IMAC Beads 6FF

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1. Product Description

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

IMAC 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 histidine tagged 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 **IMAC Beads 6FF**

Item	Description
Matrix spherical	6% high cross-linked agarose
Particle size （μm）	45–165
Max.pressure	0.3 MPa, 3 bar
Storage solution	1×PBS containing 20% ethanol
Storage	4℃-30℃

2. Chelating

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 pH 6 or higher. Fe^{3+} ions should be immobilized at low pH, approximately pH 3.0, to avoid formation of insoluble ferric compounds.

2) Wash the beads 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.

3. Purification Procedure

Sodium phosphate buffers are often used. Tris-HCl can also be used, but it may reduce the binding strength if the metal-protein affinity is very low.

The most commonly used elution method for histidine-tagged proteins is based on a competitive displacement by imidazole. In other instances, elution by reducing the pH (linear or stepwise decrease in pH) is a frequently used method. If the target proteins is strongly bound, it is advisable to check its stability in an acidic environment.

Water and chemicals used for buffer preparation should be of high purity. It is recommended to filter the buffers by passing them through a 0.22μm or 0.45 μm filter before use.

3.1 Buffer preparation

Recommended buffer for his-tagged protein purification under native conditions.

Binding Buffer: 20mM sodium phosphate, 0.5M NaCl，0–10mM imidazole, pH 8.0 .

Binding buffer is usually carried out in the pH interval of 7.0-8.5.

Wash Buffer: 20mM sodium phosphate, 0.5M NaCl，10-20mM imidazole, pH 8.0

Elution Buffer:20mM sodium phosphate, 0.5M NaCl，250mM imidazole, pH 8.0 or 20mM sodium acetate 0.5M NaCl, pH 4.0

Note: The buffers should not contain chelating agents such as EDTA or citrate and higher concentrations of competing ions such as primary amines or histidine.

Recommended buffer for his-tagged protein purification under denaturing conditions.

Binding Buffer: 20mM sodium phosphate, 0.5M NaCl, 0–10mM imidazole, 8M Urea, pH 8.0 .

Wash Buffer: 20mM sodium phosphate, 0.5M NaCl, 10-20mM imidazole, 8M Urea, pH 8.0

Elution Buffer: 20mM sodium phosphate, 0.5M NaCl, 250mM imidazole, 8M Urea, pH 8.0 or 20mM sodium acetate 0.5M NaCl, 8M Urea, pH 4.0

3.2 Sample preparation

A: Protein expressed in *E.coli*

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℃. 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℃ 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 for 10-15min at 4℃. Save supernatant. If the supernatant without EDTA, histidine and reductant, it can be purified directly, otherwise it need dialysis to binding buffer under 4℃ .

2) For a large volume of supernatant, it need to be concentrated.

C: Inclusion bodies from *E.coli*

1) Harvest cells from an appropriate volume of bacterial culture by centrifugation at 7,000rpm(7,500×g) for 10-15min at 4℃. Discard supernatant and determine weight of pellet.

2) Resuspend pellet in 1:10 ration (w/v) with Lysis Buffer. Sonicate the cell suspension/lysate on ice.

3) Centrifuge the homogenized sample at 10,000rpm(15,000×g) for 20min at 4℃ to pellet the inclusion.

4) Resuspend pellet in 1:10 ration (w/v) with denaturing Lysis Buffer(containing 8M urea). Sonicate, as needed, to redissolve the pellet.

5) Analyze the concentration of the target protein and continue with purification protocols under denaturing conditions.

3.3 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.

3.4 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 10 to 15 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.

3.5 Analysis

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

Samples containing urea can be analyzed directly by SDS-PAGE whereas samples containing Gua-HCl must be buffer-exchanged to a buffer with urea before SDS-PAGE.

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. Regeneraion

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;

After regeneration, the medium can be used immediately, otherwise, it need to be suspended in an equal volume of 1X PBS containing 20% ethanol at 4°C.

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

6. Related Products

Product	Cat. No.	Size
IMAC Beads 6FF	SA050005	5 ml
	SA050025	25 ml
	SA050100	100 ml
	SA050500	500 ml
	SA05001L	1 L
	SA05010L	10 L