



# Protein At Beads 4FF

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## 1. Product Description

**Protein At Beads 4FF** is an affinity chromatography medium designed for the purification of monoclonal antibody, polyclonal antibody and Fc tag fusion protein. The characteristics of **Protein At Beads 4FF** are summarized in Table 1. Alkali-tolerant protein A (protein At) is obtained by gene recombination technology on the base of natural protein A. **Protein At Beads 4FF** retains the capacity after 200 times repeated CIP cycles with 0.1M NaOH, approximately 80% of the initial capacity with 0.5M NaOH after 100 times repeated CIP cycles, see Figure 1. Protein At is directional immobilized to the agarose beads. The level of leakage of the ligand during elution is very low (less than 10ng ligand/mg antiodody, see Figure 2).

The base matrix of **Protein At Beads 4FF** is highly cross-linked agarose derivative with excellent chemical and physical stabilities, making it ideal for process scale applications, see Figure 3.

Table 1. Characteristics of rProtein At Beads 4FF

| Item                    | Description                                         |
|-------------------------|-----------------------------------------------------|
| Matrix Spherical        | Highly cross-linked 4% agarose beads                |
| Ligand                  | Protein At (Alkali-tolerant protein A)              |
| Static Binding Capacity | >40mg human IgG/ml medium                           |
| Particle size           | 45-165um                                            |
| pH                      | 3-12                                                |
| Chemical stability      | all reagent in the process of antibody purification |
| Cleaning-in-place       | 0.1-0.5M NaOH                                       |
| Velocity                | 50-300cm/h                                          |
| Storage                 | 1×PBS containing 20% ethanol, 2℃ - 8℃               |

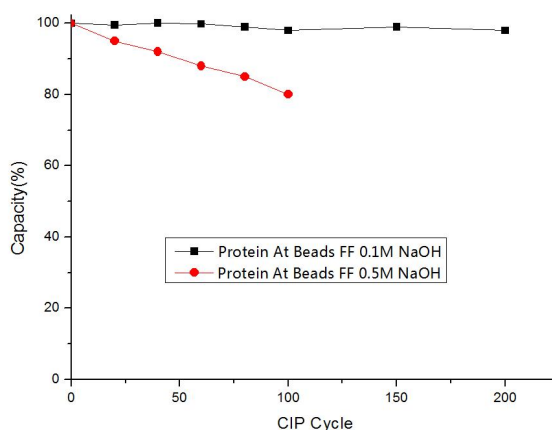


Figure 1. Dynamic binding capacity of Protein At Beads 4FF after CIP with 0.1-0.5M NaOH.



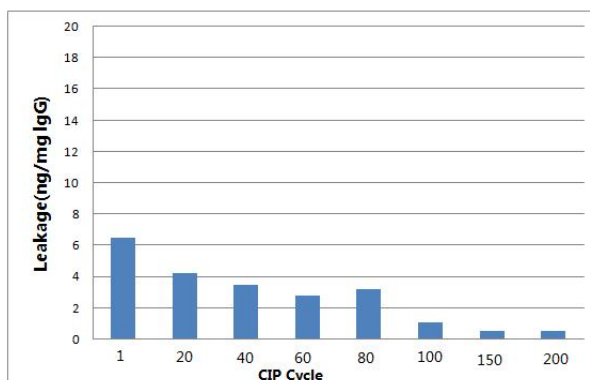


Figure 2. The leakage of the **Protein At Beads 4FF** ligand over numerous CIP cycles.

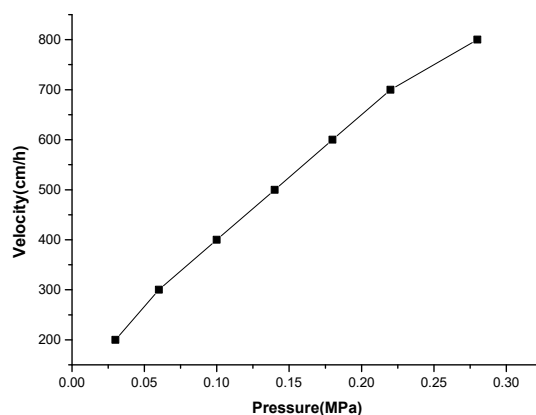


Figure 3. Pressure /flow profile for **Protein At Beads 4FF** in a packed bed (bed height 20cm i.d. 300mm) in BPG300

## 2. Purification Procedure

### 2.1 Buffer Preparation

Water and chemicals used for buffer preparation should be of high purity. It is recommended to filter the buffers by passing them through a 0.22 or 0.45  $\mu\text{m}$  filter before use.

**Binding/Wash Buffer:** 0.15 M NaCl, 20 mM  $\text{Na}_2\text{HPO}_4$ , pH 7.0

**Elution Buffer:** 0.1 M glycine, pH 3.0

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

### 2.2 Sample Preparation

To insure that proper ionic strength and pH are maintained for optimal binding, it is necessary to dilute serum samples, ascite fluid or cell culture supernatant at least 1:1 with binding/wash buffer. Alternatively, the sample may be dialyzed overnight with binding/wash buffer.

### 2.3 Packing of Column

- 1) Remove air from the column dead spaces by flushing the end-piece and adapter with packing buffer. Make sure no air has been trapped under the column net.
- 2) Close the column outlet leaving the net covered with packing buffer.
- 3) Resuspend the beads stored in its container by shaking (avoid stirring the sedimented medium). Pouring the slurry down a glass rod held against the column wall will minimize the introduction of air bubbles.

