# Con A Beads 4FF

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## 1. Product Description

**Con A Beads 4FF** is an affinity chromatography medium that conjugates Con A with agarose to purify some glycoproteins.Con A is a plant haemagglutinin separated from giant bean (Jack bean, Canavalia ensiformis) ,which can binds α - D - pyran mannose, α - D - pyran on glucose and Molecular groups related to its space position of the combination.Con A binds to carbohydrate molecules mainly in the hydroxyl part of C-3, C-4 and C-6.Con A combines better with D-pyranoid mannose than with D-pyranosaccharide.**Con A Beads 4FF** are used to isolate and purify some glycoproteins, membrane proteins, sugar lipids, polysaccharides, membrane vesicles with mannitin or glucoside residues, lgM, hormone lipoproteins and so on.Substances such as trypsin inhibitors in human serum, alkaline phosphatase, calf spleen phosphodiesterase, various variants of alpha-fetoglobulin and certain hormones such as chorionic gonadotropin (HCG) and luteinizing hormone (LH) can be purified with it.

Table 1. Characteristics of **Con A Beads 4FF**

| Item | Description |
|---|---|
| Matrix | Highly cross-linked 4% agarose |
| Ligand | Con A |
| Binding capacity | >20mg thyroglobulin /ml medium |
| Particle size (μm) | 45-165 |
| Maxi pressure | 0.3 MPa, 3 bar |
| pH stability | 4-9 |
| Storage buffer | 0.1M acetate,1MNaCl,1mM$CaCl_2$,1mM $MnCl_2$,1mM $MgCl_2$, 20% ethanol, pH6.0 |
| Storage | 2°C - 8°C |

## 2. Purification Procedure

### 2.1 Buffer Preparation

It is recommended to filter the buffers by passing them through a 0.22μm or 0.45μm filter before use.
$Mn^{2+}$ and $Ca^{2+}$ must be added to ensure adsorption activity when the buffer pH is lower than 5.0. We recommend using the following buffer for purification.
**Binding /Wash Buffer:** 20mM Tris-HCl, 0.5M NaCl, 1mM$CaCl_2$, 1mM $MnCl_2$, pH7.4
**Elution Buffer:** 20mM Tris-HCl, 0.5M NaCl, 1mM$CaCl_2$, 1mM $MnCl_2$, 0.1M-0.2M Dew α- D - methyl glucoside or α- D - methyl glucoside, pH7.4
Note: in the eluent α dew - D - methyl glucoside or α - D - methyl glucoside concentration according to physical adsorption capacity for linear or gradient elution. Mannose and glucose can also be used as eluent, but the eluent ability is weak.
For substances with strong binding capacity, the pH of the lower eluent can be eluted, but not lower than pH4.0.
Borate can be used as eluent, such as 0.1M borate, pH6.5.

### 2.2 Sample Preparation

To ensure that the sample solution has the appropriate ionic strength and pH before ascending the column, dilute the serum sample, ascites, or cell culture with a balance/wash mixture, or dialysis the sample with a balance/wash Buffer.
Samples should be centrifuged or filtered by a 0.22 μm or 0.45 μm membrane before loading to reduce impurities, improve protein purification efficiency and prevent clogging of the column.

### 2.3 Packing Columns

**Con A Beads 4FF** are widely used in industrial purification. Therefore, it involves the packing of various medium and low pressure chromatographic columns. The following describes the packing method of **Con A Beads 4FF** 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, **Con A Beads 4FF** is packed at a constant pressure of approximately 3 bar (0.3 MPa). 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 at least 5 column volumes of binding buffer.
3) Apply the sample, using a syringe fitted to the connector or by pumping it onto the column.
4) Wash with 10 to 15 column volumes of binding buffer or until no material appears in the effluent.
5) Elute with 5-10 column volumes of elution buffer. Other volumes may be required if the interaction is difficult to break.

### 2.5 Analysis

SDS-PAGE will be used to test the purification effect of the samples obtained from the purified product (including the effluents, eluted and eluted components) and the original samples.

## 3. Regeneration

In general, **Con A Beads 4FF** is 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 as follows.
Wash with 3-4 column volumes 0.1M Tris-HCl，0.5M NaCl, pH8.5 and 0.1 M NaAc, 0.5M NaCl, pH4.5, repeat the step for 3 times, then balance with 5 column volume binding buffer.

**Remove the strong binding proteins**

Wash with 2 column volumes of 0.1M borate buffer containing 1% Triton™ X-100 with pH6.5, or 20% ethanol, or 50% ethylene glycol, then immediately balance with 5 column volume binding buffer.

## 4. Related Products

| Product | Cat. No. | Size |
|---|---|---|
| Con A Beads 4FF | SA028005 | 5ml |
| | SA028025 | 25ml |
| | SA028100 | 100ml |
| | SA028500 | 500ml |
| | SA02801L | 1L |
| | SA02810L | 10L |
| PreCap Con A | SA028C11 | 1×1 ml |
| | SA028C51 | 5×1 ml |
| | SA028C15 | 1×5 ml |
| | SA028C55 | 5×5 ml |
| | SA028CS | 3×1 ml+1×5 ml |