Monofinity A Resin **Cat. No. L00433**

Technical Manual No. TM0640 **Updated 11192015**

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I. DESCRIPTION

Protein A affinity chromatography resin is the most commonly use 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 can also be used for immunoprecipitation of proteins, protein complexes or antigens. The alkali tolerant protein A Resin (Monofinity A Resin，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 Ig G.

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- Enhanced alkali stability
  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.

These features make the alkali-tolerant Protein A Resin an ideal choice for monoclonal antibody purification on an industry scale, especially at industry scale. The characteristics of the medium are summarized in Table 1.

Table 1. Product information (Characteristics) of Monofinity A Resin

Package format	Available in several package sizes of 50% slurry
Matrix	Highly-crosslinked 4% beaded agarose
Average bead size	～ 90 μm
Ligand	Alkali-Tolerant Protein A
Coupling method	Epoxy activated
Binding capacity	> 30 mg h IgG/ml resin
Chemical Stability	Stable with all commonly used reagents during the purification process
Working pH	3-12
Clean in place	0.1-0.5M NaOH
Linear flow velocity	50-300cm/h
Storage	20% ethanol， 2 - 8℃

Table 2. Chemical reagents compatible with Monofinity A Resin

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

Alkali tolerance (stability in alkaline conditions) of Monofinity A Resin was tested in terms of dynamic binding capacity using 0.1 or 0.5 M NaOH CIP. The testing data were showed in the figure below (Figure 1.).

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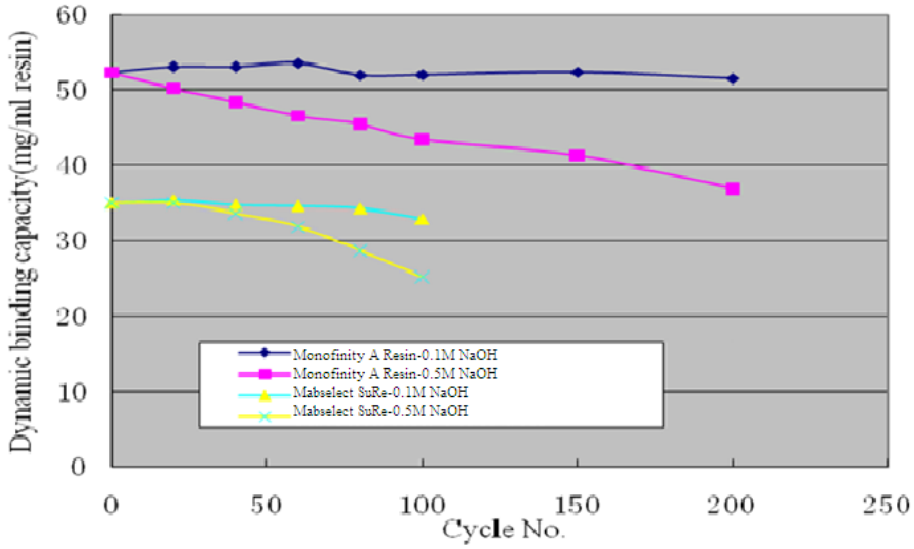
Figure 1. Dynamic change of the binding capacity of Monofinity A Resin during 200 Cleaning-in-Place cycle with 0.1 M or 0.5 M NaOH.



Each Cleaning-in-Place (CIP) cycle in figure 1 includes the following 6 steps：

1. Wash with 5-fold bed volume binding buffer
2. Load sample
3. Wash resin with 10-fold bed volume binding buffer
4. Wash with 5-fold bed volume elution buffer
5. 0.1M NaOH or 0.5M NaOH, wash 15min
6. Wash with 5-fold bed volume binding buffer

The binding capacity was tested with human IgG every 20 cycles. The binding capacity of a competitor's similar product (An alkali tolerant protein A resin from GE Healthcare-Life Science) is included for comparison.

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III. EXAMPLE

Figure 2. Profile of OD_{280} and pH monitored during the purification of a monoclonal antibody.

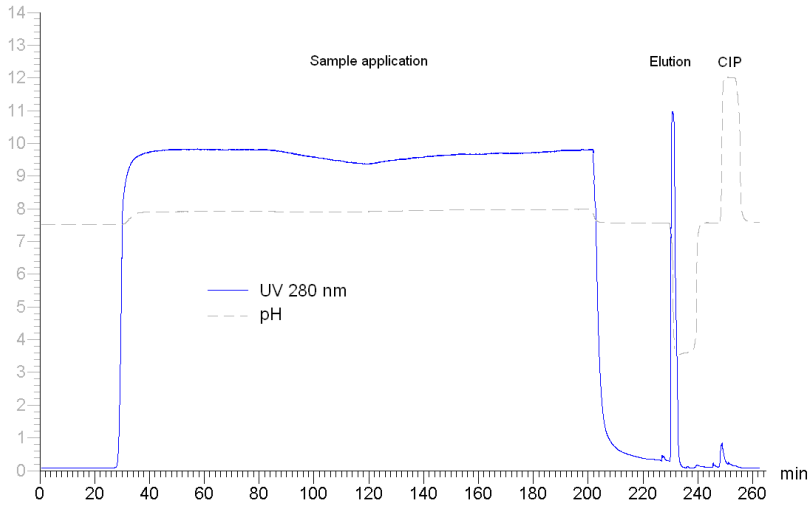
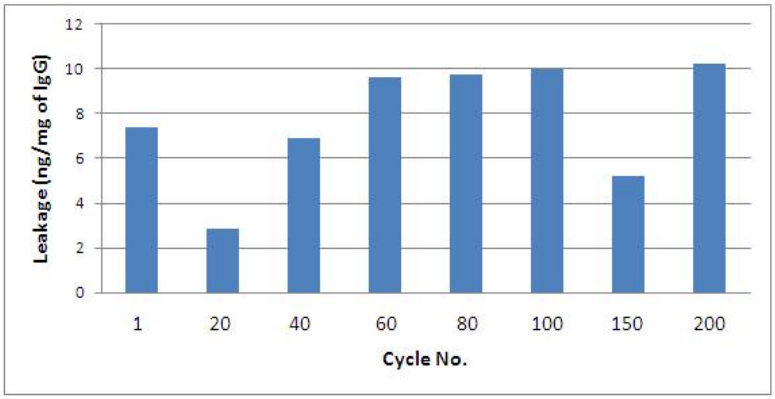


