



MabPurix A65 Resin User Manual

1 Product Introduction

MabPurix A65 affinity chromatography resin is based on highly cross-linked agarose microspheres which possess inherent high hydrophilicity. Its surface is uniquely chemically modified and bonded with an alkali-resistant recombinant Protein A ligand that is specifically designed for the separation and purification of monoclonal antibodies and recombinant protein biomolecules containing Fc fragments.

Product Characteristics

- High binding capacity
- High hydrophilicity
- Good alkali resistance and long resin lifetime
- Polymer matrix, low back pressure, fast flow rate
- High batch-to-batch reproducibility, easy to scale up

2 Safety

For information on the safe use of this product, please refer to the Safety Data Sheet (SDS).

3 Product Properties and Application

3.1 Chemical structure and technical specifications

MabPurix A65 is an affinity chromatography resin with recombinant Protein A as the ligand. A schematic diagram of its structure is shown in Figure 1, and the detailed technical specifications of the product are provided in Table 1.

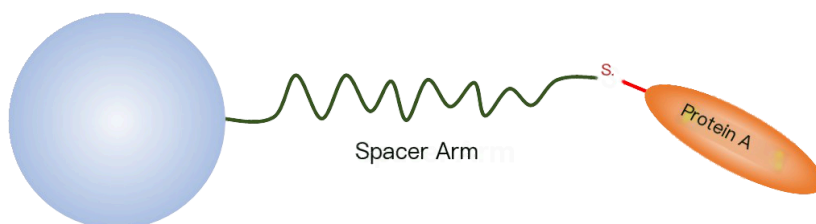


Figure 1. Schematic diagram of MabPurix A65 affinity chromatography resin structure

Table 1. MabPurix A65 Excel Affinity Resin Technical Specifications

Product Name	MabPurix A65
Matrix	Highly cross-linked agarose microspheres
Ligand	Alkaline resistant recombinant protein A
Ligand Covalent Bonding	Thioether bond
Average Particle Size*	~65 μ m
Dynamic Binding Capacity*	$\geq$ 60 mg Human IgG/mL resin (6 min residence time)
Operating Pressure	$\leq$ 0.3 MPa (3 bar)
Flow Rate *	550 cm/hr (operating pressure 2 bar)
pH Stability (operation)	3-12
pH Stability (CIP)	2-13.7
CIP	0.1-0.5 M NaOH
Operating Temperature	10-30 $^{\circ}$ C, avoid freezing
Mobile Phase Compatibility*	All commonly used buffers such as phosphoric acid and acetic



	acid. Other reagents: 8.0M urea, 6.0M guanidine hydrochloride, 20% ethanol, acetonitrile, 1.0M acetic acid, 5% isopropanol, etc
Storage Conditions	2-8 °C, 20% ethanol or 2% benzyl alcohol
Transportation Requirements	2-8 °C, 20% ethanol or 2% benzyl alcohol
Typical Applications	Antibodies, Fc fusion proteins, ADC

*Note:

1. Particle size follows a normal distribution, with the average particle size being approximately 65 µm.
2. DBC testing method: 2 mg/mL Human IgG, 6 min residence time, calculated at 10% breakthrough.
3. Testing method for flow rate: 200 mm column height, 100 mm diameter, ≤ 2.0 bar, water, 25 °C.
4. Applicable conditions for other reagents: soak resin at 25 °C for 24 hours.

3.2 MabPurix A65 Excel Purification Application Example

3.2.1 Purification of a monoclonal antibody sample - Design

Column: MabPurix A65 (6.6 × 150 mm, 5.13 mL)

Detection: UV 280 nm

Sample: Monoclonal Antibody Sample

Sample Loading: 46.4 mg/mL of resin

Purification Step	Mobile Phase	Residence Time (min)	Rinse Volume (CV)
CIP	0.1 M NaOH + 0.5 M NaCl	10	3
Equilibration	20 mM Tris-HCl + 150 mM NaCl, pH 7.2	6	5
Sample Loading	—	—	—
Post-Equilibration	20 mM Tris-HCl + 150 mM NaCl, pH 7.2	6	3
Wash 1	20 mM Tris-HCl + 1 M NaCl, pH 7.2	6	3
Wash 2	50 mM Acetic Acid (HAc) + Sodium Acetate (NaAc), pH 5.5	6	3
Elution	0.1 M Glycine-HCl + 50 mM NaCl, pH 3.5	6	3
Regeneration	0.5 M Acetic Acid (HAc)	6	3
Water Wash	H ₂ O	6	3
CIP	0.1 M NaOH + 0.5 M NaCl	10	3

