
UniMab® 50HC

Protein A Affinity Chromatography Resin

Product Instruction Manual

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Affinity Chromatography Resin

In the global pharmaceutical market, antibody drugs have ranked top in the sales list for many years, and their market competition has become increasingly fiercer. Reducing the cost of antibody production is a key factor in enhancing market competitiveness. The key to solving the bottleneck of antibody production lies in improving the first step, Protein A affinity capture. In order to meet the demands of antibody manufacturers for affinity chromatography resin with high mechanical strength, low back pressure, good chemical stability and alkaline resistance while maintaining a high dynamic adsorption capacity at high flow rate, UniMab® 50 HC Protein A affinity chromatography resin was developed.

UniMab® 50 HC is a high-performance, alkali-resistant recombinant Protein A affinity chromatography resin produced by NanoMicro Technology using proprietary patented technology. This filler is prepared by monodisperse porous polymethyl methacrylate (PMMA) microspheres as matrix using proprietary surface modification technology and epoxy coupling protein A and is suitable for the separation and purification of monoclonal antibodies and recombinant protein biomacromolecules containing Fc (function chromatography) fragments. UniMab® 50 HC has high mechanical strength, low back pressure, good chemical stability, strong alkali resistance, and can maintain a high dynamic adsorption capacity even at high flow rate, meeting various demands from laboratory preparation to pilot and industrial production.

The new generation of UniMab® 50 HC Protein A Affinity Chromatography Resin, as the antibody purification chromatography resin, can still maintain a high dynamic adsorption capacity high flow rate. It is an ideal choice for affinity purification of large-scale monoclonal antibodies and recombinant proteins containing Fc fragments and helps to reduce the production cost of enterprises. With the following unique advantages compared with similar products on the market:

Resistant to washing by 0.1 to 0.5 M (mol/L) NaOH with long service life and low production cost;

- (b) Monodisperse high-strength hydrophilic matrix of uniform particle size allowing greater operating pressure and linear flow rate;
- (c) Adoption of alkali-resistant rProtein A and unique fixed coupling technology with lower ligand shedding and stronger alkali-resistance;
- (d) With high loading capacity of 50 mg/mL (human IgG) at high flow rate, which is significantly superior to similar products, higher full-course loading capacity and lower non-specific adsorption, thus improving the production efficiency;
- (e) With highest flow rate (~ 800 cm/h) and max. pressure (~ 0.8 MPa), significantly higher than similar products;
- (f) With uniform conditions for different antibody elution, it is an ideal choice as the antibody purification process platform;
- (g) Milder elution conditions;
- (h) Good column-packing operability and batch-to-batch reproducibility.

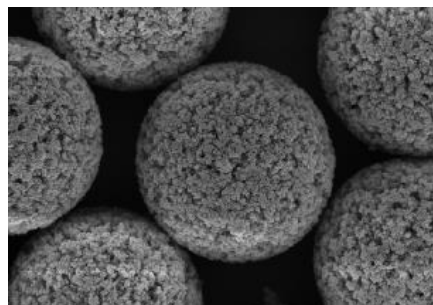


Figure 1. Electron micrograph of the UniMab® 50 HC Affinity Chromatography Resin

The technical parameters of UniMab® 50 HC are listed in the Table 1.

Table 1. Technical parameters of UniMab 50HC

Product	UniMab® 50 HC
Separation principle	Protein A affinity capture
Matrix	Polymethyl Methacrylate (PMMA)
Particle size	50 µm
Ligand coupling method	Epoxy bonding
Dynamic binding capacity*	~ 50 mg·mL ⁻¹ (human IgG, residence time of 5 min)
Maximum pressure resistance	0.8 MPa
Clean-in-place (CIP)	0.1-0.5 M NaOH
Recommended flow rate	100-800 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.
Storage	20% ethanol at 2-8°C

Alkaline resistance test

Test of the load capacity of UniMab® 50 HC after 24 hours of immersion in 0.5 M NaOH solution at 25°C:

Table 3. Experiment conditions

Equipment	Avant (NM-BD-003)
Column	NmTRAP 1mL
Sample	Monoclonal antibody, 2.83 mg/mL
Equilibrium solution	PBS
Flow rate	0.2 mL/min

Table 4. Alkaline resistance test

Load capacity (mg/mL), before immersion in alkaline	Load capacity (mg/mL), after immersion in alkaline	Reduction (%) in load capacity
53.43	43.06	19.41

Load capacity validation data

Clean in place (CIP) with 0.1M NaOH+0.5M NaOH. In each CIP cycle, the alkaline contact time is 1 hour. Afterwards, test the load capacity of UniMab® 50 HC (Figure 2)

