UniMab® 50 HC Protein A Affinity Resin

Product Manual

文件编号：NM-W-DF-0302

版本号：A0

UniMab® 50 HC

In the global pharmaceutical market, antibody drugs have occupied the top positions in the sales list for many years, and their market competition has become increasingly fierce. Reducing the cost of antibody production is a key factor in enhancing market competitiveness. The key to solving the bottleneck of antibody production is mainly to improve the first step of Protein A affinity capture. In order to meet the needs of antibody manufacturers for affinity chromatography media with high mechanical strength, low back pressure, good chemical stability and strong alkali resistance, as well as maintaining a high dynamic adsorption capacity at high flow rates, we have developed UniMab® 50 HC Protein A affinity chromatography resin.

Product Description

UniMab® 50 HC is a high-performance, alkali-resistant recombinant Protein A affinity chromatography medium developed by NanoMicro using its proprietary technologies. The resin is made with monodisperse, crosslinked polymethacrylate matrix, proprietary surface modification and epoxy coupling chemistry for bonding Protein A. It is suitable for the separation and purification of monoclonal antibodies and recombinant protein biological macromolecules containing Fc domains. UniMab® 50 HC has high mechanical strength, low back pressure, good chemical stability, and strong alkali resistance. Even at high flow rates, it can still maintain a high dynamic adsorption capacity, which can significantly improve productivity for laboratory preparation to pilot test and industrial production. Figure 1 displays a SEM picture of the resin and Table 1 lists its characteristics and specifications.

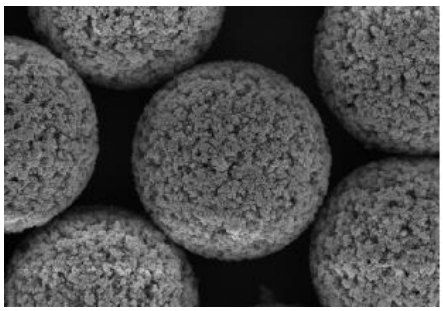

Figure 1. *SEM image of UniMab® 50 HC resin*

Product Highlights

- Monodisperse particle size and high-strength matrix, allowing greater operating pressure and linear flow rate;
- Stronger alkali resistance (0.1 to 0.5M NaOH cleaning), long life and low leachate;
- High capacity (50 mg/mL of human IgG) at a high flow rate;
- Mild elution conditions and same elution conditions for different antibodies, which is ideal for antibody purification process platforms;
- Competitive performance at affordable cost.

Table 1. *Characteristics of UniMab® 50HC resin*

Characteristics	Specifications
Matrix	Monodisperse, crosslinked polymethacrylate
Matrix	Engineered Recombinant Protein A
Particle Size	50 µm
DBC (hIgG)	~ 50 mg/mL at 4 min residence time
Maximum Pressure	8 bar (0.8 MPa)
Recommended Flow Rate	< 600 cm/h
Operation pH	3 - 12
CIP	0.1-0.5M NaOH
Protein A Leachate	Typically < 10 ppm
Storage	2 – 8 ºC in 20% ethanol

Table 2. *Characteristics of UniMab® 50HC Prepacked Columns routinely offered by NanoMicro*

Parameters	Specification
Column material	Polypropylene (PP)
Dimension（mm × mm）	7 × 25 16 × 25 7.7 × 100
DBC (hIgG)	~ 50 mg/mL Gel at 4 min residence time
Recommended Flow Rate	0.25 - 1.0 mL/min
Maximum Pressure	5 bar (0.5 MPa）

Operation Instruction

Column Packing

The concentration of the resin slurry refers to the ratio of the volume when the chromatographic medium settles to a constant volume to the total volume of the homogenate. In order to get the best protein A affinity chromatography medium column effect, we recommend 0.5 M NaCl soaking the chromatography medium, and then homogenize it before column packing. The concentration of the resin slurry is 50~70%. The specific column packing method is as follows:

1) Calculate the bed volume Vc,

$Vc = h \times \pi r^2 \times$

Vc:bed volume; h:bed height; r: column radius diameter。

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 1.1 times the desired packed bed volume.*

4) Transfer the required volume of resin to an appropriate container and replace the solvent with 0.5M NaCl through decanting (or replace the solvent in the column).

5) Adjust the slurry concentration to 50-70% (volume ratio).

