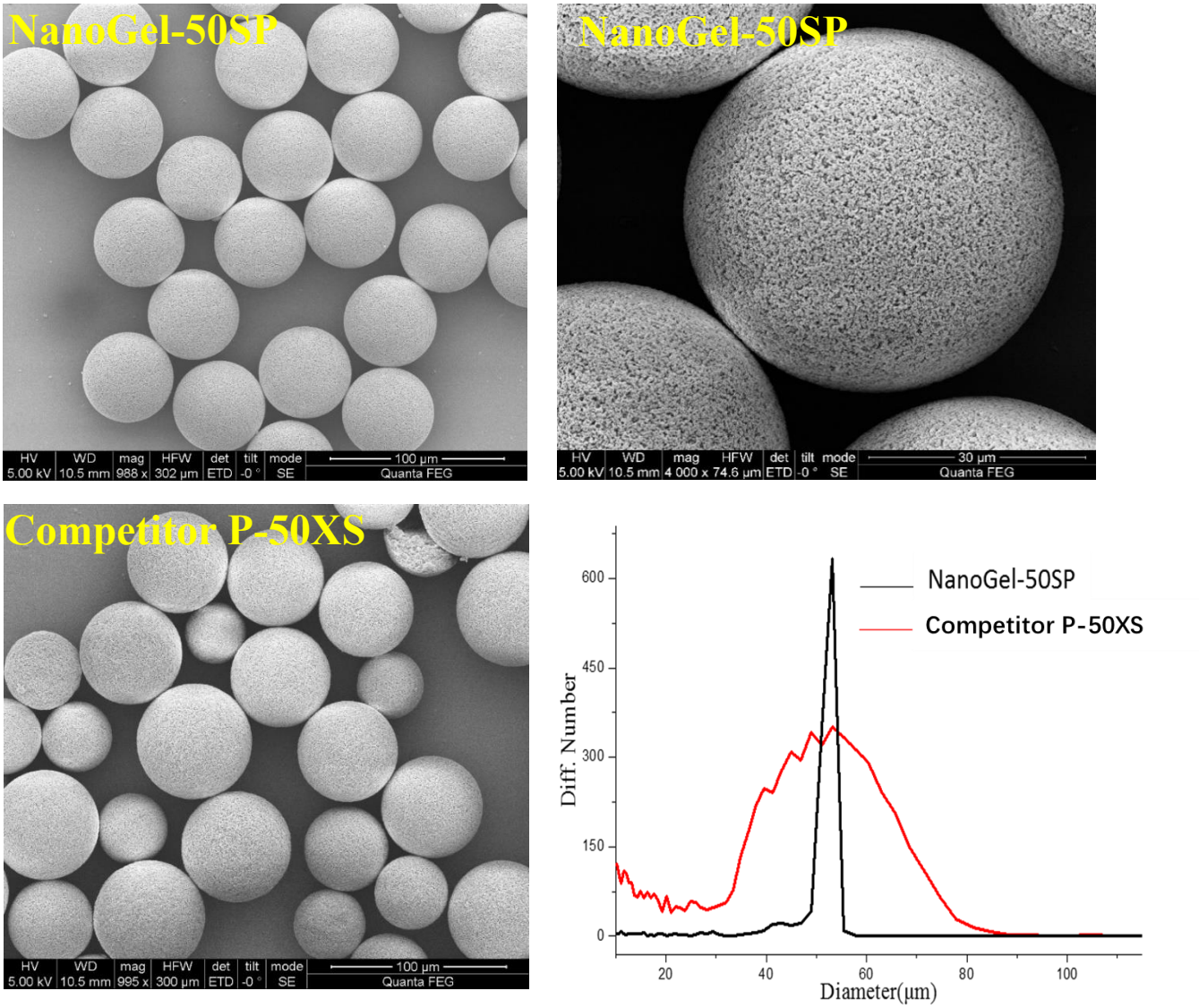


Figure 1. SEM images and Coulter particle size analysis (bottom right) of NanoGel-50SP and competitor P-50XS, a top-ranked CEX analogous in the market.