Figure 2 shows an example of purification of a monoclonal antibody from a clarified mammalian cell culture medium when using Monofinity A Resin. The antibody sample was loaded on the column at 30 mg h IgG per mL resin. The final recovery rate/yield of the antibody is 95% of highly purified antibody.

Figure 3. Ligand leakage of Monofinity A Resin during 200 washing cycles with 0.1M NaOH. The flow velocity of Monofinity A Resin under different pressures (Figure 4)



Monofinity A Resin has a characteristic of low ligand leakage (< 20 ng/mg antibody) .The leakage of ligand was measured every 20 cycles as shown in Figure 3.

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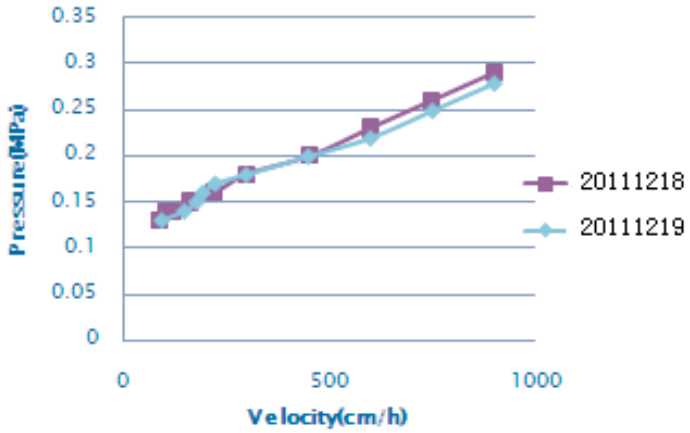
Figure 4. Pressure/flow curve for a pre-packed column with 1ml Monofinity A Resin. 20111228 and 20111229 represented different batch of the resin.



IV. PREPARATION

All solutions should be made of 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

Elution Buffer：0.1 M Glycine，pH 3.0；

Neutralization Buffer：1 M Tris-HCl buffer，pH 8.5

Purification Protocol

Here we use a column of 1 ml bed volume as an example to explain the purification procedure. The size of the column and volumes of the reagents can be scaled up according to the needed sample amount.

- **Sample Preparation**

Equilibrate all materials to room temperature. 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.

- **Packing of Column**

1. Rinse the pump and column with ddH_2O.
2. Resuspend the resin slurry completely and transfer 2 ml slurry (50%) to the clean column, in which about 2 ml Binding/Wash Buffer was added in advance.
3. Allow the resin to settle and the storage buffer to drain from the column.
4. Add 10 ml Binding/Wash Buffer onto the column with a pump to equilibrate the resin at a flow speed of about 1 ml/min, and drain the buffer.

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- **Sample Purification**

  1. Apply the sample onto the column and drain the flow-through with a flow speed of about 1 ml/min. Collect the flow-through for measuring the binding efficiency to the resin, i.e. by SDS-PAGE.
  2. Wash the column with 40 to 80 ml Binding/Wash Buffer and drain the buffer with a flow speed of about 2 ml/min**,** or until the absorbance of the effluent at 280 nm is stable.
  3. Elute the immunoglobulins with 10-15 ml Elution Buffer and drain the eluate with a flow speed of about 1 ml/min. Collect and immediately neutralize the eluate to pH 7.4 with Neutralization Buffer (1/10 volume of total eluate).

- **Sample test**

  1. Measure the antibody concentration in eluate。
  2. Take some flow-through, effluent, and elute and inspect the purity of the antibody using 15% SDS-PAGE electrophoresis.

V. Cleaning-in-Place (CIP)

Cleaning-in-lace (CIP) is 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 future use of the column. For Monofinity A Resin, 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 by customers who use the native protein A resin, because NaOH can dissolve proteins and saponify fats well.

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 neutral.

*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.

VI. STORAGE

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

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VII.TOUBLESHOOTING

High ligand leakage during the 1st purification cycle	Run a blank cycle, including CIP, before the first purification cycle of real sample on a new column.
Decrease in yield	• Sample overloaded. Reduce the sample load. • Antibodies Precipitate 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 as needed.

VIII. ORDERING INFORMATION

Product Name	**Cat. No.**
Monofinity A Resin	L00433
Protein A Resin	L00210
Ultra Protein A Resin	L00400
Protein A MagBeads	L00273

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