6) 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 3. *Conditions for 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.*

Pressure-Flow Characteristics

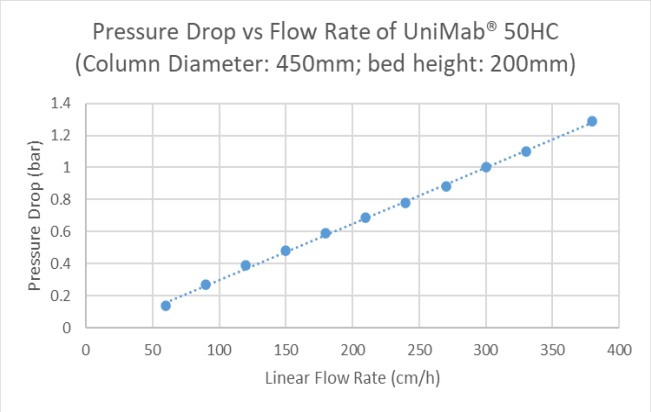

Figure 2. UniMab® 50HC's Pressure-flow curve of 45cmID×20cm column

UniMab®50HC Life Cycle Study

The dynamic binding capacity (DBC) of the UniMab® 50HC 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. CIP uses 0.1M NaOH for 1 hour exposure and uses 0.5M NaOH after every 10 cycles. As shown in figure 3, during the continuous purification process, the mAb dynamic binding capacity of UniMab® 50HC (DBC is measured once every 10 cycles) has no obvious downward trend.

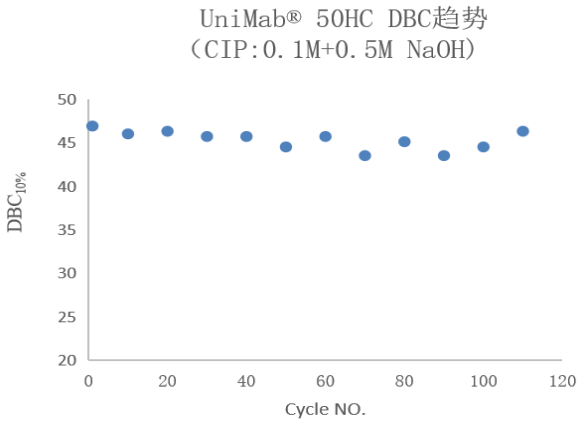

***Figure 3**. DBC lifecycle of UniMab® 50HC*

mAb Purification Example

The following example displays UniMab® 50HC 's performance of a mAb supernatant culture medium. The resin demonstrates highly competitive performance in terms of mAb purity, recovery yield, HCP clearance and protein A leaching levels.

Test conditions:

Instrument: *AKTA avant;*
Column Dimension: *7 mm × 25 mm*
Loading Sample: *a mAb of 3.056 mg/mL;*
Equilibration: *20 mM PBS, pH=7.4*
Washing: *20 mM HAc-NaAC, pH=5.5*
Elution: *20mM NaAC, pH=3.5*
CIP: *0.1M NaOH;*
Re-equilibration: 20mM PBS pH7.4
Residence Time: 5 min

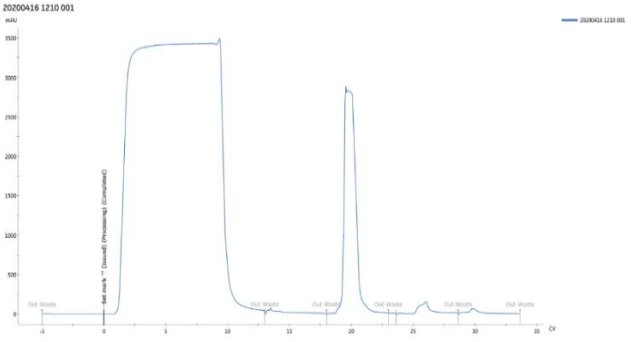

Figure 4. mAb capture using UniMab® 50 HC

Table 4. *Example of mAb Purification Using UniMab®50HC*

Resin Name	UniMab ® 50HC
DBC $_{10\%}$ of mAb（mg/ml）	50.23
Recovery yield (%)	95.56
SEC Purity	98.50%
Elution（CVs）	1.41

Ordering Information

Product Name	Packing Size	Product Code
UniMab® 50 HC	30 mL	17010-250100-2030
	100 mL	17010-250100-2100
	500 mL	17010-250100-2500
	1 L	17010-250100-1001
	5 L	17010-250100-1005
	10 L	17010-250100-1010

Note：UniMab® 50HC can be offered as prepacked columns of 7 mm × 25 mm, 16 mm ×25 mm, and 7.7 mm×100 mm or customized configurations.

Suzhou NanoMicro Technology Company Ltd.

2 Baichuan Street, Suzhou Industrial Park
Jiangsu 215123, China
Tel: (86) 512-6295 6000

NanoMicro Technologies Inc.

196 E Main Street, Suite 2C-210
Milford, MA 01757, USA
Tel: +01-(508) 338-3051

NanoMicro India Pvt. Ltd

1033 10th Floor Ithum Bldg. Tower A;
Noida Sector 64, 201301 INDIA
Tel: +91 120 424 7445

email：info@nanomicrotech.com