3.2.2 MabPurix A65 Purification of Monoclonal Antibody Sample (Figure 2)

The sample loading capacity was 46.4 mg/mL, achieving a SEC purity of 98.45% after purification, with a yield of 96.87%. Protein A leaching was 2.04 ppm, and HCP residue was 415.26 ppm.

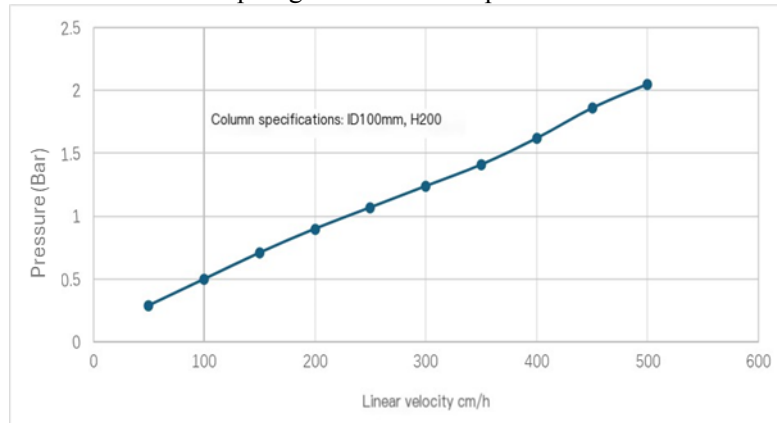
MabPurix A65 effectively enhances target sample purity while ensuring high yield and low Protein A leaching.

Figure 2. Purification chromatogram of a monoclonal antibody using MabPurix A65



4 Column Packing Recommendation

Chromatography resin requires different column packing methods for various scenarios. The packing methods for laboratory-scale columns differ significantly from those used in large-scale production. Column packing is related to the pressure-flow rate relationship. Figure 3 shows the pressure-flow rate curve of MabPurix A65.



(Mobile phase: 20 mM Na phosphate, 150 mM NaCl, pH 7.4)

Figure 3. MabPurix A65 Pressure Flow Rate Curve

4.1 Laboratory scale column packing (ID 6.6 mm - 25 mm)

4.1.1 Preparation

4.1.1.1 Packing equipment: Ensure the FPLC system (e.g. AKTA Pure25) functions normally, especially the pressure detection module and conductivity detection module.

4.1.1.2 Solution preparation: Prepare 0.1 M NaCl aqueous solution and 1 M NaCl aqueous solution.

4.1.2 Replace storage solvent

MabPurix A65 resin is stored in 20% ethanol at a 50% volume ratio when shipped. Before column packing, the 20% ethanol must be replaced with purified water. Quick Replacement Method: Mix the resin thoroughly, take the required amount of slurry, and pour it into the column tube. Install the column head and flush with purified water for 5 CV. Then, transfer the resin into a wide-mouth bottle and add purified water until the resin volume ratio reaches 50-70%. Alternatively, the storage solvent can be replaced using a sedimentation method.

4.1.3 Calculation of slurry ratio

Quick Calculation Method: Mix the slurry thoroughly until the mixture is homogenous, take 20 mL, and add it into a glass column such as a Generik FPLC 10×400 mm glass column. Open the bottom stopper to allow water to drain out until the resin settles and the bed height is stable. Measure the bed height using a ruler and calculate the resin volume. For example, if the bed height is 14 cm, the resin volume is calculated as $14 \times 0.7854 = 10.9956 \text{ mL}$, resulting in a slurry ratio of 54.98%.

Alternatively, the slurry ratio can be calculated using an overnight sedimentation method in a graduated cylinder with a resin settling time of 14–16 hours.

