

UniMab® EXE

Protein A Affinity Chromatography Resin

PRODUCT INSTRUCTION MANUAL



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UniMab® EXE is the latest high-performance protein A affinity chromatography resin meeting the increasing productivity demand of bioprocessing. It is built on NanoMicro's proprietary core-shell technology (Figure 1), through modifying the rigid monodisperse base particles with polysaccharide dextran. With outstanding mechanical strength and optimal density of the alkaline-stable recombinant Protein A ligand, this macroporous affinity resin offers robust performance in antibody capturing and extended column lifetime. Non-specific protein absorption is minimal on the hydrophilic surface of the UniMab® EXE resin, and the bound monoclonal antibodies (mAbs) and bispecific antibodies (BsAbs) can often be eluted at mild acidic pH for maximal recovery of mAb monomers. The technical specifications of UniMab® EXE are listed in Table 1.

UniMab® EXE overcomes the pressure-flow issues often associated with the traditional agarose-based Protein A affinity chromatography resins. It can significantly improve productivity, provide a cost-effective solution to today's process-scale production of mAbs, bispecific antibodies, Fc fusion proteins and antibody-drug conjugates. UniMab® EXE resin come with a full regulatory support file (RSF).

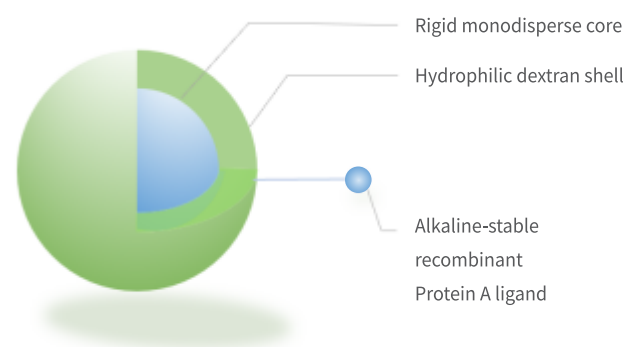


Figure 1: Schematic representation of UniMab® EXE resin design.

Table 1. Specifications

Chromatography technique	Antibody affinity chromatography
Matrix	Polymethyl methacrylate (PMMA) with dextran surface extenders, spherical
Ligand	Alkaline stable recombinant Protein A ¹
Ligand coupling method	Epoxy
Particle size (d[4,3] ²)	~ 55 µm
Dynamic binding capacity ³	55-65 mg · mL ⁻¹
Protein A leaked	~ 10 ppm
Maximum operation back pressure	≤ 0.8 MPa
Recommended flow velocity (cm/hr)	100 ~ 400
CIP	0.1-0.5 M NaOH
Delivery condition	50% Slurry in 20% Ethanol
Storage condition	2~8 °C
Shelf-life	24 months in unopened container under recommended storage condition

¹ Overproduced in *E. coli* without the use of materials from animal origin.

² Determined by MAZ3000.

³ 10% Breakthrough capacity (DBC_{10%}) at 4 min residence time, determined with 2.77 mg · mL⁻¹ human IgG in 20 mM PB + 150 mM NaCl (pH 7.4). Column size 8 x 100 mm.

Attributes of UniMab® EXE Resin

- Alkali resistant at 0.1-0.5 M NaOH; less than 10 % loss of DBC_{10%} after incubation in 0.5 M NaOH for 24 hr at room temperature;
- Consistent dynamic binding capacity for mAbs (55-65 mg · mL⁻¹ at 4 min residence time) and BsAb (>40 mg · mL⁻¹ at 5 min residence time) ;
- Efficient clearance of host cell proteins (HCPs): typically less than 1000 ppm;
- Excellent recovery of antibodies: >90 %;
- Highly pure antibodies in eluate: >99 %;
- Mild elution condition ideal for sensitive antibody molecules.

Excellent Pressure-flow Property

UniMab® EXE is designed to meet the productivity requirement of today's downstream manufacturing of monoclonal antibodies at large scale. With superb mechanical property, UniMab® EXE chromatography can be operated at high linear velocity for the reduction of processing time and minimal exposure to proteases present in cell culture, the latter is an additional benefit for labile or susceptible antibody molecules (Figure 2).

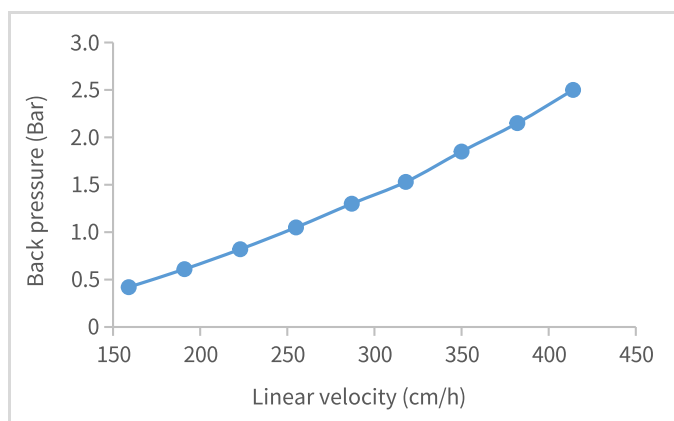


Figure 2. Pressure-flow performance of UniMab® EXE. Resin slurry prepared in 0.5M NaCl was packed into a 200x185 mm column with a compression factor of 1.15.