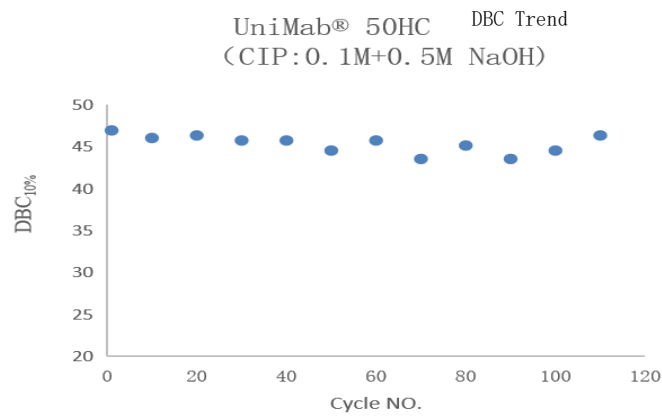


Figure 2. Load capacity data for the HCCF of XX monoclonal antibody

Overall performance evaluation

The experiment results of the purification of Antibody 3 using NanoMicro UniMab® 50 HC Affinity Chromatography Resin are presented below. The experiment showed that NanoMicro UniMab® 50 HC has excellent affinity capture ability of monoclonal antibodies.

Table 5. Experiment conditions

Equipment	Avant (NM-BD-003)
Column	NmTRAP 1 mL
Sample	Monoclonal antibody, 3.056 mg/mL
Flow rate	0.2 mL/min
Equilibrium solution	PBS
Washing solution	20 mM NaAC, pH 5.5
Eluent	20mM NaAC, pH 3.5
Regeneration	0.1M NaOH
Residence time	5 min

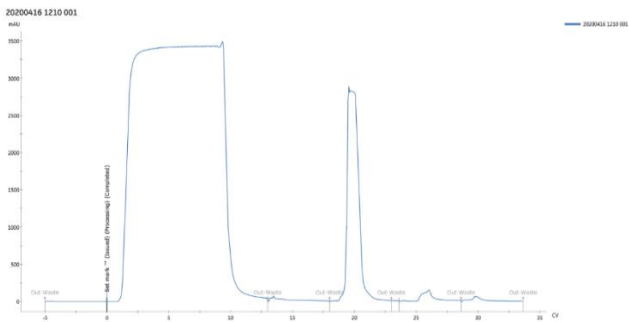


Figure 3. Affinity capture ability performance of UniMab® 50 HC in purification of Antibody 3

Table 6. Antibody purification results with UniMab®50HC

DBC 10% load capacity (mg/mL)	Recovery (%)	Purity (%)	Elution (CVs)
50.23	95.56	98.50	1.41

Operation Guidelines

Homogenate concentration determination

UniMab® 50HC 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 chromatography media to the total volume of the homogenate. For accurate determination of Cs, shake the media 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 media homogenate with 0.5 M NaCl solution.

Pretreatment of media

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

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

h: column height; r: column radius

Calculate the required homogenate volume (Vs): In general, the chromatography media 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 1.05-1.1.

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

Preparation of column loading media homogenate: Shake the chromatography media in the original container, measure the required volume of the original solution Vs into a suitable container, allow the media particles to settle naturally, then tilt the container to pour off the supernatant; wash the media 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.

Chromatographic column loading method: (using NmXK 16/20 empty chromatographic column as an example)

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 media, 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.

Column Efficiency Evaluation

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 UniMab® 50HC chromatographic column

Sample	5% (v/v) acetone aqueous solution or 2 M NaCl
Sample injection	1-5 % of column volume
Mobile phase	Deionized water or 0.5 M NaCl
Linear flow rate	50 ~ 200 cm/h
Detection	5% acetone loading: UV @ 280 nm 2 M NaCl loading: Conductivity meter
Recommended qualification specifications	As: 0.8-1.5; Plates (N/m): >2500

How to use

Rinsing and equilibration:

Rinse and equilibrate the UniMab®50HC 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 load does not exceed 0.8 times the medium DBC10%;

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 medium 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 with the UniMab® 50HC, 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 chromatography media.
	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 #
UniMab® 50HC	30 mL	17010-250100-2030
	50 mL	17010-250100-2050
	100 mL	17010-250100-2100
	300 mL	17010-250100-2300
	500 mL	17010-250100-2500
	1 L	17010-250100-1001
	5 L	17010-250100-1005
	10 L	17010-250100-1010
	50 L	17010-250100-1050
	100 L	17010-250100-1100

Note: Chromatographic prepacked columns of 7.7 mm × 22 mm, 16 mm × 25 mm, 7.7 mm × 100 mm are available. Other sizes and custom configurations are also available. Please contact us for further details.

NanoMicro Technologies, Inc.

National hotline: 400-828-1622

Chinese website: www.nanomicro.com

Headquarters address: No. 2 Baichuan Street, Suzhou Industrial Park 215123

Email: info@nanomicrotech.com

English website: en.nanomicro.com

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