
NMab[®]

Protein A Affinity Chromatographic Resin

Product Instruction Manual

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Affinity Chromatographic Resin

Protein A affinity chromatography is designed to separate and purify the antibody utilizing the specific binding effect of Protein A ligand to the target antibody. Protein A affinity chromatography greatly simplifies the downstream separation and purification processes of antibodies and becomes the gold standard for antibody separation and purification. At present, the marketed Protein A affinity chromatographic resins are mainly divided into two categories: those using polysaccharides (agarose, dextran and cellulose) as the matrix and those using synthetic polymers (polyacrylate, acrylamide) as the matrix. Agarose matrix has a network structure in the swelling state with large specific surface area, so the affinity loading capacity is relatively high, but the mechanical strength is low with low pressure resistance.

Suzhou NanoMicro Technology Co., Ltd. has developed the world's leading precision manufacturing technology of microspheres through long-term R&D and innovation, which enables accurate fine-tuning of the material composition, particle size, particle size homogeneity, pore size and surface properties of microspheres. By virtue of this technology platform, Suzhou NanoMicro Technology Co., Ltd. firstly developed the monodisperse Protein A affinity chromatographic medium UniMab®. UniMab® is a polyacrylate-based filler with high mechanical strength, good pressure resistance, stable column bed, rapid mass transfer, and durable properties, thus it is well received among many antibody clients, especially in the field of antibody continuous flow purification.

With the advancement of upstream fermentation technology, the antibody expression level is getting higher, and downstream affinity capture has become a bottleneck in antibody production, posing higher requirements for the loading capacity of downstream Protein A affinity chromatographic resin. To meet this need, Suzhou NanoMicro Technology Co., Ltd. used agarose as the base, utilized the unique microsphere modification technology to enhance the mechanical strength, and successfully developed the NMab® Protein A affinity chromatographic resin with higher loading capacity than UniMab®, based on the Protein A ligand technology with independent property rights. In addition to high loading capacity, NMab® also has excellent binding specificity, alkaline resistance and pressure-flow rate characteristics, which is an excellent choice for antibody clients to reduce the purification cost of monoclonal antibodies.

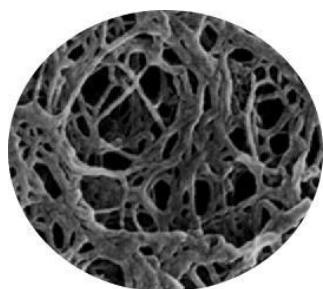


Figure 1. Electron micrograph of NMab® Protein A affinity chromatographic resin

It has the following unique advantages compared with similar products on the market:

- (a) High loading capacity: Average dynamic loading capacity in general monoclonal antibody projects is about 55 mg/mL;
- (b) Strong alkali resistance: Loading capacity decreased by only 11% after immersion in 0.5 M NaOH for 24 hours;
- (c) Low ligand detachment: Less than 10 ppm;
- (d) Low residual host protein (HCP): Generally within 1000 ppm, not inferior to SuRe LX in performance;
- (e) High recovery rate: Greater than 90%;
- (f) High purity: The purity of affinity eluent is more than 98%;
- (g) Good pressure-flow rate: Significantly better than the traditional 4FF/6FF series resin;

Table 1. Technical parameters of NMab® Protein A affinity chromatographic resin from NanoMicro Technology

Product model	NMab®
Chromatography technique	Antibody affinity chromatography
Matrix	Agarose
Particle size	90 μm
Ligand coupling method	Epoxy
Dynamic binding capacity*	~ 55 mg·mL ⁻¹ (human IgG, residence time of 4 min)
Maximum pressure resistance	0.3 MPa
Clean-in-place (CIP)	0.1-0.5 M NaOH
Recommended flow velocity	100-500 cm/h
pH stability	2-12
Chemical stability	All commonly used buffers, 1 M acetic acid, 1 M hydrochloric acid, 100% ethanol, isopropanol and other commonly used organic solvents; long-term contact with strong acids and bases should be avoided.
Temperature	2-40 °C
Storage	20% ethanol or 2% benzyl alcohol at 2-8°C

Excellent Pressure-Flow Property

The results of pressure comparison test are shown as below. The pressure of NMab® at 300 cm/h was only 0.05 MPa, which was similar to that of SuRe LX, an internationally renowned protein A chromatographic resin. This pressure-flow rate profile can meet the requirements of industrial production for the flow rate of fillers, which is superior to the traditional 4FF/6FF gel series fillers. Test column type: XK16, H14.5 cm; mobile phase: 0.5 M sodium chloride solution; the blue curve represents NMab® Protein A resin from NanoMicro Technology, and the red curve represents the resin of a foreign brand.