4.1.4 Calculation of slurry volume

$$V_{\text{slurry}} = V / P = S \times H \times F / P$$

V_{slurry} : Required slurry volume

V: Target column volume

P: Slurry ratio

H: Target column height

S: Cross sectional area of column

F: Compression factor

For example, if the cross-sectional area of a column with an inner diameter of 10mm is 0.7854 cm^2 and the target bed height for column is 18 cm, the required volume of resin is:

$$V_{\text{slurry}} = 0.7854 \text{ cm}^2 \times 18 \text{ cm} \times 1.15 / 54.98\% = 29.6 \text{ mL}$$

***Note:** A compression factor of 1.15 is applied under lab-scale conditions with water as the packing solution.

Packing steps (e.g., 10mm x 180mm column)

4.1.4.1 Use a pipette to add 29.6mL of slurry to a Generik FPLC 10 x 250mm AF chromatography column tube (using a column mounting ring).

4.1.4.2 Start the flow rate at 100 cm/h, tighten the upper column head onto the column tube, and start the pump to compress the bed at a linear flow rate of 100cm/hr. Gradually increase the flow rate by increments of 100cm/hr and maintain each flow rate for 3 min. Once the column pressure reaches 2-3 bar, maintain that flow rate for 15 min. Then stop the pump, lower the upper column head down to the target bed height at a compression factor of 1.04, and the column packing is complete.



4.2 Pilot scale column packing (ID 100 mm - 300 mm)

4.2.1 Preparation

- 4.2.1.1 Location: Clean and dust-free, temperature of 18°C -35°C, humidity of 45% -65%.
- 4.2.1.2 Packing equipment and pipelines: Wash the FPLC system and pipelines thoroughly, connect all pipelines, and check whether there is any leakage in the pipelines. If necessary, perform leak tests and verify that all parameters including pressure are displayed correctly.
- 4.2.1.3 Air Removal: Clean the column thoroughly and remove any bubbles from the upper and lower frits for later use.
- 4.2.1.4 QC testing equipment: Low pressure chromatography system.
- 4.2.1.5 Solutions: prepare 0.1 M NaCl aqueous solution and 1 M NaCl aqueous solution.

4.2.2 Replace storage solvent

MabPurix A65 resin is stored in 20% ethanol at a 50% volume ratio when shipped. Before column packing, the 20% ethanol must be replaced with 0.1 M NaCl. For manually packing a column with an inner diameter ranging from 100 mm to 300 mm, the exchange method is as follows: Mix the resin thoroughly, take the required amount of slurry, and pour it into the column tube. Open the outlet valve to allow the 20% ethanol to drain out and replace it three times with 0.1 M NaCl.

4.2.3 Calculation of slurry volume

$$V_{\text{slurry}} = V/50\% = S \times H \times F/50\%$$

V_{slurry} : Required Slurry Amount

V: Target column volume

H: Target bed height

S: Cross sectional area of column

F: Compression factor

For example, if the cross-sectional area of a column with an inner diameter of 200mm is 314cm² and the target height for column is 18 cm, the required volume of slurry is

$$V_{\text{slurry}} = 314 \text{ cm}^2 \times 18 \text{ cm} \times 1.20/50\% = 13.6 \text{ L}$$

***Note:** Compression factor of 1.20 is applied, resin is stored in 20% ethanol.

4.2.4 Packing method 1 (e.g., a 200mm inner diameter column)

4.2.4.1 After completing the replacement of the resin storage solution, resuspend the resin in the column tube and make sure the mixture is homogenous. Close the bottom valve and allow the resin to settle naturally. Once the resin bed surface has settled more than 5 cm below the column top, install the column head and make sure there are no air bubbles. Tighten the column sealing ring and open the bottom column valve. Using water as the mobile phase, start the pump and set the flow rate to 100 cm/h to accelerate the resin settling. Continue to flow until the bed height stabilizes. Once the column bed stabilizes, gradually increase the flow rate by increments of 50cm/hr to compress the column bed (e.g., 150 cm/h, 200 cm/h, etc.), maintaining each flow rate for 3 minutes. Continue until the inlet pressure reaches 2 bar $\pm$ 0.1 bar and then maintain this pressure for 30 minutes. Once the column bed has stabilized, mark the column bed height.

4.2.4.2 Stop the pump, wait for the column pressure to drop to zero, and then close the bottom valve. Rotate the four-way valve on the column head to the discharge pipe and lower the column head to the target height using a compression factor of 1.03 (based on the marked column bed height).

