NMab™ Pro Protein A Affinity Resin

Product Manual

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Product Introduction

Protein A affinity chromatography uses immobilized Protein A ligand to specifically bind antibody to the resin beads and achieve of separation and purification of antibody. Protein A affinity chromatography greatly simplifies the downstream separation and purification process of antibodies and has become the standard for antibody separation and purification. Protein A affinity chromatography media currently on the market are mainly divided into two categories: polysaccharides (agarose, dextran, cellulose) as the matrix and synthetic polymers (polyacrylate, acrylamide) as the matrix. The agarose matrix has a network structure in the swollen state, and the specific surface area is large, so the affinity capacity is relatively high, but the mechanical strength is not high, and the pressure resistance is low.

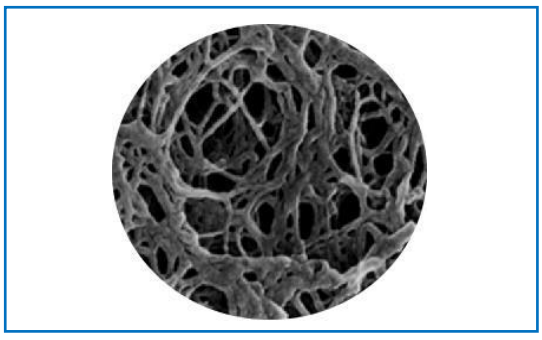


Figure 1. *SEM Image of NMab™ Pro Protein A Resin*

With the advancement of upstream fermentation technology, antibody expression levels are getting higher and higher, and downstream affinity capture has become the bottleneck of antibody production. Therefore, the loading capacity requirements for downstream Protein A affinity chromatography media are getting higher and higher. In order to meet this demand, NanoMicro Technology has developed the high performance affinity resin, NMab™ Pro that combines a unique, highly cross-linked agarose matrix with an engineered Protein A ligand having enhanced chemical stability. NMab™ Pro has excellent binding capacity and specificity, extended alkali resistance and superior pressure-flow rate characteristics. Complete characteristics of NMab™ Pro are displayed in Table 1. NMab™ Pro is the best choice to improve process productivity and reduce the purification cost of antibodies.

Table 1*. Characteristics of NMab™ Pro*

Characteristics	Specification
Matrix	Agarose
Ligand	Engineered Protein A
Ligand coupling chemistry	Expoxy
Particle Size	69 μm
Dynamic Binding Capacity （5 min residence time）	60~80 mg/mL
Protein A leachate	< 10 ng/mL
Recommended Pressure	≤ 0.3 MPa
CIP conditions	0.1-0.5 M NaOH

Pressure-Flow Rate Characteristics

NMab™ Pro has better mechanic strength than conventional agarose resins due to its unique, extensive crosslinking chemistry, providing good pressure-flow rate characteristics in process scale chromatography columns (Figure 2).

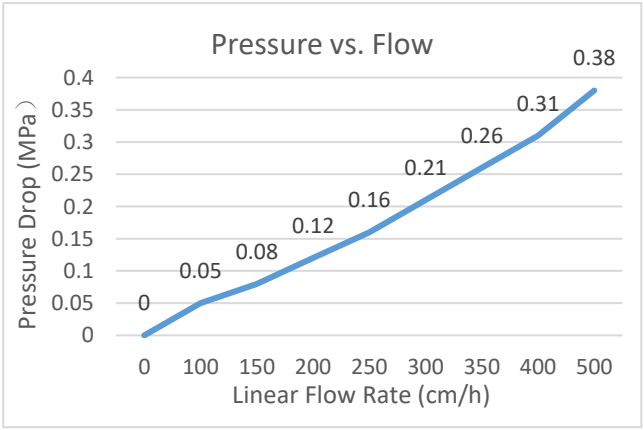
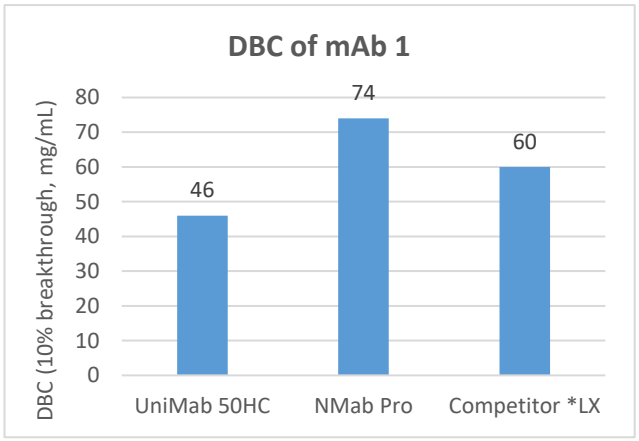


Figure 2. *Pressure-flow curve of NMab™Pro (Column: 200mm D ×180mm L; Mobile phase: 0.5M NaCl)*

Dynamic Binding Capacity

NMab™ Pro exhibits highly competitive dynamic binding capacity of mAbs (Figure 3) and bispecific antibodies (Figure 4).