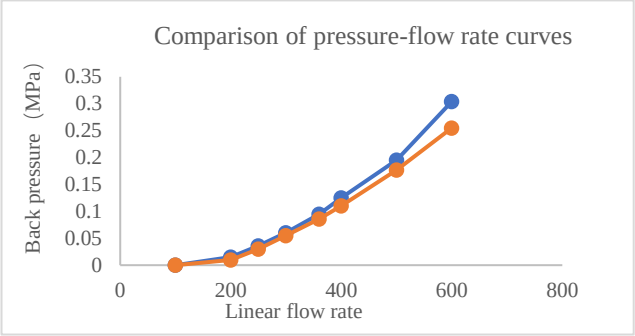


Figure 2. Comparison diagram of pressure-flow rate curves of NMabT® medium from NanoMicro Technology

Higher Binding Capacity

The sample loading test on multiple monoclonal antibody feed liquids showed that the mean dynamic loading capacity (10% flow-through, residence time of 5 minutes) of NMab® was about 55 mg/mL, which was close to that of an internationally renowned protein A chromatographic resin, SuRe LX, and the high loading capacity performance was excellent.

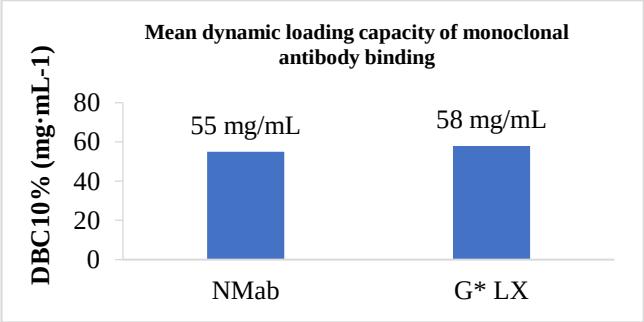


Figure 3. Comparison diagram of dynamic loading capacity of NMab resin from NanoMicro Technology

Low ligand detachment

In several monoclonal antibody projects, the ligand detachment of NMab® Protein A was all less than 10 ppm.

Monoclonal antibody project	Ligand detachment (ppm)
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mAb1	5.06
mAb2	0.68

Low residual host protein

In several monoclonal antibody purification projects, the HCP residue of NMab® Protein A resin was within 1000 ppm, which was equivalent to that of the internationally renowned protein A chromatography resins, SuRe LX and UniMab.

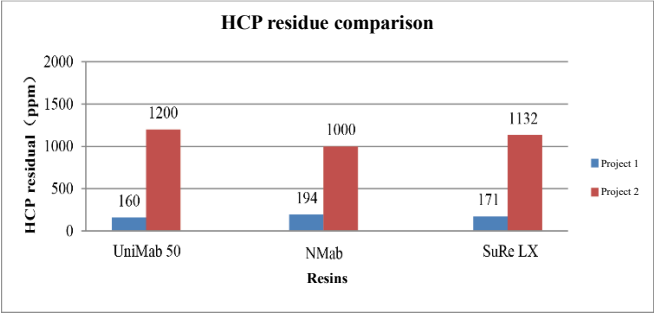


Figure 4. Comparison diagram of residual host protein of NMab resin from NanoMicro Technology

Alkaline resistance test

The filler was immersed in 0.5 M sodium hydroxide for 24 h at 25°C. The dynamic binding capacity (DBC) of affinity filler was measured before and after strong alkali immersion using an antibody B, and the decrease ratio of DBC after immersion to that before immersion was calculated as an index of alkaline resistance. Smaller decrease ratio of DBC indicates better alkaline resistance.

