
NMab Titan

Protein A affinity chromatography resin

Instruction

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Protein A affinity resin

NMab Titan is a third-generation protein A chromatography resin with high binding capacity and improved alkaline stability, making it an excellent choice for the downstream purification of monoclonal antibodies (mAbs), bispecific antibodies (bsAbs), antibody-drug conjugates (ADCs), and other Fc-containing biomolecules. Prepared by immobilizing recombinant protein A ligands onto a rigid, highly cross-linked agarose matrix, NMab Titan features low back pressure and high dynamic binding capacity at high flow rates, making it suitable for a range of application needs, from laboratory preparation to industrial production.

Table 1. Characteristics of NMab Titan.

Product name	NMab Titan
Chromatography technique	Protein A affinity
Matrix	Highly cross-linked agarose
Particle size	~70 μm
Ligand coupling	Epoxy
DBC	~75 mg · mL ⁻¹ (human IgG, 6 min retention time)
Maximum pressure	0.5 MPa
CIP	0.1-0.5 M NaOH
Maximum operating flow velocity	400 cm/h (I.D.: 600 mm, H: 250mm)
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

The key features of NMab Titan are:

- Superior dynamic binding capacity for higher mass throughput of processed biomolecules.
- Advanced alkaline stability allowing better process economy.
- Robust mechanical properties with low back pressure for industrial-scale purification.
- Low non-specific absorption, leading to a higher yield.

Superior dynamic binding capacity to mAbs

NMab Titan is a protein A resin specifically designed for antibodies capturing with high productivity. As illustrated in Figure 1, NMab Titan features a superior dynamic binding capacity towards various cell culture harvests of mAbs, thanks to its custom-designation. The productivity improvement and cost reduction offer a competitive advantage in the purification process.

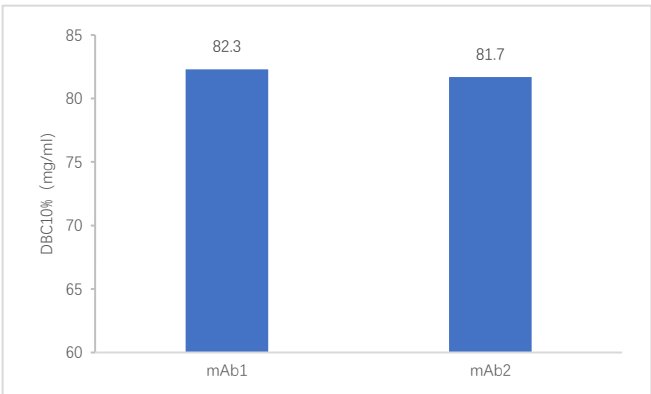


Figure 1. NMab Titan is designed for high productivity of mAbs.

Effective Capture of bispecific antibodies

Upon assessing multiple supernatants of bsAbs culture, NMab Titan has consistently shown a dynamic binding capacity of approximately $40 \text{ mg} \cdot \text{mL}^{-1}$, enabling equal outputs with less resin, thereby streamlining sample processing efficiency.

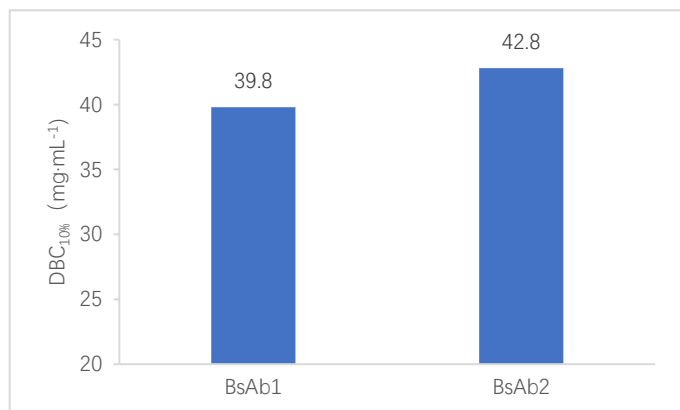


Figure 2. NMab Titan is designed for high productivity of bsAbs.

Improved alkaline stability allows better process

economy

After incubation in 0.5 M NaOH at 25°C for 24 hours, the binding capacity (mAb) of NMab Titan decreased about 10%, indicating excellent alkali resistance. This significantly improves the resin lifetime in antibody purification, enhances production efficiency, and reduces production costs.

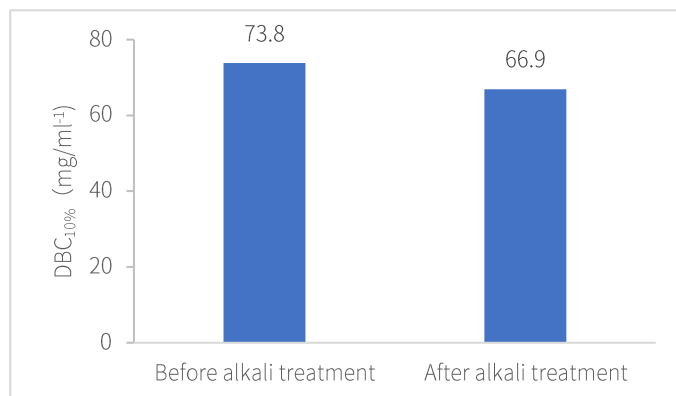


Figure 3. Alkaline stability study of NMab Titan by using 0.5 M NaOH at 25°C for 24 hours.

