Protein At Beads 4FF Gravity Column

Index

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 antidody, 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.

Protein At Beads 4FF Gravity Column are packed with 1ml and 5ml of **Protein At Beads 4FF**. It is easy to operate.

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℃

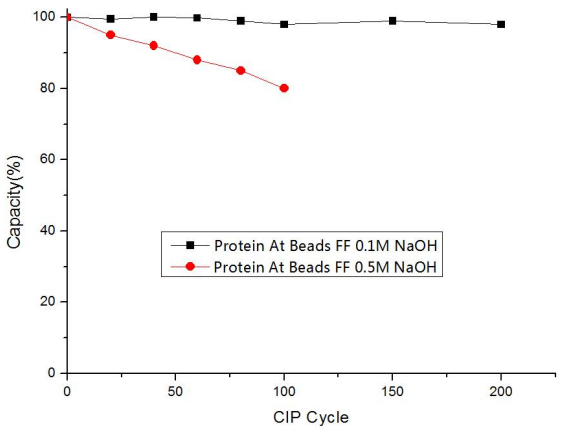

Figure 1. Dynamic binding capacity of Protein At Beads 4FF after CIP with 0.1-0.5M NaOH.

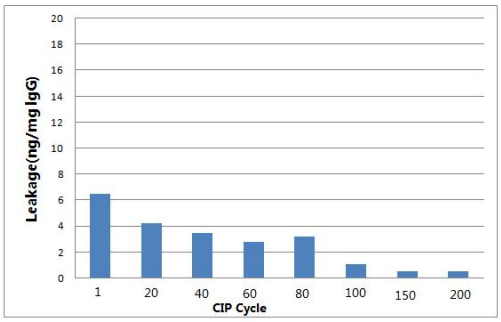

Figure 2. The leakage of the **Protein At Beads 4FF** ligand over numerous CIP cycles.

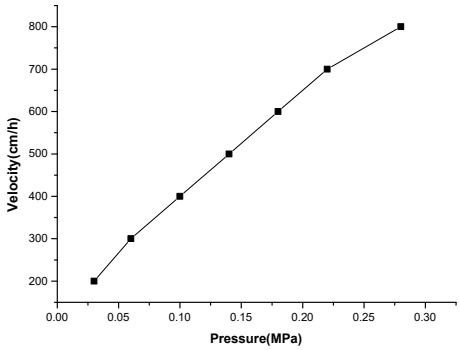

Figure 3. Pressure /flow profile for **Protein At Beads 4FF** in a packed bed （bed height 20cm i.d. 300mm） in BPG300

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 μm filter before use.

Binding/Wash Buffer: 0.15 M NaCl, 20 mM Na_2HPO_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.

2.4Column Purification

For the use of **Protein At Beads 4FF Gravity Column**, please refer to the following instructions. The amount of each solution is calculated according to the volume of the column (e.g., for 2 CV, the 1 ml size corresponds to 2 ml of solution, and the 5 ml size corresponds to 10 ml of solution). The entire purification process takes about 30 min (depending on the sample volume and the viscosity of the solution). Please refer to Figure 1 for the procedure.

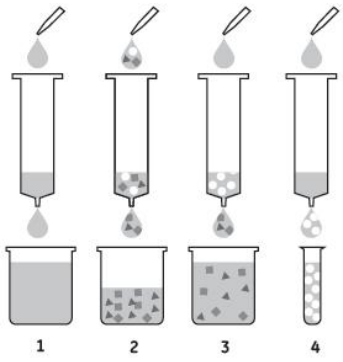

Figure 1. Schematic diagram of the protein purification process using Protein At Beads 4FF gravity columns

Step 1: Column equilibration, Step 2: Sample loading, Step 3: Washing, Step 4: Elution

1) Fix **Protein At Beads 4FF Gravity Column** on the iron stand, remove the lower and upper plugs in turn, and drain the buffer from the column. Add 5 column volumes Lysis Buffer onto the column to equilibrate the beads.
2) Apply the sample to the column and drain the flow-through. Collect the flow-through for measuring the binding efficiency to the beads, i.e. by SDS-PAGE.
3) Wash the column with 10-15 column volumes.
4) Elute the sample with 5-10 column volumes Elution Buffer and collect the eluate.
5) Add 10µl Neutralization Buffer to each 100 µl of eluate to neutralize the pH.
6) Equilibrate the column with 3 column volumes of Lysis Buffer , 5 column volumes of distilled water. Finally store the resin with 1×PBS containing 20% ethanol at 2℃-8℃.

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 a 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 increases 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 Sulfolink 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 Gravity Column	SA023GC01	1 ml
	SA023GC05	5 ml