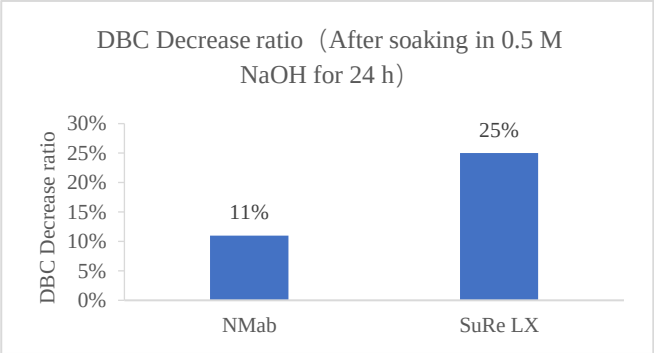


Figure 5. Comparison diagram of alkaline resistance of NMab resin from NanoMicro Technology

Life Cycle

NMab filler were tested for dynamic binding capacity (DBC), purity, pressure, and changes in filler performance over a certain number of uses on 150 cycles of monoclonal antibodies. Each cycle includes mAb cell culture fluid loading, washing, elution, CIP, and re-equilibration steps. Figure 6, Figure 7, Figure 8, and Figure 9 show the changes in chromatograms UV280, DBC, purity, and pressure, respectively, over the various cycles of use to determine the life cycle of the filler.

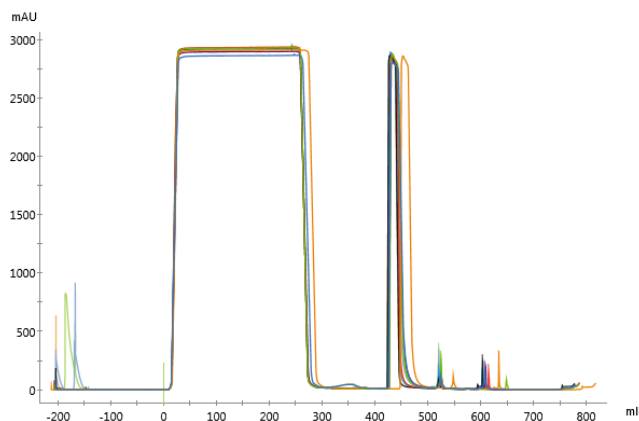


Figure 6. UV280 profile in NMab® life cycle study for every 10 times of data overlay