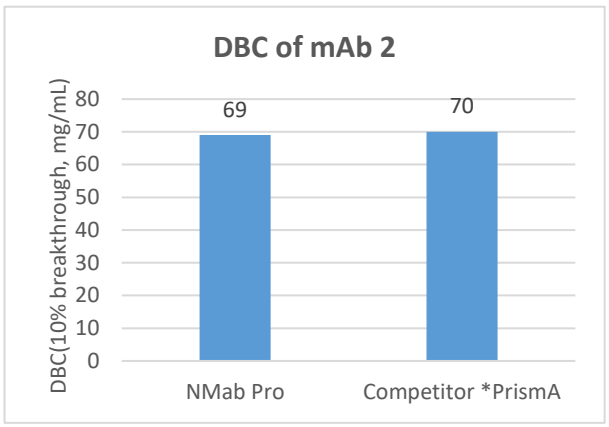


Figure 3. *Benchmarking of mAb dynamic binding capacity (10% breakthrough; 5min residence time)*

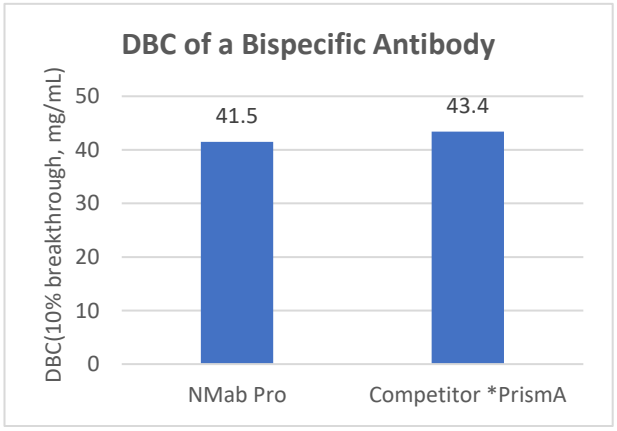


Figure 4. *Benchmarking of a bispecific antibody dynamic binding capacity (10% breakthrough; 5min residence time)*

Alkaline Resistance

Due to the use of NanoMicro's proprietary, engineered protein A ligand (Patent CN202012747812.8), NMab™ Pro exhibits excellent alkaline resistance and can tolerate routine CIP with 0.5M NaOH.. Dynamic binding capacity remains relatively constant through ca. 100 purification cycles which employ a 15 minute CIP of 0.5 M NaOH after each cycle (Figure 5).

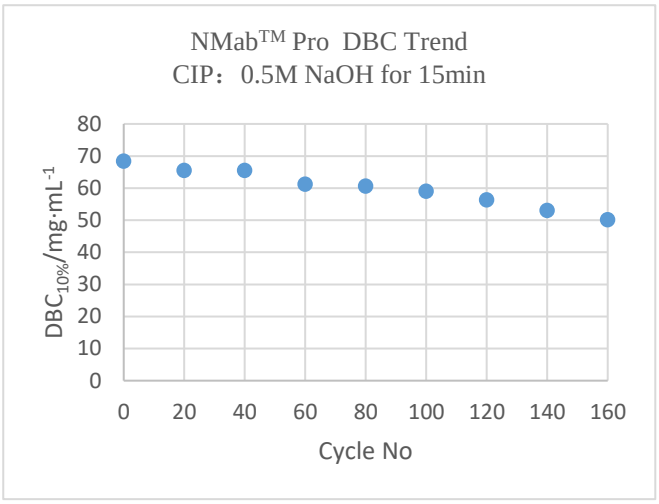


Figure 5. *DBC of HCCF that employ harsh CIP treatment (0.5M NaOH exposure for 15min) after each cycle.*

mAb Purification Performance

Figure 6, Figure 7 and Table 3 display the results of protein A affinity purification of a mAb supernatant culture medium. NMab™ Pro shows highly competitive performance in terms of mAb purity, recovery yield, HCP clearance and protein A leaching levels.