Table 1. Characteristics of NanoGel-50SP and NanoGel-50Q

	NanoGel-50SP	NanoGel-50Q
Support Matrix	Monodisperse Poly(styrene-divinylbenzene) Bead	
Particle Size	50 µm	50 µm
Ion Exchange Type	Strong Cation	Strong Anion
Charged Group	$-CH_2CH_2CH_2SO_3^-$	$-N^+(CH_3)_3$
Total Ionic Capacity	~0.16 mmol/ml	~0.20 mmol/ml
Dynamic Binding Capacity	>100mg/ml (hIgG)	>100mg/ml (BSA)
Operating Pressure	< 20 bar (2 MPa)	< 20 bar (2 MPa)
pH Range	1 to 14	1 to 14
Operating Temperature	4 to 30 °C	4 to 30 °C
Chemical Stability	All common aqueous buffers, 1M acetic acid, 1M NaOH, 1M HCl, ethanol, IPA and other common organic solvents; Do not expose to stronger oxidizers	

Product Main Features

- **High flow and low backpressure**

High flow velocities allow increased productivity of large-scale bioprocessing operations and processing of larger volumes in shorter working shift. Lower cycle times also reduce exposure of the target protein to proteases. NanoGel resins are based on highly rigid and monodisperse PS-DVB microspheres, and hence enable very high flow rate operation at low backpressure. Figure 2 displays the typical pressure-flow rate curves of NanoGel-50SP and NanoGel-50Q resins.

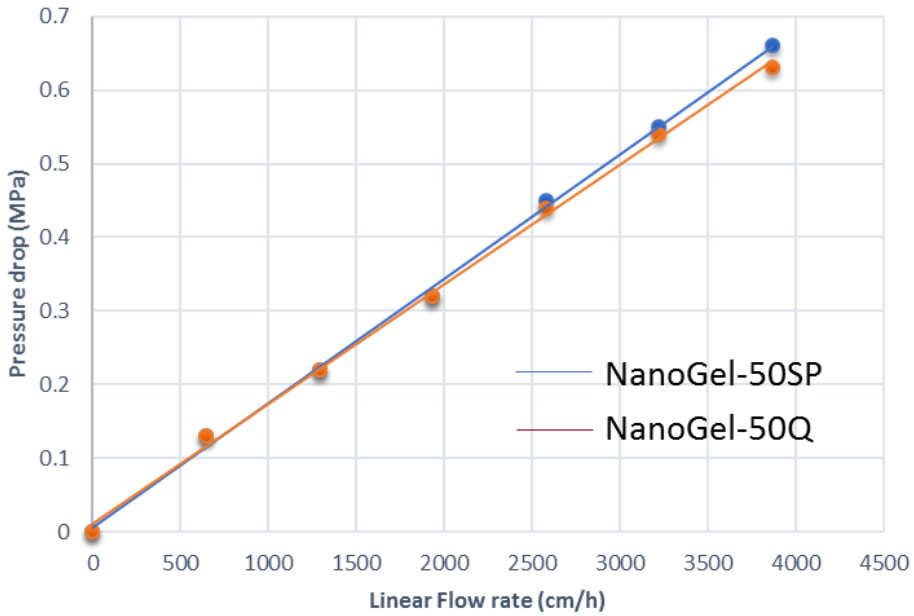


Figure 2. Pressure/flow curves of NannoGel-50SP and NanoGel-50Q (Test column: 7.7×100mm; mobile phase: water; temperature:25°C). System/tubing pressure is excluded.

- **Superior resolution**

Due to their monodisperse 50µm particle size as well as the high mass transfer associated with their large and open pore structure, both NanoGel-50SP and NanoGel-50Q provide superior resolution performance. As an example, NanoGel 50SP exhibits better separation resolution of model proteins than a top ranked CEX analogous in the market (Figure 3).

