Protein A CIP Resin **Cat. No. L00433**

Technical Manual No. TM0640 **Version 09182012**

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I. Description

Protein A affinity chromatography resin is the most commonly applied method for the 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. The alkali tolerant protein A Resin (Protein A CIP 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) procedure 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 requirements of industry-scale customers.

II. Key Features

- **High IgG binding capacity**

  Binding capacity of > 50 mg human IgG/ml of settled resin.

- **Good pressure resistance**

  Made with rigid base matrix, the resin can withstand pressure up to 0.3 MPa

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- **Low ligand leakage during elution**

  The leakage is less than 15 ng ligand (protein A)/ mg purified human Ig G.

- **Enhanced alkali stability**

  There is no significant decrease of binding capacity after CIP with 0.1M NaOH for 200 cycles; After CIP with 0.5M NaOH for 100 cycles, the resin retains 80% of its initial binding capacity.

The combination of these four key features makes the Protein A CIP Resin an ideal choice for monoclonal antibody purification, especially at industrial scale. The characteristics of the medium are summarized in Table 1.

Table 1. **Product Characteristics of Protein A CIP Resin**

Package format	Available in several package sizes of 50% slurry
Matrix	Highly-crosslinked 4% beaded agarose
Average bead size	～ 90 μm
Ligand	Recombinant protein A
Binding capacity	> 50 mg human IgG/ml settled 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 Protein A CIP Resin

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

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

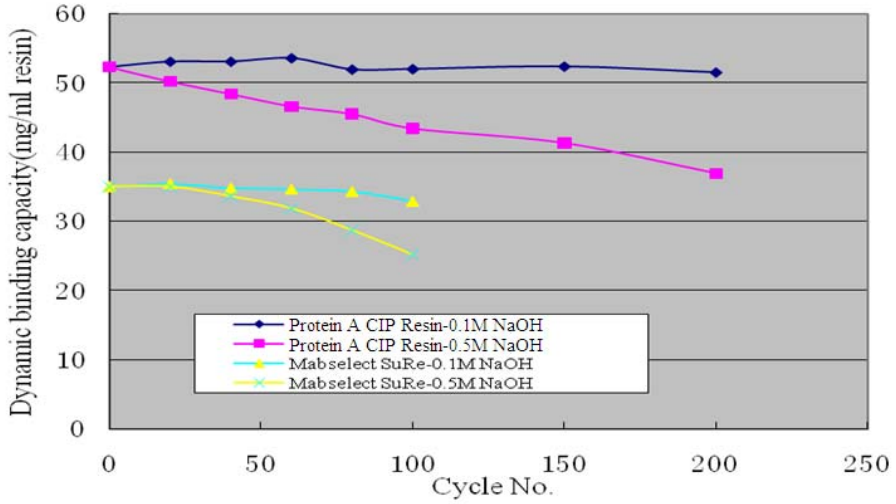

Figure 1. Changes of the dynamic binding capacity of Protein A CIP Resin during 200 Cleaning-in-place cycles with 0.1 M or 0.5 M NaOH.

Each Cleaning-in-place (CIP） cycle in figure 1 includes the following 4 steps：

- Wash with 5-fold (5x) column volume binding buffer;
- Loading sample;
- Wash resin with 10x column volume of binding buffer;
- Wash with 5x column volume of elution buffer;
- Wash with 0.1M NaOH or 0.5M NaOH， for 15min;
- Wash with 5x column volume of binding buffer;

The binding capacity was tested with human IgG every 20 cycles. The binding capacity of MabSelect SuRe (An alkali tolerant protein A resin from GE Healthcare-Life Science) is also included in the comparison.

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Example

Figure 2 shows an example of purification of a monoclonal antibody from a clarified mammalian cell culture medium when using Protein A CIP Resin. The antibody sample was loaded on the column at 30 mg human IgG per mL settled resin. The final recovery rate of the antibody is over 95%.

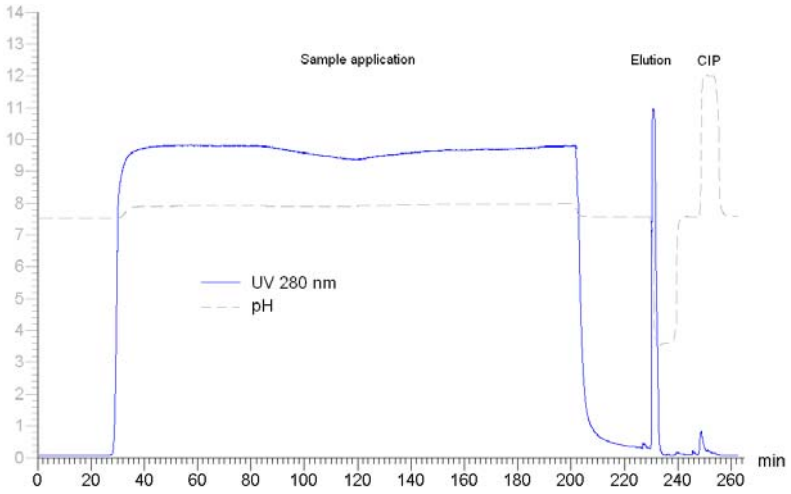

Figure 2. Profile of A_{280} and pH monitored during the purification of a monoclonal antibody.