Experiment conditions：

Instrument：AKTA Avant150

Column：1.1mL MILLIPORE column

Sample：a mAb supernatant culture medium

Equilibration: 20 mM PBS pH7.4

Elution: 20 mM NaAc-HAc pH3.6

Residence Time：5 min

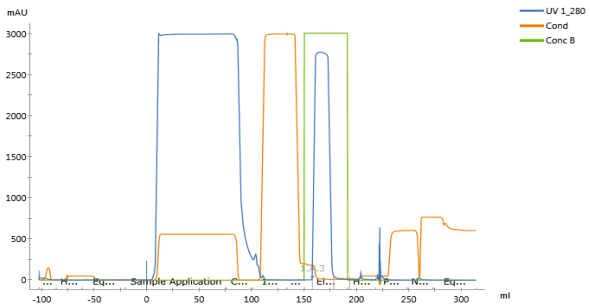


Figure 6. *Affinity chromatogram of a mAb using Competitor *PrismA resin*

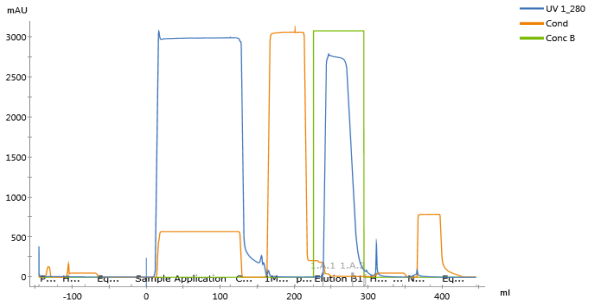


Figure 7. *Affinity chromatogram of a mAb using NMab™ Pro resin.*

Table 3. *Benchmarking results of a mAb affinity purification*

Resin	mAb SEC purity	Yield	HCP（ppm）	ProA（ppm）
NMab™ Pro	99%	99.2%	354.8	13.0
*PrismA	99%	98.8%	500.2	6.0

Life Cycle Study

The dynamic binding capacity (DBC), purity, and recovery performance of the NMab Pro resin have been tested in monoclonal antibody purification for 100 cycles. Each cycle includes cell culture medium load, wash, elution, CIP and re-equilibration steps. As shown in figure 8-10, during the continuous purification process, the mAb dynamic binding capacity of NMab™ Pro (DBC is measured once every 20 cycles) has no obvious downward trend. Recovery is maintained at more than 95% and the purity of the eluted sample (the value is measured once every 10 cycles) is >99%.