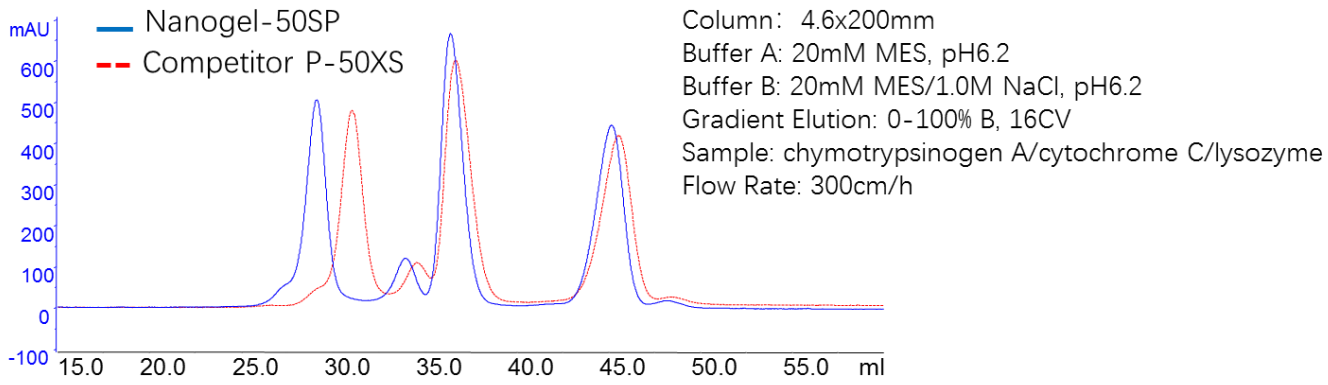


Figure 3. Comparison of model protein separation of NanoGel-50SP with competitor P-50XS, a top ranked CEX analogous in the market.

- **High dynamic binding capacity over a wide range of process conditions**

NanoGel ion-exchange resins provide high dynamic binding capacity (DBC) due to their optimized pores and surface functionalization chemistry. More importantly, they exhibit high DBC over a range of process conditions such as varied residence time and effluent conductivities. This allows target-molecule binding and impurity removal over a wide range of process conditions, thereby increasing process development flexibility and manufacturing throughput. As examples, Figure 4 displays robust DBC performance of NanoGel-50Q at varied residence time (or flow rate). Figure 5 shows that NanoGel-50SP exhibits excellent DBC performance over a range of salt concentration.

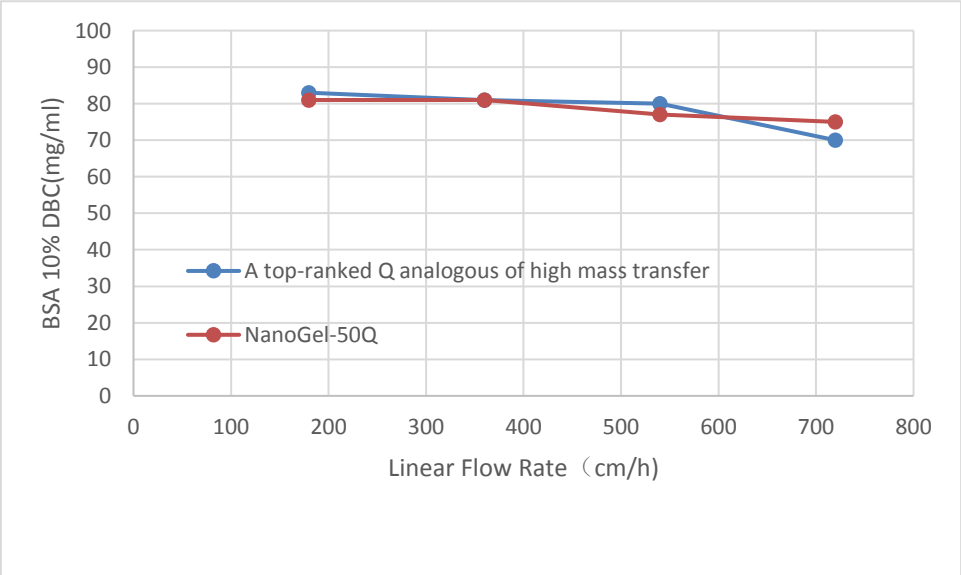


Figure 4. NanoGel-50Q exhibits comparable robust DBC performance at varied flow rate with a top-ranked AEX analogous of high mass transfer.

