
NMab Titan HC

Protein A affinity chromatography resin

Instruction

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Protein A affinity resin

In the global pharmaceutical market, antibody drugs continue to lead in sales, and market competition is becoming increasingly fierce. Reducing antibody production costs is one of the key factors for enhancing market competitiveness. In the downstream purification sector, Protein A affinity chromatography is not only a critical step in determining drug quality but also a key lever for cost control. To meet the high-capacity, high-rigidity, high-mass transfer, and high-alkali-resistance demands that antibody enterprises have for affinity chromatography media in high-efficiency production, NanoMicro Technologies has introduced an upgraded version based on its original NMab Titan — the NMab Titan HC.

NMab Titan HC is a next-generation high-performance Protein A affinity chromatography medium developed by NanoMicro Technologies. Featuring a highly rigid matrix combined with an optimized pore structure design, this resin is suitable for the separation and purification of monoclonal antibodies, bispecific antibodies, polyclonal antibodies, and recombinant protein biologics containing Fc fragments. It demonstrates exceptional performance particularly in the purification of bispecific antibodies and large-molecular-weight antibody molecules. With its four core advantages — high binding capacity, high rigidity, high mass transfer, and high alkali resistance — NMab Titan HC maintains excellent dynamic binding capacity even under high-speed operation, meeting the diverse needs from laboratory-scale preparation to pilot-scale and industrial-scale production, helping enterprises achieve cost reduction and efficiency gains while ensuring product quality.

Table 1. Characteristics of NMab Titan HC.

Product name	NMab Titan HC
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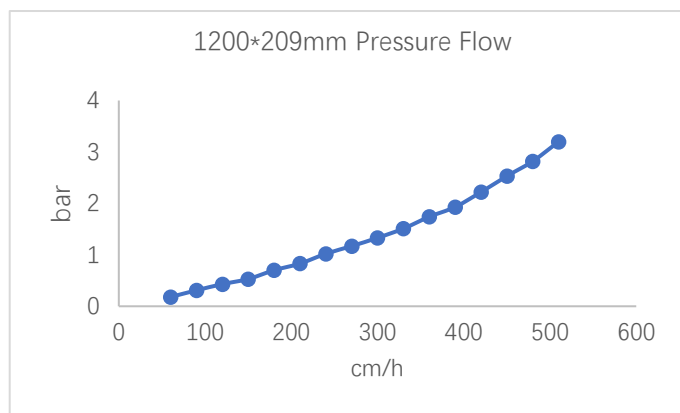
Chromatography technique	Protein A affinity
Matrix	Highly cross-linked agarose
Particle size	~60 μm
Ligand coupling	Epoxy
DBC*	≥80 mg·mL ⁻¹ (human IgG, 6 min retention time)
Maximum pressure	0.5 MPa
CIP	0.1-0.5 M NaOH
Recommended Flow Rate	100 - 400 cm/h
pH stability	3-12 (operational), 2-14 (CIP)
Chemical stability	Stable in commonly used aqueous buffers in Protein A chromatography.
Storage	20% Ethanol or 2% benzyl alcohol, 2-8 °C

*Dynamic binding capacity is measured at 10% antibody breakthrough.