Application in purification of mAb

Experimental condition:

Sample: supernatant of mAb culture

Equilibrium: 20 mM PB+ 150 mM NaCl, pH 7.4

Washing: 20mM HAC-NaAc, pH 5.5

Elution: 20mM HAC-NaAc, pH 3.6

Regeneration: 0.1 M NaOH

Retention time: 5 min

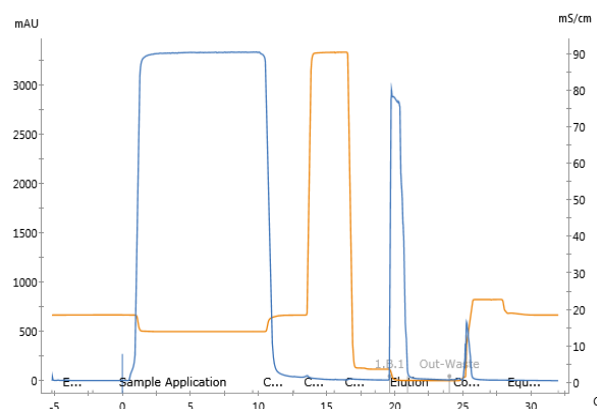


Figure 5. Capturing of monoclonal antibodies from clarified cell culture harvest using NMab Titan.

Table 2. Result of NMab Titan in purification of mAb.

Purity (%)	Yield (%)	Elution (CVs)	HCP (ppm)	Pro A (ppm)
98.1	97.27	1.99	1053.14	2.71

Column packing instruction

Determination of slurry

NMab Titan 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.

Concentration of slurry (Cs) refers to the ratio of settled resin volume (Vr) to the total volume of the slurry. To determine Cs 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 (Vr) and calculate the slurry percentage (Cs) according to Eq. 1.

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

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

Packing preparation

Calculate the column volume (Vc):

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

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

Determination of slurry volume (Vs) needed for packing a column of desired bed height: the recommended compression factor (CF) for packing NMab Titan resin is 1.15 -1.2. The required slurry volume can be calculated according to Eq. 3.

$$\text{Eq. 3 } Vs = 100 \times (Vc \times CF)/Cs$$

Column packing method (as NmXK 16/20 column)

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
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

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.

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

Troubleshoot

Problems that occasionally occur during NAb Titan affinity 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 Causes	Solution
Column clogging	Insufficient clarification of feed stream or buffers	Filter with 0.45 mm membrane; Use a 0.22 mm membrane if needed
No antibodies in eluate	Strong antibody binding; insufficient elution stringency	Lower elution pH; use different elution buffer species; include additives in elution buffer (e.g., L-arginine)
Low antibody yield in elution	Insufficient elution stringency; antibody denaturation/precipitation on column	Optimize elution pH; use buffer additives to improve antibody stability (e.g., L-arginine, glycerol, and/or L-glutamic acid etc.)
Antibody aggregation/degradation	Unstable molecules	Optimize elution buffer pH; include buffer additives to improve antibody stability (e.g., L-arginine, glycerol, and/or L-glutamic acid etc.)
High level of contaminants	Insufficient post-loading wash; association of contaminants with antibody molecules or recombinant protein A ligands	Vary wash buffer NaCl concentration and/or pH; use buffer additives such as non-ionic detergents (e.g., 0.2% Tween-20), L-arginine, glycerol, GnHCl, urea, and/or low concentration of alcohol (e.g., ethanol and isopropanol);
Protein deposit on column	Accumulation of hydrophobic proteins and lipid-protein complexes; precipitation of denatured protein impurities	Remove tightly bound substances with non-ionic detergents (e.g., 0.1% Tergitol 15-S-9 or Triton X-100 reduced), or 6 M GnHCl, or 50mM NaOH + 1M NaCl; perform CIP with 0.5M NaOH; then re-equilibrate column to neutral pH
Target antibodies in flow-through fractions	Overloading column	Load no more than 80% of pre-determined DBC _{10%} for target antibodies
	Denaturation of antibodies	Optimize expression and harvest conditions to protect the integrity of target antibodies

Ordering information

Product name	Package	Cat. No.
NMab Titan	30 mL	17013-080100-2030
	50 mL	17013-080100-2050
	100 mL	17013-080100-2100
	300 mL	17013-080100-2300
	500 mL	17013-080100-2500
	1 L	17013-080100-1001
	5 L	17013-080100-1005
	10 L	17013-080100-1010
	50 L	17013-080100-1050
	100 L	17013-080100-1100

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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