4.2.5 Packing method 2 (e.g., a 200mm inner diameter column)

4.2.5.1 As described in the process for replacing the storage solvent, resuspend the resin thoroughly, close the bottom column valve, and allow the resin to settle naturally. Once the resin bed surface has settled more than 5 cm below the column top, install the column head and make sure there are no air bubbles. Tighten the column sealing ring and open the bottom column valve. Using purified water as the mobile phase, start the pump and set the flow rate to 100 cm/h to accelerate the resin settling. Continue to flow until the bed height stabilizes and then mark the final bed height.

4.2.5.2 Turn off the chromatography system and wait for the column pressure to drop to zero before closing the bottom valve. Rotate the four-way valve on the column head to the liquid discharge pipe, then lower the column head to the target bed height using a compression factor of 1.15 (based on the marked column bed height).

4.3 Production scale column packing (ID 450mm and above, automatic column packing)

4.3.1 Preparation

- 4.3.1.1 Location: Clean and dust-free, temperature of 18°C-35°C, humidity of 45%-65%.
- 4.3.1.2 Packing equipment and pipelines: Wash the FPLC system and pipelines thoroughly, connect all pipelines, and check whether there is any leakage in the pipelines. If necessary, perform leak tests and verify that all parameters including pressure are displayed correctly.
- 4.3.1.3 Air Removal: Clean the column thoroughly and remove any bubbles from the upper and lower frits for later use.



4.3.1.4 QC testing equipment: Low pressure chromatography system.

4.3.1.5 Solutions: prepare 0.1 M NaCl aqueous solution and 1 M NaCl

aqueous solution.

4.3.2 Replace storage solvent

MabPurix A65 resin is stored in 20% ethanol at a 50% volume ratio when shipped. Before column packing, the 20% ethanol must be replaced with water. Calculate the required amount of slurry using the method below, pour it into the slurry tank, and allow it to settle. After settling, decant the supernatant, add an equal volume of 0.1 M NaCl, mix thoroughly, and allow it to settle again. Repeat this replacement process three times.

4.3.3 Calculation of slurry volume:

$$V_{\text{slurry}} = V/50\% = S \times H \times F/50\%$$

V_{slurry} : Required slurry volume

V: Target column volume

H: Target column height

S: Cross sectional area of column

F: Compression factor

For example, if the cross-sectional area of a column with an inner diameter of 600 mm is 2826 cm² and the target bed height for column is 18 cm, the required volume of slurry is

$$V_{\text{slurry}} = 2826 \text{ cm}^2 \times 18 \text{ cm} \times 1.20/50\% \approx 122 \text{ L.}$$

**Note:* Compression factor of 1.20 is applied, resin is stored in 20% ethanol.

4.3.4 Packing steps (e.g., a 600 mm inner diameter electric column)

4.3.4.1 Eliminate air bubbles in the resin pipeline.

4.3.4.2 Start the pump and set the linear velocity to 300 cm/h to draw the required amount of resin.

4.3.4.3 After resin transfer is complete, flush the resin remaining in the pipeline with water.

4.3.4.4 Column Compression: Lower the column head at a linear velocity of 60 cm/h to compress the resin. When the column head is 2–3 cm above the resin surface, record the resin bed height and compress the column bed using a compression factor of 1.14.

4.4 Reference standards for column efficiency testing and evaluation

After packing the MabPurix A65 column, the column efficiency testing method and evaluation standards can be referred to in Table 2.

Sample	1.0 M NaCl
Sample Volume	1.0-2.0% CV
Mobile Phase	0.1-0.5 M NaCl
Flow Rate	60-180 cm/h
Detector	Cond.
Qualified Standards	Asymmetry: 0.8-1.8; Theoretical plate number: $\geq 2000/m$

Table 2. Column Efficiency Testing Methods and Evaluation Standards

4.5 Solutions to non-ideal column efficiency

4.5.1 Tailing peak: reduce slurry concentration; increase compression factor

4.5.2 Leading peak: increase slurry concentration; decrease compression factor

4.5.3 Low column efficiency: repack column, reduce the column efficiency test flow rate

4.5.4 Peak splitting: clean or replace the frits, check test sample for impurities, degradation products, or insoluble particles

4.5.5 Cracking in packed resin bed: increase packing pressure, check if the mobile phase is degassed, and fully eliminate bubbles when connecting column head

5 Troubleshooting

During the actual purification process, the following common issues may arise. Suggestions for common problems and their solutions are provided in Table 3. These recommendations can be used to identify and resolve corresponding issues, ensuring smooth progress in method development or production.