Pressure-Flow Rate Curve Test

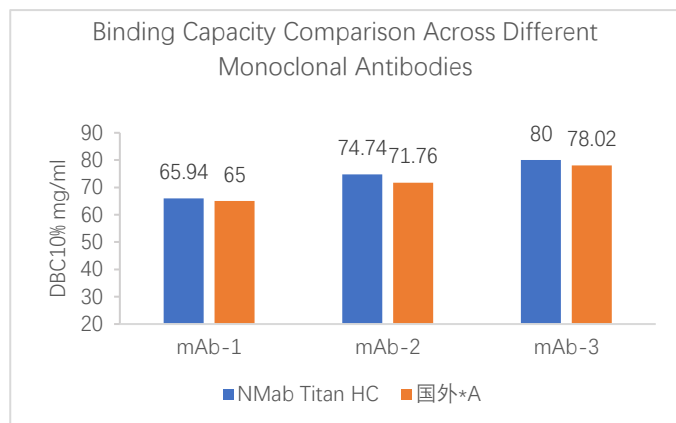
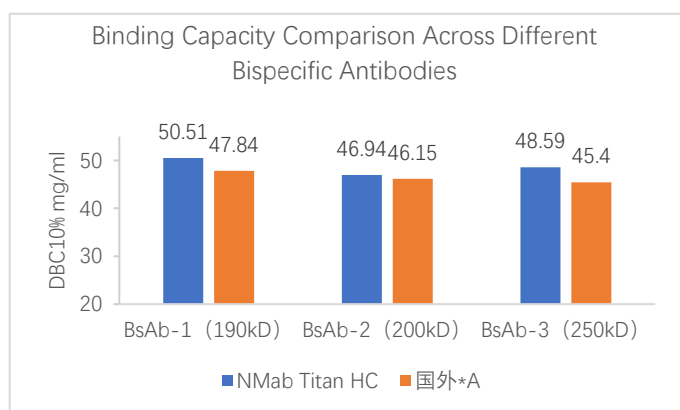
The mechanical strength and pressure resistance of chromatography media are critical for ensuring production efficiency. Excellent mechanical properties not only enable high-flow-rate operation, significantly improving production throughput, but also reduce the risk of performance degradation and product loss caused by media compression or breakage. (Test

conditions: 1200 mm column, 209 mm bed height, compression factor: 1.15, test solution: pure water)



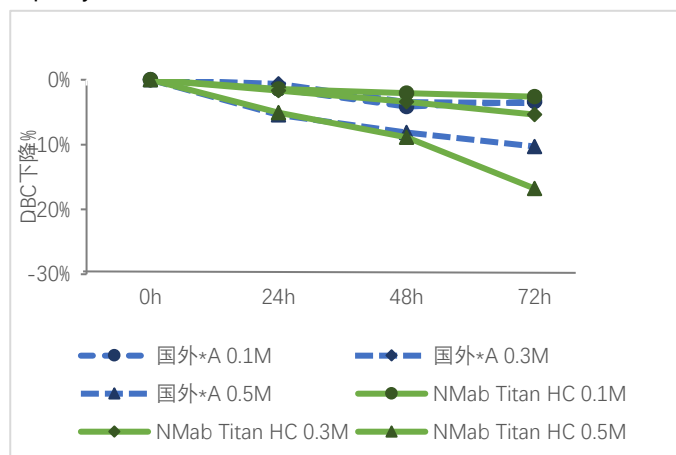
Dynamic Binding Capacity (DBC)

Dynamic binding capacity (DBC) directly impacts the processing capability of chromatography media. A higher DBC value means that efficient purification can be achieved with a smaller column volume, thereby reducing equipment investment and media costs. For example, across different monoclonal and bispecific antibody feed streams, NMab Titan HC demonstrates superior dynamic binding capacity under identical conditions (compared to leading international competitor A*), enabling reduced media usage during process scale-up and optimized production costs.



Alkali Resistance

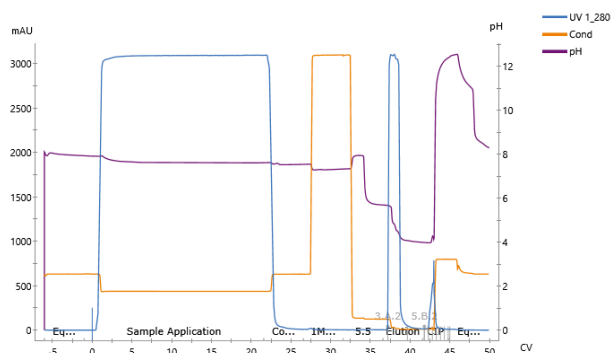
Protein A resins require periodic alkaline cleaning to remove impurities and microorganisms, making alkali resistance a direct factor in determining their service life. In accelerated alkali resistance tests at different concentrations (0.1 M / 0.3 M / 0.5 M NaOH), NMab Titan HC retains more than 80% of its initial binding capacity after 72 hours of immersion.