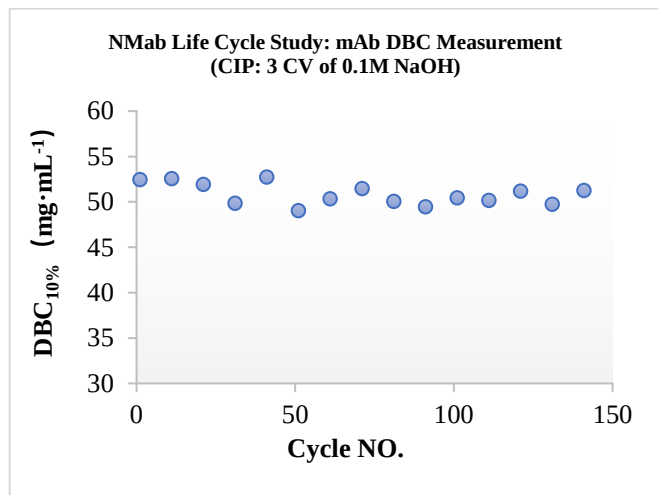


Figure 7. DBC_{10%} trend in NMab® life cycle study

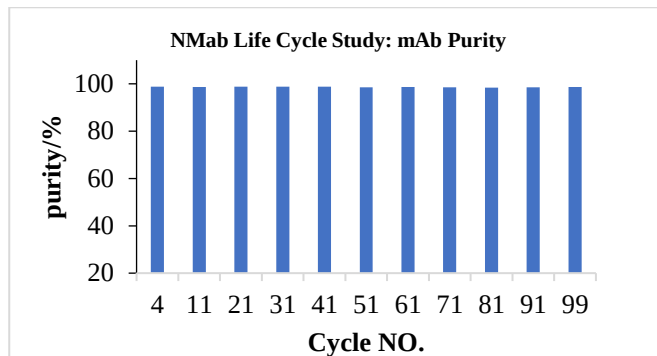


Figure 8. Purity test data of elution samples in NMab® life cycle study

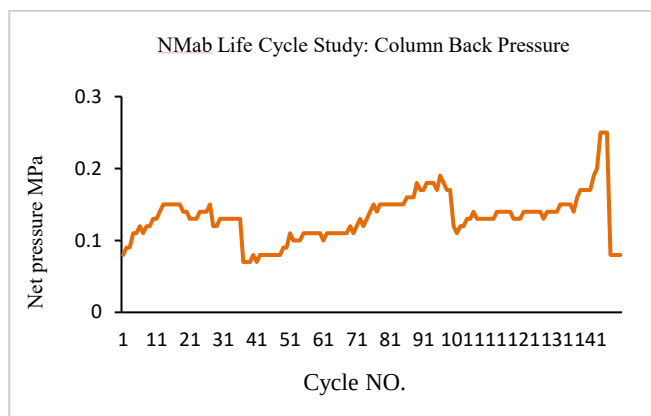


Figure 9. Data of column pressure in NMab® life cycle study

Conclusion

During the life cycle study with 150 cycles, the loading capacity of fillers was consistently greater than 90% of the initial loading capacity, and the loading capacity decrease was relatively slow. The separation performance and pressure-flow rate performance were stable, and the filler could be reused for more than 100 times, indicating satisfactory life cycles.

Column Packing Instruction

Homogenate concentration determination

NMab® affinity chromatography resin is supplied in 20 % ethanol solution bottled at a homogenization concentration (Cs) of approximately 65% (v/v). The homogenate concentration is the ratio of the constant settling volume of the chromatographic medium to the total volume of the homogenate. For accurate determination of Cs, shake the medium in the original container, then transfer 10 mL of homogenate in a measuring cylinder and allow it to stand overnight, read the settling volume Vr, and calculate the homogenate concentration:

$$\text{Eq. 1 } C_s (\%) = 100 \times (V_r/10) = 10 V_r$$

For the best loading of the column, it is recommended to prepare 50-70 % of the resin homogenate with 0.5 M NaCl solution.

Packing Preparation

Calculate the column volume (V_c) of the loaded column:

$$\text{Eq. 2} \quad V_c = h \times \pi r^2$$

h : column height; r : column radius

Calculate the required homogenate volume (V_s): In general, the chromatographic resin will be compressed under pressure, resulting in volume shrinkage. In order to obtain a tight column bed, it is recommended that the filler volume is prepared in excess, generally with a compression factor (CF) of 1.15-1.2.

$$\text{Eq. 3} \quad V_s = 100 \times (V_c \times \text{CF})/C_s$$

Preparation of column loading resin homogenate: Shake the chromatographic resin in the original container, measure the required volume of the original solution V_s into a suitable container, allow the medium particles to settle naturally, then tilt the container to pour off the supernatant; wash the resin with more than 5 times the column volume of the loading solution, such as 0.5 M NaCl, to remove the original preservation solution, and then adjust the homogenate concentration to 50-70% (v/v) with the loading solution.

Packing NMAb in NmXK 16/20 Column

Quickly rinse the connector at the end of the column with the loading solution to remove air bubbles, then close the column outlet, with 1-2 cm of loading solution remaining at the bottom of the column.

Remove air bubbles from the upper column head pipeline with the loading solution.

Resuspend the resin, divert the flow with a glass rod against the inner wall of the column, pour the gel suspension continuously into the column, clean the column wall with the loading solution and fill the column tube, and install the upper column head properly. Avoid the introduction of air bubbles during the operation.

Open the outlet at the bottom of the column, turn on the chromatographic system pump and press the column with constant flow or constant pressure within the recommended pressure range. Mark the gel interface properly when the column bed is stabilized.