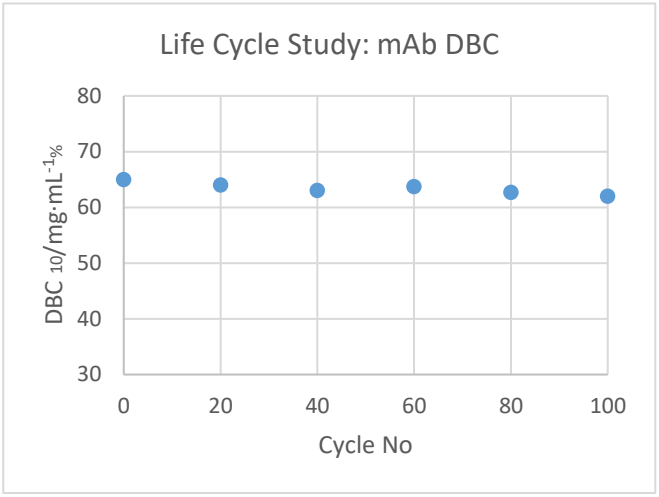


Figure 8. *NMab™ Pro life cycle DBC trend*

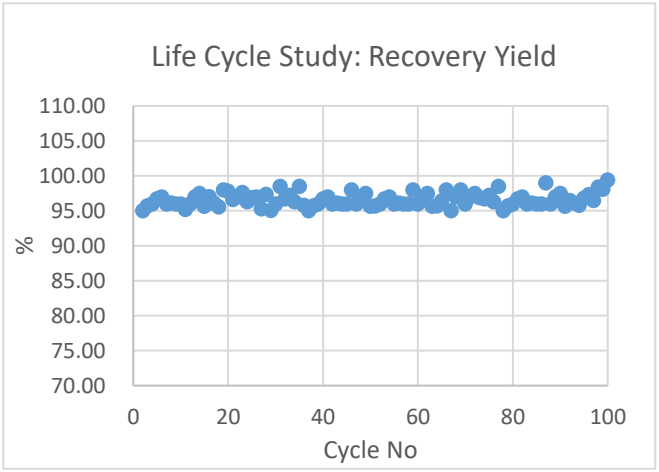


Figure 9. *NMab™ Pro life cycle recovery yield trend*

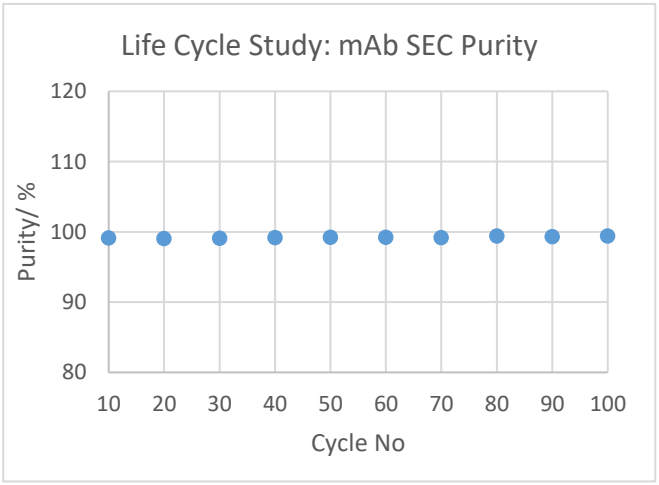


Figure 10. *NMab™ Pro life cycle SEC purity trend*

Operation Instructions

Column Packing

The NMab™ Pro resin is delivered in 20% ethanol at 60% slurry concentration. In order to get the best packing, we recommend pure water as the slurry solvent for column packing. The specific column packing method is as follows:

1) First calculate the column volume (Vc) of the desired chromatographic column: $Vc = h \times \pi r^2$
   - h: the height of the resin bed;
   - r: the radius of the column.
2) Gently agitate the resin in the original container to make it homogeneously dispersed in the liquid. Measure the volume of the required stock solution*;

   **Under normal circumstances, the chromatographic medium will be compressed under pressure to cause volume shrinkage. In order to obtain a compact column bed, it is recommended that the volume of the packing slurry be, generally about 1.15-1.2 times the desired packed bed volume.*
3) Transfer the required volume of resin to an appropriate container and replace the solvent with pure water through decanting (or replace the solvent in the column).
4) Adjust the slurry concentration to 50-70% (volume ratio).
5) Use a pump to pack the column within the recommended pressure range. It is recommended to pack the column with a low flow rate and constant flow first, and then pack the column with a high flow rate and constant pressure. Stop the pump, move the piston to the bed surface, and then press down 5 mm.

Column Efficiency Evaluation

After the chromatographic column is packed, equilibrate it with 0.5 M NaCl at a flow rate of 50~200 cm/h and conduct a column efficiency test using a isocratic chromatographic run with 2M NaCl as the injection sample. The specific test parameters are shown in the following table:

***Table 4. NMab™ Pro** column efficiency test*

Sample	2 M NaCl
Sample injection	1 %~5 % of bed volume
Mobile phase	0.5 M NaCl
Linear flow rate	50~200 cm/h
Detection	Conductivity meter

Column Operation

1）Washing and equilibration： Before use, replace the 20% ethanol storage solution in the chromatography column with the equilibration buffer; wash sequentially with the eluent (such as 100 mM Gly, pH=3.0) and the equilibration solution (such as 20 mM PBS, 150 mM NaCl, pH=7.0);

2) Sample loading: The sample is an antibody fermentation broth. Recommended loading amount is 0.8 times the DBC (@10% breakthrough);

3) Washing: Use the equilibration buffer (such as 20 mM PBS, 150 mM NaCl, pH=7.0) to wash 5 CVs;

4) Elution: Use citric acid, acetic acid or glycine (such as 100 mM Gly, pH=3.0) as the eluent ca. 5 CVs to the or until baseline is achieved;

5) Strip: 1 M acetic acid ca. 5 CVs;

6) SIP/CIP: 0.1-0.5 M NaOH solution ca. 3-5 CVs;

7) Re-equilibration: Use an equilibration buffer (such as 20 mM PBS, 150 mM NaCl, pH=7.0) to wash 5 CVs to achieve stable baseline;

8) Storage: After use, first replace the buffer salt in the column with pure water, and then equilibrate with 20% ethanol and store.

**Note: During use, all samples and mobile phases must be filtered with a 0.45 μm filter.*

Ordering Information

Product Name	Packaging Size	Product Code
NMab™ Pro	30 mL	17010-150100-2030
	100 mL	17010-150100-2100
	500 mL	17010-150100-2500
	1 L	17010-150100-1001
	5L	17010-150100-1005
	10 L	17010-150100-1010

Note：NMab™ Pro can be offered as prepacked columns of 7 mm × 2 5 mm 、16 mm ×25 mm 、7.7 mm×100 mm or customized configurations.

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