High Binding Capacity for Monoclonal Antibodies

The open-pore structure of UniMab® EXE facilitates efficient mass transfer of large biomolecules. It exhibits exceptional $DBC_{10\%}$ for monoclonal antibodies of diverse structures and applications, which demonstrates its wide utility in large-scale production of these protein therapeutics (Figure 3).

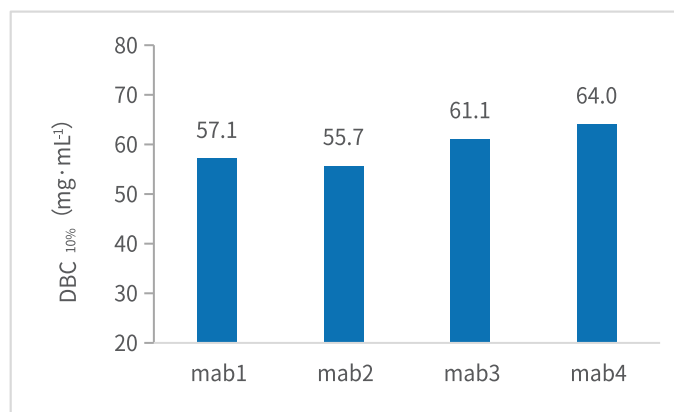


Figure 3. UniMab® EXE's dynamic binding capacity for various monoclonal antibodies.

Effective Capturing of Bispecific Antibodies

The purification of bispecific antibodies is challenging due to their unique conformations and often compromised stability. The optimized surface properties and open-pore structure of UniMab® EXE makes it the resin of choice for a variety of bispecific antibodies (Figure 4).

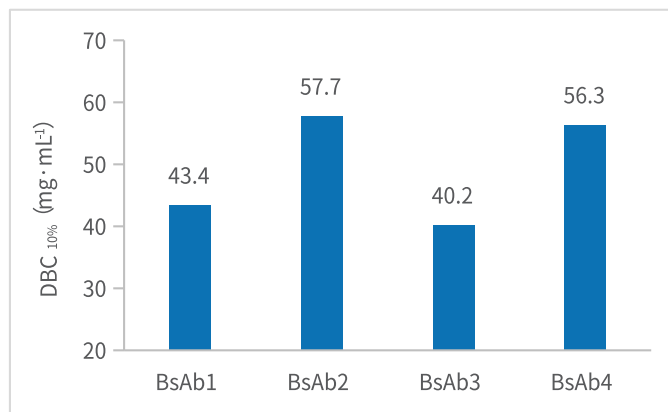


Figure 4. UniMab® EXE's dynamic binding capacity for various bispecific antibodies.

Robust Purification Performance

UniMab® EXE can be used to capture monoclonal antibodies from clarified cell culture harvest (Figure 5). Its purification performance, including the purity and recovery of target mAb molecules, the clearance of host-cell proteins, and level of leached recombinant protein A fragment (rPA) are summarized in Table 2. The hydrophilic resin surface as well as the unique presentation of affinity ligand allows the elution of bound antibodies from UniMab® EXE under mild acidic condition (~ pH 4.0) for minimal aggregation and/or degradation, which helps protect the integrity of target molecules.

Chromatography Method

Feedstream: Clarified cell culture harvest containing target monoclonal antibodies

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

Post-loading wash: 20 mM NaAc-HAc, pH 5.5

Elution: 20 mM NaAc-HAc, pH 4.0

Regeneration: 0.1 M NaOH

Residence time: 5 min

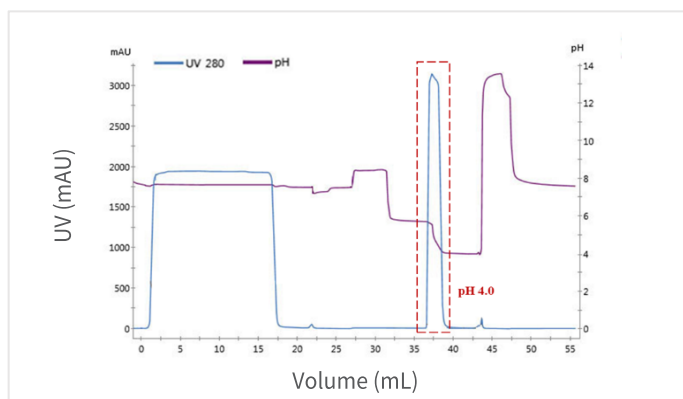


Figure 5. Capture of monoclonal antibodies from clarified cell culture harvest using UniMab® EXE affinity chromatography.