Turn off the pump and close the column outlet, unscrew the upper column inlet pipeline, push the column bed with the upper column head to 2-3 mm below the mark line, and then tighten the upper column inlet pipeline.

Performing Column Qualification Test

The loaded column is first equilibrated with 2 CV of 0.5 M NaCl solution and then tested with 2.0 M NaCl at a flow rate of 100 cm/h. The column can also be equilibrated with deionized water and tested with acetone solution. See Table 3 below for specific test parameters:

Table 7. Column efficiency test conditions for NMAb® chromatographic column

Probe	5% (v/v) acetone aqueous solution or 2 M NaCl
Probe volume	1-5 % of Column volume

Mobile phase	Nanopure water or 0.5 M NaCl
Linear velocity	50 ~ 200 cm/h
Detection	5% acetone as probe: UV @ 280 nm 2 M NaCl as probe: Conductivity meter
Recommended qualification specifications	As: 0.8-1.5; Plates (N/m): >2500

Generic Protocol for NMAb Affinity Chromatography

Rinsing and equilibration:

Rinse and equilibrate the NMAb column with a proper eluent (e.g. 100 mM glycine, pH 3.0) and a proper equilibration solution (e.g. 20 mM PB + 150 mM NaCl, pH 7.4) in sequence before use;

Sample injection:

The sample loading does not exceed 0.8 times the resin DBC_{10%};

Cleaning:

5 CV equilibrium solution (e.g. 20 mM PB + 150 mM NaCl, pH 7.4);

Elution:

5 CV citric acid, acetic acid or glycine, pH 3-4;

Cleaning:

5 CV 1 M acetic acid;

Regeneration (Cleaning-in-place, CIP):

Clean with 3 - 5 CV 0.1 - 0.5 M NaOH solution, and extend the soaking time if necessary;

Re-equilibrium:

Wash with 5 CV equilibration solution (e.g., 20 mM PB + 150 mM NaCl, pH 7.4) to baseline;

Storage:

Replace the buffer salt in the chromatographic column with purified water at the end of use and store in 20% ethanol.

Storage

Seal and store the column/resin in 20 % ethanol or 2% benzyl alcohol at a recommended storage temperature of 2-8°C. To prevent ethanol evaporation and microbial growth, it is recommended to change 20% ethanol once every 3 months.

Note: All samples and mobile phases must be filtered with a 0.45-μm filter during use.

Trouble Shooting Guide

If you have any problems when using NMab®, 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.
	Clogged on-line filter of instrument	Remove impurities and wash or replace the filter, and filter the sample and buffer prior to use.
	Column bed compressed	Repack the column.
	Long service time of the chromatographic column	Replace the chromatographic column or replace chromatographic medium.
	Valve between pump and collector not opened	Open the valve.
Precipitation observed on the column	Accumulation of hydrophobic proteins, lipoproteins or lipids	Rinse with a nonionic surfactant (e.g., 0.1% Tergitol 15-S-9 or Triton X-100 reduced/reduced), or 0.5 M NaOH, or 1 M guanidine hydrochloride; then rinse with at least 5 times the column volume of sterilized and filtered binding buffer (pH 7-8).
	Protein denaturation and precipitation	Rinse the column with 2 times the column volume of 50 mM NaOH, or 50 mM NaOH and 1.0 M NaCl, or 0.1 M H ₃ PO ₄ , or 6 M guanidine hydrochloride and 10 mM NaOH for at least 10 min; then rinse with at least 5 times the column volume of sterilized and filtered binding buffer (pH 7-8)

Order Information

Product	Size	Catalog#
NMab [®]	30 mL	17013-090100-2030
	50 mL	17013-090100-2050
	100 mL	17013-090100-2100
	300 mL	17013-090100-2300
	500 mL	17013-090100-2500
	1 L	17013-090100-1001
	5 L	17013-090100-1005
	10 L	17013-090100-1010
	50 L	17013-090100-1050
	100 L	17013-090100-1100

Note: Chromatographic preloaded columns of 7.7 mm × 22 mm, 16 mm × 25 mm, 7.7 mm × 100 mm are available. Please contact us for further details.

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