If using a packing reservoir, immediately fill the remainder of the column and reservoir with packing buffer. Mount the adapter or lid of the packing reservoir and connect the column to a pump. Avoid trapping air bubbles under the adapter or in the inlet tubing.

- 4) Open the bottom outlet of the column and set the pump to run at the desired flow velocity. Ideally, **Protein At Beads 4FF** is packed at a constant pressure of approximately 3bar (0.3MPa). If the packing equipment does not include a pressure gauge, use a packing flow velocity of approximately 400 cm/h (10 cm bed height, 25°C, low viscosity buffer). If the recommended pressure or flow velocity can not be obtained, use the maximum flow velocity the pump can deliver. This should also give a reasonable well-packed bed. Do not exceed 75% of the packing flow velocity in subsequent chromatographic procedures.





5) When the bed has stabilized, close the bottom outlet and stop the pump.

If using a packing reservoir, disconnect the reservoir and fit the adapter to the column. If using the column, carefully place the top filter on top of the bed before fitting the adapter.

6) With the adapter inlet disconnected, push the adapter down, approximately 2 mm into the bed, allowing the packing solution to flush the adapter inlet.

7) Connect the pump, open the bottom outlet and continue packing. The bed will be further compressed at this point and a space will be formed between the bed surface and the adapter.

8) Close the bottom outlet. Disconnect the column inlet and lower the adapter approximately 2 mm into the bed. Connect the pump. The column is now ready to use.

## 2.4 Sample Purification

1) Fill the syringe or pump tubing with binding buffer. Remove the stopper and connect the column to the syringe (with the provided connector), or pump tubing, "drop to drop" to avoid introducing air into the column. Remove the snap-off end at the column outlet.

2) Wash the column with 10 column volumes of binding buffer.

3) Load the sample by using a syringe fitted to the connector or by pumping it onto the column.

4) Wash the column with 5 to 10 column volumes of binding buffer or until no material appears in the effluent.

5) Elute with 5 column volumes of elution buffer. Other volumes may be required if the interaction is difficult to break.

6) Add 10µl Neutralization Buffer to each 100 µl of eluate to neutralize the pH.

## 2.5 Analysis

Identify the fractions using UV absorbance, SDS-PAGE, or western blot.

## 3. Removal of leached ligand

The ligand leakage of **Protein At Beads 4FF** is generally low (less than 10ng ligand/mg antibody). However, in many monoclonal antibody applications it is a requirement to eliminate leached ligand from the final product. There are a number of chromatographic solutions, such as cation exchange chromatography, anion exchange chromatography, or size exclusion chromatography.

## 4. Cleaning-in-place

In general, **Protein At Beads 4FF** are well suited for reuse a number of times. When precipitation and protein aggregation cause the loss of velocity and combined loads, you need to clean the medium.

**Protein At Beads 4FF** is an alkali tolerant medium and allows the use of 0.1-0.5 M NaOH for cleaning-in-place(CIP), which improves product quality and reduces overall costs. Each cycle in figure 10 consisted of:

- 3 column volumes binding buffer, pH7.4;
- 0.1 or 0.5 M NaOH, 15min contact time;
- 5 column volumes binding buffer, pH7.4

**Note:** The viscosity of 0.1–0.5 M NaOH may increase the column pressure. Customers can reverse flushing according to need.

## 5. Troubleshooting

| Problem                                            | Possible Cause                               | Solution                                                                                                                                                                          |
|----------------------------------------------------|----------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Back pressure is too high                          | The filter is blocked.                       | Clean or replace the filter.                                                                                                                                                      |
|                                                    | Column is clogged.                           | Cleaning in place(part 4).                                                                                                                                                        |
|                                                    |                                              | Filter the sample solution by passing them through a 0.22µm or 0.45µm filter.                                                                                                     |
| The curve is not stable during sample purification | There are air bubbles in buffer or sample.   | De-gas buffers and samples. Do not allow the column to dry.                                                                                                                       |
| No antibody in the elute.                          | The antibody can not be eluted.              | Reduce the pH of the elution buffer.                                                                                                                                              |
|                                                    | The antibody is unstable at low pH.          | Neutralize the eluted fractions with neutralization buffer immediately after elution.                                                                                             |
|                                                    | The IgG subclass does not bind to protein A. | Try to use the other affinity chromatography media, such as rProtein G Beads, rProtein A/G Beads or PabPur Sulfalink Beads(the specific antigen can be immobilized to the beads). |
| The recovery rate gradually decreases.             | The sample is overloaded.                    | Reduce the loading volume.                                                                                                                                                        |
|                                                    | The reduced performance of the medium.       | Cleaning in place(part 4).                                                                                                                                                        |





## 6. Related Products

| Product              | Cat. No. | Size          |
|----------------------|----------|---------------|
| Protein At Beads 4FF | SA023005 | 5 ml          |
|                      | SA023025 | 25 ml         |
|                      | SA023100 | 100 ml        |
|                      | SA023500 | 500 ml        |
|                      | SA02301L | 1 L           |
|                      | SA02310L | 10 L          |
| MabCap At 4FF        | SA023C11 | 1X1 ml        |
|                      | SA023C51 | 5X1 ml        |
|                      | SA023C15 | 1X5 ml        |
|                      | SA023C55 | 5X5 ml        |
|                      | SA023CS  | 3X1 ml+1X5 ml |

