



Chealting Beads 6FF

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1. Product Description

Chealting Beads 6FF is immobilized metal ion affinity chromatography .**Chealting Beads 6FF** with a suitable immobilized metal ion can be used to purify 6xHis- tagged proteins expressed in series of expression vectors, such as *E.coli.*, yeast, insect cells and mammalian cells. **Chealting Beads 6FF** consists of iminodiacetic acid (IDA) coupled to 6% high cross linked agarose beads.

Chealting Beads 6FF can chelated with different transition metal ions such as Cu^{2+} , Zn^{2+} , Co^{2+} , Ni^{2+} and Fe^{3+} . When choosing the desired metal ion, consider the structural requirements underlying the basis of metal chelate-protein recognition.

Ni^{2+} is usually the first choice metal ion when purifying most histidinetagged

recombinant proteins from cellular contaminants. Some histidine-tagged proteins might therefore be easier to purify with ions other than Ni^{2+} , e.g. Zn^{2+} and Co^{2+} .

In other cases, i.e. for non-tagged proteins, Cu^{2+} and Zn^{2+} metal ions are the most frequently used. Cu^{2+} ions bind strongly to a wide range of proteins and some proteins will only bind to them. Zn^{2+} ions generally give a weaker binding and in some cases this can be exploited to achieve selective elution of a target protein. In some applications, Co^{2+} , Fe^{3+} and Ca^{2+} have also been used.

Table 1. Characteristics of **Chealting Beads 6FF**

Item	Description
Matrix spherical	6% high cross-linked agarose
ion capacity	>30 μmol Cu^{2+} /ml medium
Particle size (μm)	45–165
Max.pressure	0.3 MPa, 3 bar
Storage solution	20% ethanol
Storage	4°C-30°C

2. Immobilized Metal Ion Affinity Chromatography (IMAC)

2.1 Charging the column with metal ions

1) Prepare a 0.1 M solution of the desired metal ion (Cu^{2+} , Zn^{2+} , Ni^{2+} , Co^{2+} , Fe^{3+} etc.) in distilled water.

Note:Solutions of Zn^{2+} ions should have a pH of approximately 5.5 or lower to avoid solubility problems that arise at pH6 or higher. Fe^{3+} ions should be immobilized at low pH, approximately pH3.0, to avoid formation of insoluble ferric compounds.

2) Wash the column with 3 column volumes (CV) of distilled water.

3) Apply approximately 3 CV of the metal ion solution to the column.

4) Wash the column with at least 5 CV of distilled water to remove excess of metal ions.

5) Wash the column with at least 5 CV of an acidic buffer (0.02 M sodium acetate, 0.5–1.0 M NaCl, pH 4.0)or until the pH of the effluent is 4.0. This will elute loosely bound ions that might otherwise leak out during desorption / desorption phase of the actual chromatographic step.

6) Equilibrate the column with at least 3CV of the binding buffer and the column is now ready for use.

Note: In neutral aqueous solutions, Fe^{3+} ions are easily reduced to form insoluble compounds that can be hard to remove. Columns loaded with Fe^{3+} should therefore not be left for a longer period of time in neutral solutions.

2.2. Buffer and sample preparation

2.2.1 Buffer preparation

Binding buffer: 20mM sodium phosphate, 0.5–1.0 M NaCl, 0–50mM imidazole, pH 7.0 . Binding buffer is usually carried out in the pH





interval of 5.5-8.5.

Elution Buffer: 20mM sodium phosphate, 0.5–1.0 M NaCl, 250mM imidazole, pH 7.0 or 20mM sodium acetate 0.5–1.0 M NaCl, pH 4.0

Note: The buffer and 100 mM NaH₂PO₄, 100 mM Tris·HCl, 8M Urea, pH 8.0 sample should not contain chelating agents such as EDTA or citrate and higher concentrations of competing ions such as primary amines or imidazole/histidine.

2.2.2 Sample preparation

A: Protein expressed in *E.coli* or yeast

- 1) Single colonies were cultured in LB medium. According to the instruction, adding the inducers for a period of time.
- 2) Harvest cells from an appropriate volume of bacterial culture by centrifugation at 7,000rpm(7,500×g) for 10-15min at 4°C. Discard supernatant and determine weight of pellet. Resuspend pellet in 1:10 ration(w/v) in lysis buffer and add lysozyme (0.2-0.4mg/ml cell paste ,if the host cell containing pLysS or pLysE ,it can be without lysozyme) and PMSF (1mM/ml cell paste) .
- 3) If high concentration of cell suspension ,it is consider to add 10µg/ml RNase A and 5µg/ml DNase I . Sonicate the cell suspension/lysate on ice.
- 4) Centrifuge the homogenized lysate at 15,000rpm(15,000×g) for 20min at 4°C to clarify sample. Save supernatant.

B: Protein expressed in yeast, insect or mammalian cells

- 1) Harvest the cells from an appropriate volume of culture by centrifugation at 5,000rpm(3,800×g) for 10-15min at 4°C. Save supernatant. If the supernatant without EDTA, histidine and reductant, it can be purified directly, otherwise it need dialysis to binding buffer under 4°C .
- 2) For a large volume of supernatant, it need to be concentrated.

2.3 Packing columns

- 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, the medium is packed at a constant pressure. 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 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 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.

2.5 Analysis

Identify the fractions containing the target protein. Use UV absorbance, SDS-PAGE, or western blot.

3. Regeneration

Before the medium is immobilized with a new metal ion, the medium must be stripped or regenerated.

Wash the column with one of the following solutions.





- 1) Rinse with 5 column volumes of distilled water;
- 2) 100 mM EDTA (pH 8.0), 5 column volumes;
- 3) Rinse with 10 column volumes of distilled water;
- 4) 0.5M NaOH ,5 column volumes, contacting for 10-15 min;
- 5) Rinse with 10 column volumes of distilled water;
- 6) 100mM metal ion(Cu^{2+} , Zn^{2+} , Ni^{2+} , Co^{2+} , Fe^{3+} etc.) , 3-5 column volumes;
- 7) Rinse with 10 column volumes of distilled water;
- 8) After regeneration, the medium can be used immediately , otherwise, it need to be suspended in an equal volume of 20% ethanol.

Note: Strongly bound ferric ions and ferric compounds can be removed by leaving the medium in 50mM EDTA overnight.

4. Clean-In-Place

A column used to purify protein from cell extract usually contains some soluble substances and cell debris that are nonspecifically absorbed onto the matrix. Cleaning-in-Place eliminates material not removed by regeneration and prevents progressive buildup of contaminants. If the column is to be reused , these contaminants should be cleaned from the column, as they were not completely removed during the sample clarification steps.

Remove the strong hydrophobic binding protein, lipoprotein and lipid

Wash the column with 5-10 column volumes 30% isopropanol contacting for 15-20min. Or you can choose the 2 column volumes acidic or alkaline solution containing detergents, for example, 0.1 M acetic acid solution contains 0.1-0.5% non-ionic detergent, contacting for 1-2 hours .

Finally wash the column with 10 column volumes distilled water.

Remove the proteins combined with ion interacting

Wash the column with 1.5M NaCl solution contacting for 10-15min.

Finally wash the column with 10 column volumes distilled water.

5. Related Products

Product	Cat. No.	Size
Chelating Beads 6FF	SA051005	5 ml
	SA051025	25 ml
	SA051100	100 ml
	SA051500	500 ml
	SA05101L	1 L
	SA05110L	10 L