Table 2. Purification performance of UniMab® EXE

Purity	Recovery	HCP	rPA
99.2%	97.9%	876.5 ppm	6.6 ppm

Outstanding Alkaline Stability

The recombinant Protein A ligand on UniMab® EXE is alkaline resistant. After exposure to 0.5 M NaOH at 25 °C for 24 hrs, UniMab® EXE maintained 95.8% of its original dynamic binding capacity for a test mAb (Figure 6). Stringent condition can be used for cleaning and sanitization, offering effective bioburden control and column fouling prevention. The extended lifetime of a UniMab® EXE column will also improve overall process economics.

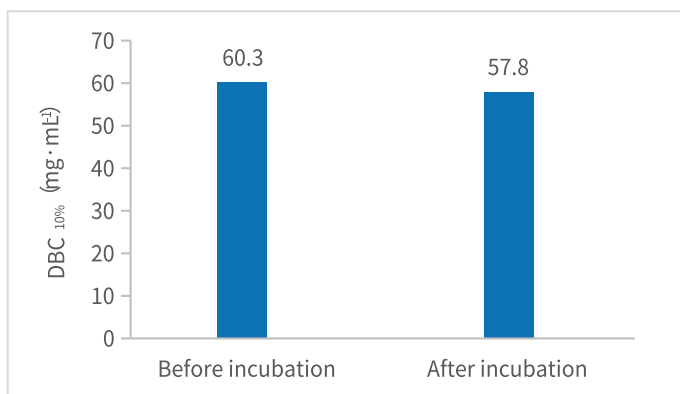


Figure 6. Stability of UniMab® EXE after incubation in 0.5 M NaOH at 25°C for 24 hrs.

Column Packing Instruction

UniMab® EXE affinity chromatography resin is supplied fully hydrated in 20 % ethanol as a ~65 % slurry (v/v). Replacement of storage solution with 5 volumes of 0.5 M NaCl solution prior to column packing is recommended.

Determination of Slurry Percentage and Resin Volume for Column Packing

Resin slurry percentage (Cs) refers to the ratio of settled resin volume (Vr) to the total volume of the slurry. To determine Cs of a resin slurry, gently resuspend resin in an appropriate vessel, then pour 10 ml of the homogeneous slurry into a calibrated cylinder and allow to settle overnight (>12 hrs). Record the volume of the settled resin bed (Vr) and calculate the slurry percentage (Cs) using Eq. 1.

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

For best results, using 50~70 % resin slurry in 0.5 M NaCl solution is recommended for packing a UniMab® EXE column.

Packing Preparation

- 1) Calculate the column volume (Vc):

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

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

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

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

Packing Unimab® EXE in An 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 solution and remove air; leave 1~2 cm of solution in the column and close the bottom outlet.
- 2) Flush column top adaptor with packing solution 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 solution. 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.

Performing Column Qualification Test

A packed column should be equilibrated with 2 CV of eluent at test velocity, such as 100 cm/hr, before the probe solution is injected. Refer to Table 3 for detailed test conditions.

Table 3. Recommended test conditions for UniMab® EXE column qualification

Probe	5 % (v/v) Acetone in nanopure water or 2 M NaCl solution
Probe volume	1 ~ 5 % Column volume
Eluent (Mobile phase)	Nanopure water or 0.5 M NaCl solution
Linear velocity	50 ~ 200 cm/h
Detector	5 % Acetone as probe: UV @ 280 nm 2 M NaCl as probe: Conductivity meter
Recommended qualification specifications	As: 0.8-1.5; Plates (N/m): >2500

Generic Protocol for Unimab® EXE Affinity Chromatography

- 1) 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);
- 2) Sample loading: No more than 80 % of pre-determined DBC_{10%} for target antibody;
- 3) Post-loading wash: 5 CV of equilibration buffer;
- 4) Elution: 5 CV of elution buffer (e.g., Sodium citrate, Sodium acetate, or Glycine, pH 3 – 4);
- 5) Regeneration: 5 CV of 1 M Acetate;
- 6) 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);
- 7) Re-equilibration: 5 CV of equilibration buffer.

Storage

Rinse column/resin fully with nanopure 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.

Trouble Shooting Guide

Problems that occasionally occur during UniMab® EXE 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 feedstream or buffers	Filter with 0.45 µm membrane; Use a 0.22 µm 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 recovery 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 50 mM NaOH + 1 M NaCl; perform CIP with 0.5 M 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

UniMab® EXE resin is available in the following sizes and formats

Format	Size	Catalog #
Bottled Resin	30 mL	17010-450100-2030
	100 mL	17010-450100-2100
	500 mL	17010-450100-2500
	1 L	17010-450100-1001
	5 L	17010-450100-1005
	10 L	17010-450100-1010
	50 L	17010-450100-1050
	100 L	17010-450100-1100
Prepacked Columns ¹	7.7 x 22 mm	77221-17010-450100
	16 x 25 mm	16255-17010-450100
	7.7 x 100 mm	77105-17010-450100

¹ Other sizes and custom configurations are also available. Please contact us for further details.



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