Monofinity A Resin Prepacked Column

Cat. No. L00433 Version 06162017

I Product Description

Protein A affinity chromatography resin is the most commonly used method for isolation and purification of IgG. Protein A is a cell wall component found in several strains of *Staphylococcus aureus*. It has five high affinity binding sites capable of binding specifically to the Fc region of immunoglobulin molecules from several species. Covalently immobilized Protein A matrices have been extensively used to purify IgG from several species of mammals. Monofinity A Resin Prepacked Column can also be used for immunoprecipitation of proteins, protein complexes or antigens. The alkali tolerant protein A Resin (Monofinity A Resin Prepacked Column, Cat. No. L00433) is made of recombinant protein A as ligand, which not only keeps the specific binding capacity to Fc region of immunoglobulin molecules, but also tolerates alkaline conditions. It can withstand rigorous Cleaning-in-place (CIP) procedures with 0.1 to 0.5 M NaOH. As a high quality antibody purification resin, it can be used for large scale antibody purification, and meet the purification requirement of industry-scale customers.

II Key Features

- High IgG binding capacity
  Each milliliter of the resin can bind over 30 mg human IgG (h IgG)
- Good pressure resistance
  Made of rigid base matrix, the resin can withstand a pressure up to 0.3 MPa
- Low ligand leakage during elution
  The leakage is less than 15 ng ligand (protein A)/ mg purified H IgG.
- Enhanced alkali stability

Column compatibility: The 5 mL and 1 mL columns are compatible with ÄKTA™ chromatography systems. Additional adaptors may be needed for other systems. The optimal flow rate is 1 mL/min for the 1 mL column, and 5 mL/min for the 5 mL column. For preserving the column integrity, please do not exceed a flow rate of 4 mL/min for the 1 mL column and 15 mL/min for the 5 mL column (The mobile phase of the test is aqueous buffers at room temperature and the alarm pressure is 0.3 MPa).

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Table 1. Characteristics of Monofinity A Resin Prepacked Column Prepacked Column

Resin Volume	1 mL x 1 (L00433-11); 1 mL x 5 (L00433-15); 5 mL x 1 (L00433-51); 5 mL x 5 (L00433-55)
Ligand	Alkali-Tolerant Protein A
Number of IgG binding sites	2 per ligand
M.W. of ligand	Approximately 14 kDa
Dynamic binding capacity	> 30 mg human IgG/ mL resin
Matrix component	Highly-crosslinked 4% beaded agarose
Average particle size	90 μm (45-165 μm)
Storage solution	20% ethanol
Shelf life	18 months when stored unopened
Clean in place	0.1-0.5M NaOH
Storage conditions	2-8 °C

Table 2. Chemical reagents compatible with Monofinity A Resin Prepacked Column

Detergents	Reductants	Chelates	Others
1% NP-40	2 mM	20mM EDTA	1mM PMSF
1% Triton X-100	β-Mercaptoethanol		5% glycerol
0.1% SDS			

III Purification protocol

Buffer Preparation

All solutions must be made with double deionized water. It is recommended to filter the buffers and samples through a 0.45 μm filter before use.
Binding /Wash buffer: 0.15 M NaCl, 20 mM Na_2HPO_4, pH7.0
Elution Buffer: 0.1 M Glycine, pH 3.0;
Neutralization Buffer: 1 M Tris-HCl buffer, pH 8.5

Sample Preparation

To insure a proper sample ionic strength and pH for optimal binding, it is necessary to dilute serum samples, ascites fluid or cell culture supernatant at 1:1 or higher ratio with Binding/Wash Buffer. Alternatively, dialyze the sample overnight against Binding/Wash Buffer.

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Column Purification

We recommend using flow rate of 1 mL/min for the 1 mL column and 5 mL/min for the 5 mL column.

1) Wash out the ethanol preservative with at least 5 column volumes of distilled water or binding buffer.
2) Equilibrate the column with 5 to 10 column volumes of binding buffer.
3) Apply the sample onto the column. Collect the flow-through for measuring the binding efficiency to the resin by SDS-PAGE.
4) Wash the column with 5 to 10 column volumes of Binding/Wash Buffer or until the absorbance of the effluent at 280 nm is stable.
5) Elute the immunoglobulins with 2 to 5 column volumes of Elution Buffer but other volumes (or different elution buffer) will be required to elute strongly bound IgG
6) Collect the eluate and immediately neutralize to pH 7.0 with Neutralization Buffer (1/10 volume of total eluate).

IV Cleaning-in-Place (CIP)

Cleaning-in-Place (CIP) procedure is used to remove very tightly bound, precipitated or denatured substances from the purification system. The accumulation of such contaminants may affect the chromatographic properties of the column, reduce the capacity of the column and, potentially, come off in subsequent runs and contaminate the purified antibody. For native protein A resin, detergents are commonly used to clean and regenerate the column. However, some contaminants cannot be removed under those conditions, and will affect the future use of the column. For Monofinity A Resin Prepacked Column, 0.1-0.5 M NaOH wash is recommended for CIP due to its enhanced alkali tolerance. Using NaOH as CIP agent can resolve most of the resin contamination problems faced while using native protein A resin, because NaOH can dissolve proteins and saponify fats well.
There is no significant decrease of binding capacity after CIP with 0.1M NaOH for 200 cycles. When using 0.5M NaOH for 100 cycles, the resin can still remain 80% of its binding capacity.

CIP protocol*

1) Wash the column with 3 column volumes of binding/wash buffer.
2) Wash with at least 2 column volumes of 0.1 or 0.5 M NaOH with a contact time of 10-15 minutes.
3) Wash immediately with at least 5 column volumes of sterile and filtered binding buffer to neutralize.

*CIP is usually performed immediately after the elution. In general, we recommend cleaning the column at least every 5 cycles during normal use. A commonly adopted CIP protocol is to use 0.1 M NaOH every cycle and 0.5 M NaOH every 10 cycles.

V Storage

Store the Monofinity A Resin Prepacked Column in Binding/Wash Buffer containing 20% ethanol at 2°C to 8°C.
Do not freeze.

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VI Troubleshooting

Decrease in the rate of antibody recovery Lower than expected antibody yield	• Sample overloaded. Reduce the sample load. • Antibodies precipitated during elution. Change the elution conditions. • Insufficient elution and CIP. Optimize the elution conditions, perform CIP more frequently.
High backpressure during the run	• Blocked/clogged column. Perform CIP to clean the column. • Clogged adapter net/filter. Replace the net/filter.

VII Ordering Information

Product Name	Cat. No.
Monofinity A Resin Prepacked Column	L00433
Protein A Resin FF	L00464
Protein G Resin FF	L00664
Protein L Resin	L00239
Protein G Resin FF Prepacked Column	L00681
Protein A MagBeads MX	L00672
Protein G MagBeads MX	L00673

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