Table 3. Common Issues and Recommended Solutions in the Purification Process

Common Issue	Possible Root Cause and Recommended Solution
High back pressure	- Blockage in the column frit: Clean or replace the frit. - Contaminants clogging resin pores: Perform CIP (Clean-In-Place) operation.



Widened elution peak	- Resin aging due to repeated use resulting in reduced column efficiency: Repack the column or replace the resin. - Insufficient elution strength of the mobile phase or accumulation of contaminants in the column: Optimize elution conditions, improve CIP protocol or perform CIP more frequently. - Low flow rate or column temperature: Optimize flow rate and temperature.
Decreased yield	- Overloading the sample: Reduce the sample loading volume. - Blockage in the column: Clean the resin promptly. - Ligand leaching as the resin approaches its lifetime: Replace the resin. - Aggregation of the sample during elution: Optimize elution conditions, improve CIP protocol, or perform CIP more frequently.
Increasing CIP peaks	- Insufficient elution or CIP: Optimize elution conditions, improve CIP protocol, or perform CIP more frequently.
High protein A ligand leaching during initial purification	- Conduct a blank run of the purification process, including the CIP step, prior to initiating the first purification.

6 Cleaning In Place (CIP)

During use, chromatography resin can accumulate contaminants. The strip step and CIP effectively remove attached contaminants, maintaining the resin's cleanliness without compromising performance. Customers can adjust the intensity and duration of the strip and CIP based on the level of contamination observed during use. Below is the standard strip and CIP method for MabPurix A65 chromatography resin which can be modified as needed:

- 1) Rinse the chromatography column with 0.5-1.0 M HAC for 3 CV with a residence time of 5-8 min;
- 2) Rinse the chromatography column with water for 3 CV with a residence time of 5-8 min;
- 3) Rinse the chromatography column with 0.1 M NaOH for 3 CV with a residence time of 10 min. If the column is heavily contaminated, 0.1 M NaOH+0.5-1.0 M NaCl can be used for enhanced cleaning, or 0.5 M NaOH can be used every 10 cycles for enhanced cleaning;
- 4) Rinse the chromatography column with equilibration solution for 5 CV with a residence time of 5-8 min.

7 Sterilization

Since the 20% ethanol or 2% benzyl alcohol storage solution does not provide sterilization or pyrogen removal, it is recommended to minimize the risk of microbial contamination by treating MabPurix A65 media with either 0.1 M NaOH for 30 minutes or 0.5 M NaOH for 15 minutes before and during use.

8 Storage Guidelines

MabPurix A65 resin is sold in a storage solution of 20% ethanol or 2% benzyl alcohol. Upon receiving the resin, please store it under the following conditions:

Unopened resin:

- Can be stored long-term at 2–8°C. The shelf life is 60 months.

Used Resin:

- Short-term storage in the packed column: If the packed chromatography column will be used again in the short term, it is recommended to store the resin in the column. Flush the column with 5 CV of a storage solution consisting of 0.1 M NaAc-HAc, 2% benzyl alcohol, pH 5.0. Seal the column and store at $\leq 25^{\circ}\text{C}$. Replace the storage solution every two months.
- Storage of resin removed from the column: If the packed chromatography column will not be used for an extended period, flush the column with 5 CV of 20% ethanol, disassemble the column, and transfer the resin to a container. Seal the container and store it at 2–8°C. To prevent ethanol evaporation or microbial growth, replace the storage solution with fresh solution every three months.

9 Disposal and Recycling

Since the MabPurix A65 resin is difficult to degrade naturally, it is recommended to dispose of it through incineration or by outsourcing to a third-party disposal service.

10 Ordering Product

Product Name	Type	Particle Size	Product Number
MabPurix A65	Affinity	65 μm	270765990

Prepacked column specifications: 5.0 mL; Packaging specifications: 1 L, 5 L, 10 L, 50 L



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