Purification Chromatogram Comparison

Purification data for a representative bispecific antibody feed stream — all performance metrics are comparable to leading international competitor A.

Competitor A Purification Chromatogram:



Concentration of slurry (C_s) refers to the ratio of settled resin volume (V_r) to the total volume of the slurry. To determine C_s of a slurry, gently resuspend resin in an appropriate vessel, then pour 10 ml of the slurry into a calibrated cylinder and allow to settle overnight (>12 hours). Record the volume of the settled resin bed (V_r) and calculate the slurry percentage (C_s) according to Eq. 1.

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

It is recommended to prepare 50~70% resin slurry in 0.5M NaCl solution for packing a NMac Titan HC column.

Packing preparation

Calculate the column volume (V_c):

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

h : Bed height of column; r : Radius of column

Determination of slurry volume (V_s) needed for packing a column of desired bed height: the recommended compression factor (CF) for packing NMac Titan HC resin is 1.10 -1.25. The required slurry volume can be calculated according to Eq. 3.

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

NMac Titan HC Purification Chromatogram:

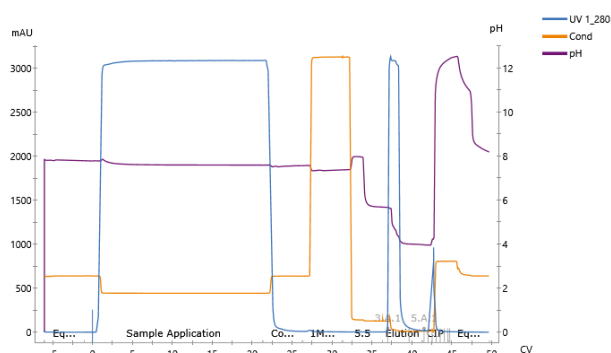


Table 2. Purification Test Data is as Follows:

Resin	DBC mg/ml	Yield %	Elu cv	Purity %	HCP ppm	ProA ppm
NMac Titan HC	40.28	92.92	2.68	96.67	1344.66	6.12
国外*A	40.96	92.55	2.46	96.54	1235.41	16.78

Column packing method (as NmXK 16/200 column)

Material Confirmation

NmLOAD 16/200 Pre-packed Column: Inner diameter 1.6 cm, column tube height 30 cm, bed height 20 cm.

Theoretical resin volume:

$$BV (\text{Theoretical}) = 3.14 \times (1.6/2)^2 \times 20 = 40.2 \text{ mL}$$

Actual resin volume (including compression factor 1.09 and transfer loss 0.02, total factor 1.11):

$$BV (\text{Actual}) = 3.14 \times (1.6/2)^2 \times 20 \times 1.11 = 44.6 \text{ mL}$$

- Suspend the resin thoroughly. If no concentration label is provided, take 2 mL of the homogenate for rapid centrifugation to verify.
- Measure the calculated volume into a graduated cylinder, and allow sedimentation for at least 4 hours for accurate quantification.

Column packing instruction

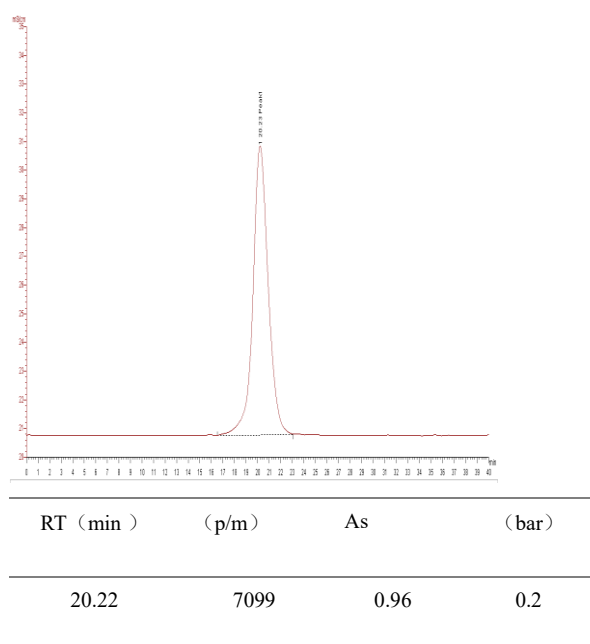
Determination of slurry

NMac Titan HC resin is supplied in 20% ethanol in original package as a ~65% slurry (v/v). Replacement of storage solution with 5 column volumes of 0.5 M NaCl solution prior to column packing is recommended.