Protein A CIP Resin has a characteristic of low ligand leakage (< 15 ng/mg antibody) .The leakage of ligand was measured every 20 cycles as shown in Figure 3.

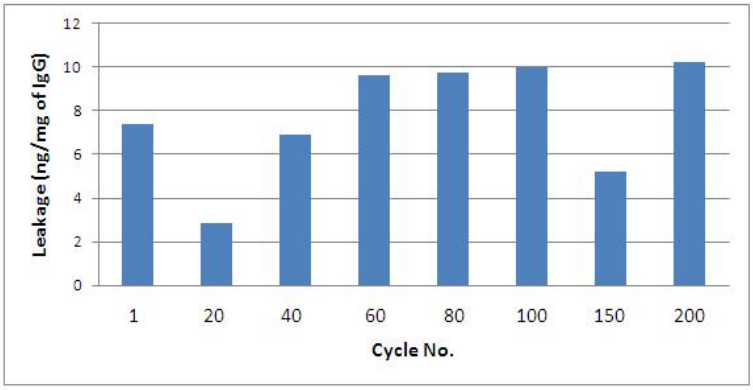

Figure 3. Ligand leakage of Protein A CIP Resin during 200 washing cycles with 0.1M NaOH.

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The linear flow velocity of Protein A CIP Resin under different pressure (Figure 4).

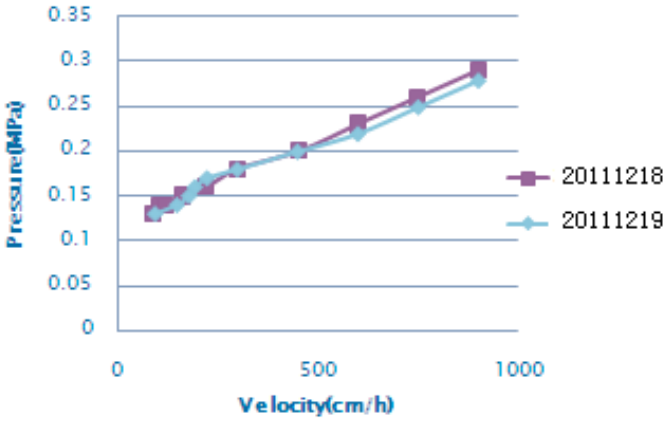

Figure 4. Pressure/flow rate correlation for a pre-packed column with 1ml Protein A CIP Resin. 20111228 and 20111229 represented different batches of the resin.

III. Buffer Preparation

All solution 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，pH7.0；

Elution Buffer：0.1 M Glycine，pH 3.0；

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

IV. Purification Protocol

The follow protocol is for a packed column of 1 ml bed volume. The size of the column and volumes of the reagents can be scaled up accordingly.

Sample Preparation

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

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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, but do not let the resin run dry.

4) Add 10 ml Binding/Wash Buffer onto the column with a pump to equilibrate the resin at a flow rate of about 1 ml/min.

Sample Purification

1) Apply the sample to the column with a flow rate of about 1 ml/min. Collect the flow-through to monitor binding efficiency, such as by SDS-PAGE.

2) Wash the column with 40 to 80 ml Binding/Wash Buffer with a flow rate 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 with a flow rate of about 1 ml/min. Collect and immediately neutralize the eluate to pH 7.4 by adding 1/10 volume of Neutralization Buffer to the total eluate.

Sample evaluation

1) Measure the antibody concentration in eluate.

2) Prepare samples of starting material, flow-through, and eluate. Evaluate purification efficiency and purity of the antibody by SDS-PAGE (e.g 15% SDS-PAGE gel).

V. Cleaning-in-Place （CIP）

Cleaning-in-place (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

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to clean and regenerate the column. However, some contaminants cannot be removed under these conditions, which will affect future use of the column. For Protein A CIP 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 at least 10 to15 minutes.

3) Wash immediately with at least 5 column volumes of sterile and filtered Binding Buffer to neutralize the resin.

*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 Protein A CIP Resin in Binding/Wash Buffer containing 20% ethanol at 2°C to 8°C. **Do not freeze**.

VII. Trouble shooting

High ligand leakage during the 1st purification cycle	Run a blank cycle, including CIP, before the first purification cycle before applying 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.

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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.
Protein A CIP Resin	L00433
Protein A Resin	L00210
Ultra Protein A Resin	L00400
Protein A MagBeads	L00273
Protein A ELISA Kit	L00430

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