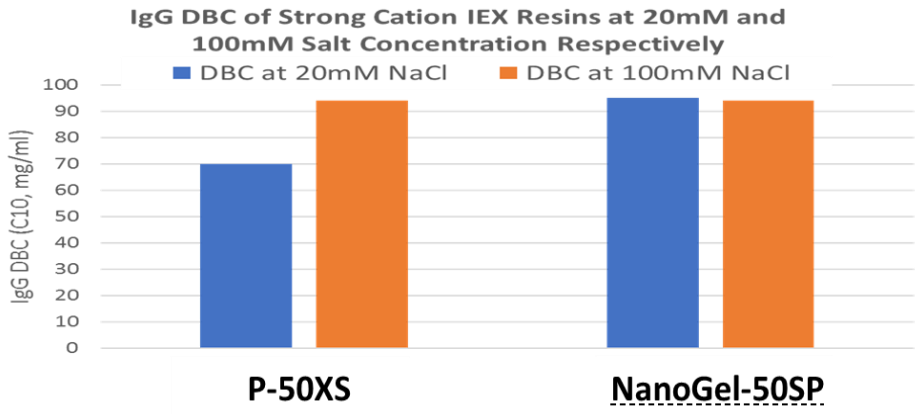
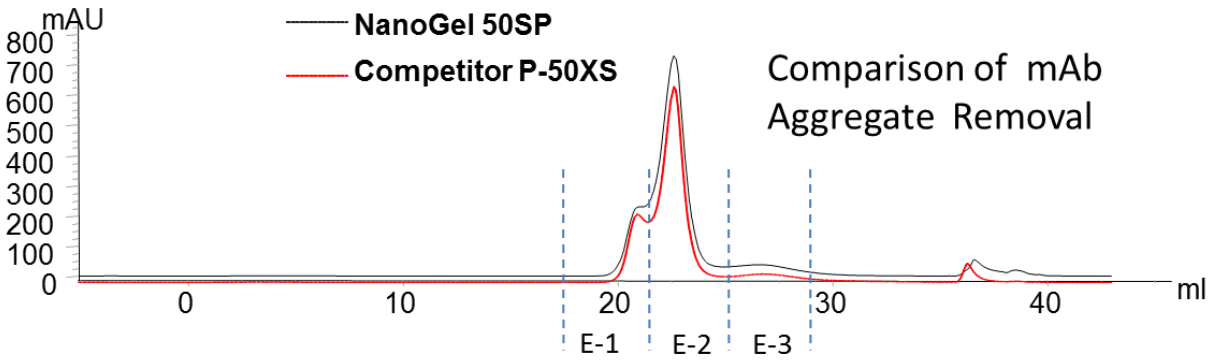


Figure 5. Comparison of DBC at varied salt concentration of NanoGel-50SP vs competitor P-50XS, a top-ranked CEX analogous. NanoGel-50SP exhibits high DBC at both low and high salt concentration.

Applications

Due to their large and open pores, NanoGel 50Sp and NanoGel 50Q are especially suitable for the purification of many large biomolecules such as antibodies, viruses, and vaccines. The combination of high volume throughput and high capacity makes NanoGel resins the optimal choice for processing large amounts of protein in a fast and efficient way. In addition, their high resolution and high mass transfer properties provide superior performance for many unprecedented impurity clearance independent of scale and flow rate. NanoGel 50SP and NanoGel 50Q provide high performance in both bind-elute and flow-through modes. As an example, Figure 6 displays NanoGel 50SP's competitive performance in the application of monoclonal antibody (mAb) aggregate removal using a bind-elute mode. .



	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	

Figure 6. Comparison of NanoGel 50SP with P-50XS, a top ranked CEX analogous, in the performance of mAb aggregate removal using bind-elute mode. Top are the conductivity gradient elute chromatograms; bottom table displays the analysis results of the elution collections.