- Aspirate the supernatant or perform buffer exchange using a sintered glass funnel, and fully equilibrate the resin into the packing homogenate buffer.
- Inspect the homogenate for foreign particles and impurities to ensure the medium is pure.
- Mix the resin and homogenate buffer completely before column packing.

Mobile phase	Pure water or 0.5 M NaCl solution
Linear flow rate	50 ~ 200 cm/h
Detector	5 % Acetone: UV@280 nm 2 M NaCl: Conductivity meter
Recommended qualification specifications	As: 0.8-1.5; Plates (N/m): >2500

Column Efficiency Results



Please thoroughly review manufacturers instruction manuals for column and chromatography system before performing the following procedure.

- 1) Wet the column bottom frit with packing buffer and remove air; leave 1~2 cm of packing buffer in the column and close the bottom outlet.
- 2) Flush column top adaptor with packing buffer to remove trapped air.
- 3) Transfer an appropriate amount of fully resuspended resin slurry (Vs) into the column in a single operation; use an extension tube or a packing reservoir as necessary. Rinse the column inner wall with packing buffer. When a clear supernatant of 2~3 cm has developed above the column bed, insert the top adaptor into the column and tighten the seal. Avoid trapping air bubbles during this process.
- 4) Connect the top adaptor to a chromatography system, open the bottom outlet, and turn on the system pump to consolidate the resin using constant flow rate or pressure. When a stable consolidated column bed is formed, stop the system pump and close the column bottom outlet.
- 5) Loosen the column top adaptor seal slightly and push the adaptor 2-3 mm down slowly, then tighten the seal.

Column qualification

A packed column should be equilibrated with 2 CV of 0.5 M NaCl, before the sample is injected. Refer to Table 3 for detailed test conditions.

Table 3. Column qualification test condition

Sample	5% (v/v) Acetone in pure water or 2 M NaCl solution
Injection volume	1 ~ 5 % Column volume

Attention: Sample, buffers and solutions should be filtered with a 0.45 µm membrane prior to chromatography operation.

Generic chromatography protocol

Resin pretreatment and equilibration:

5 CV of elution buffer (e.g., 100 mM Glycine, pH 3.0) followed by

5 CV of equilibration buffer (e.g., 20 mM PB + 150 mM NaCl, pH 7.4);

Sample loading:

No more than 80% of pre-determined DBC_{10%} for target antibody;

Post-loading wash:

5 CV of equilibration buffer;

Elution:

5 CV of elution buffer (e.g.; Sodium citrate, Sodium acetate, or Glycine, pH 3 – 4);

Regeneration:

5 CV of 1 M acetate;

Cleaning-in-place (CIP):

3 - 5 CV of 0.1 - 0.5 M NaOH; recommended contact time is 15 min at room temperature per cycle (Adjust contact time as needed);

Re-equilibration:

5 CV of equilibration buffer.

Storage

Rinse column/resin fully with pure water to remove residual buffer, then equilibrate the column/resin with 20% ethanol or 2% benzyl alcohol. Store column/resin at 2 ~ 8°C. Replacement of storage solution every three months is recommended to prevent column/resin from drying out or growth of microorganisms.

Ordering information

Product name	Package	Cat. No.
NMab Titan HC	30 mL	17080-060100-2030
	50 mL	17080-060100-2050
	100 mL	17080-060100-2100
	300 mL	17080-060100-2300
	500 mL	17080-060100-2500
	1 L	17080-060100-1001
	5 L	17080-060100-1005
	10 L	17080-060100-1010
	50 L	17080-060100-1050
	100 L	17080-060100-1100

Note: Prepacked 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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