Column Packing

Both NanoGel-50SP and NanoGel-50Q are supplied as suspension in 20% ethanol in a ratio of 1.0 liter of resin per 1.5 kg of suspension slurry, i.e., the total slurry mass containing a liter of resin is 1.5 kg. Resins are mechanically rigid and incompressible and can be packed effectively in low-pressure glass columns and in high-pressure stainless steel columns. Columns can be packed with traditional flow pack, axial compression, or pack-in-place/stall pack packing methods. The 1.05 packing factor is recommended to account for the difference in bed volume between a gravity-settled bed in 0.1 M sodium chloride and a 1- to 3-bar pressure-packed bed. This factor, along with the slurry ratio, is used to determine the volume of slurry required to yield the intended final column volume (CV). Standard 10–23 µm screens (frits) can be used. Recommended buffer for column packing is 0.5 M NaCl solution. Use the following procedure to pack a column of NanoGel IEX resins.

1. Determine the required slurry mass:
   Required slurry mass = target CV × packing factor × 1.5 kg/L of resin
   Example for a 40 cmD × 20 cmL 25-L column using slurry:
   25 L × 1.05 ×1.5kg/L = 39.4 kg of slurry required
   Note: To ensure the measurement accuracy of resin amount, the suspension slurry should be homogenized before weighing through stirring with plastic spatula or mechanic stirring of <50rpm.
2. Prepare slurry: lab scale columns (column diameter ≤ 50 mm)
   Transfer the required mass of slurry to a bottle-top filter or a sintered glass filter. Apply vacuum to remove the shipping solution (20% ethanol). Resuspend the resin cake to the starting resin slurry volume with the packing solution, 0.5 M NaCl. Mix with a plastic spatula. Repeat the vacuum and resuspension steps for a total of three exchanges. Resuspend the exchanged resin to the original slurry concentration then proceed to step 3 for column packing.

   Prepare slurry: larger columns (column diameter > 50 mm)
   Transfer the required mass of slurry to a mixing container. Allow the resin to settle for at least 2 hours. Carefully decant the supernatant. Do not disturb the bed. Some particles/turbidity may be present in the decant. Replace the supernatant with the same volume of the desired packing solution. Resuspend the resin by gentle agitation with paddle or mixer, then allow the resin to settle for at least 2 hours. Repeat the decanting and resuspending steps 2-3 times to thoroughly exchange into the 0.5 M NaCl packing solution.

   Note: During the slurry preparation, avoid using magnetic stirrer or gear pump as they can abrade the resin particles to cause fines to form.
3. Resuspend the resin slurry and deliver it into the column to pack.
4. Start packing using 0.5 M NaCl as mobile phase. You may observe some turbidity in the eluent as packing starts. Turbidity will clear as packing proceeds and 1–2 bed volumes of packing buffer pass.
5. Increase the flow rate to the maximum or desired flow rate and pressure obtainable with the equipment used. The recommended maximum flow rate is about twice of the desired operation flow rate.
6. After the bed is formed, bring the adapter into contact with the top of the bed without pushing the resin over the O-ring by closing the column outlet and displacing liquid through the top of the adapter to the waste container through the bypass line. NanoGel resin does not shrink or swell, so an open headspace is not recommended.
7. After the column is packed, flow 2–3 CVs of packing solution through the packed bed at the operating flow rate to stabilize the bed. The flow rate used should generate no more than 80% of the maximum packing pressure.

Resin Cleaning and Storage

Clean the Column

Cleaning-in-place (CIP) is a cleaning procedure that removes contaminants such as lipids, precipitates, or denatured proteins that may remain in the packed column after regeneration. Regular CIP also prevents the build-up of these contaminants in the media bed and helps to maintain the capacity, flow properties and general performance of the media. A specific CIP protocol should be designed for each process according to the type of contaminants present. The frequency of CIP depends of the nature and the condition of the starting material.

General protocol to clean the NanoGel 50SP:
Clean the resin with 3 to 5 CVs of 1–2 M NaCl followed by 3 to 5 CVs of 0.5–1 M NaOH.

General protocol to clean the NanoGel 50Q:
Clean the resin with 5 CVs of 1-2 M NaCl, 5CVs of 0.5-1M NaOH, and 5CVs of water followed by 5 CVs of 1M acetic acid.

Resin storage guidelines

Store bulk resin at 2–30°C in 20% ethanol.

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