Ni IDA Beads 6FF

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1. Product Description

Ni IDA Beads 6FF can be used to purify 6xHis-tagged proteins expressed in series of expression systems, such as *E.coli.*, yeast, insect cells and mammalian cells. **Ni IDA Beads 6FF** consists of 90μm beads of highly cross-linked 6% agarose, to which iminodiacetic acid (IDA) has been coupled. The chelating group has then been charged with nickel ions (Ni^{2+}). This form is relatively stable plane structure, which can be destroyed by other competitive small molecule. The structure of Ni-IDA has more sites to bind histidine-tagged proteins. **Ni IDA Beads 6FF** has the advantages of higher binding capacity and cost-effective. Its high flow properties make it excellent for scale-up. See table 1.

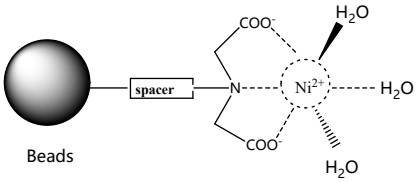

Fig.1. The chemical structures of Ni IDA Beads 6FF

Table 1. Characteristics of Ni IDA Beads 6FF

Item	Description
Matrix Spherical	Highly cross-linked 6% agarose
Static Binding Capacity	＞40 mg 6×His-tagged protein/ml medium
Particle size	45-165um
Maximum Pressure	0.3MPa, 3bar
Storage Solution	1×PBS containing 20% ethanol
Storage Temperature	4-30℃

2. Purification Procedure

2.1 Buffer Preparation

The basic principle of the following recommended buffer and other buffer is low concentration of imidazole in Lysis and wash buffer and high in elution buffer. Water and chemicals used for buffer preparation should be of high purity. It is recommended filtering the buffers by passing them through a 0.22μm or 0.45 μm filter before use. The recommended buffer is shown in table 2.

Table 2. Recommended buffer of his-tigged protein purification under native conditions

Name	Volume	Ingredient
Lysis Buffer	1L	50 mM NaH2PO4 （7.80 g NaH2PO4·2H2O） 300 mM NaCl （17.54 g NaCl） 10 mM imidazole （0.68 g imidazole） Adjust the buffer pH to 8.0 with NaOH solution.
Wash Buffer	1L	50 mM NaH_2PO_4 （7.80 g $NaH_2PO_4·2H_2O$） 300 mM NaCl （17.54 g NaCl） 20 mM imidazole（1.36 g imidazole） Adjust the buffer pH to 8.0 with NaOH solution.
Elution Buffer	1L	50 mM NaH_2PO_4 （7.80 g $NaH_2PO_4·2H_2O$） 300 mM NaCl （17.54 g NaCl） 250 mM imidazole （17.0 g imidazole） Adjust the buffer pH to 8.0 with NaOH solution.

2.2 Sample Preparation

2.2.1 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 7,000 rpm(7,500×g) for 10-15 min 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 10 μg/ml RNase A 和 5 μg/ml DNase I .Sonicate the cell suspension/lysate on ice.

4) Centrifuge the homogenized lysate at 10,000 rpm(15,000×g) for 20 min at 4℃ to clarify sample. Save supernatant.

2.2.2 Protein expressed in yeast, insect or mammalian cells

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

2) For a large volume of supernatant, it need precipitation by adding ammonium sulfate and dialysis to1XPBS under 4℃.

2.3 Packing Columns

Ni IDA Beads 6FF is easy to pack and use, and its high flow properties make it excellent for industrial scaling-up. Here we describe the packing procedure of **Ni IDA Beads 6FF** to medium pressure chromatography 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, **Ni IDA Beads 6FF** 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) Maintain packing flow velocity for at least 3 bed volumes. When the bed has stabilized, mark the bed height on the column and 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 into the column until it reaches the mark, allowing the packing solution to flush the adapter inlet. Lock the adapter in position.

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 distilled water. 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 3-5 column volumes of distilled water.

3) Equilibrate the column with at least 5 column volumes Lysis Buffer.

4) Apply the pre-treated sample, using a Loop fitted to the connector or by pumping it onto the column.

5) Wash with Wash Buffer until the absorbance reaches the baseline or no material appears in the effluent(Generally at least 10-15 column volumes).

6) Elute with elution buffer using a stepwise or linear gradient. For one-step elution, 5 column volumes are usually enough. Other volumes may be required if the interaction is difficult to break. Linear gradient elution can be used to separate proteins of different binding strengths with a small gradient, such as 20 column volumes or more.

2.5 Analysis

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

3. Cleaning-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 15-20 min. 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 10-15 min.Finally wash the column with 10 column volumes distilled water.

4. Regenerating the medium

In general, **Ni IDA Beads 6FF** may be reused before it becomes necessary to recharge them with metal ions. When the back pressure is too highor the capacity significantly lower, it need to strip the metal ions and recharge the **Ni IDA Beads 6FF** as the following procedure. Wash the column with one of the following solutions.

1) 5 column volumes of distilled water;
2) 5 column volumes of 100 mM EDTA (pH 8.0);
3) 10 column volumes of distilled water;
4) 5 column volumes of 0.5 M NaOH , contacting for 10-15 min;
5) 10 column volumes of distilled water;
6) 3-5 column volumes of 100 mM $NiSO_4$;
7) 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.

5. Troubleshooting

Problem	Probable Cause	Solution
Back pressure exceeds 3 bar	Column is clogged	Cleaning in place(part 3).
		Increase the centrifugation speed or filtering the sample.
	Sample is too viscous	Increase sonication or add DNase I (5 μg/ml with 1mM Mg2+. Incubate on ice for 15min.
	Buffer is too viscous	Dilute sample by adding more homogenization buffer.
No protein is eluted	Expression of target protein in extract is very low	Check expression level of protein by estimating the amount in the extract, flow through, elute fraction and pellet upon centrifugation. Or apply large sample volume.
	Target protein is found in the flow through	Reduce imidazole concentration in lysis buffer sample and wash buffer. Or increase buffer pH.
	Elution conditions are too mild.	Increase imidazole concentration in Elution buffer. Or decrease buffer pH.
		Strip nickel ion by using 10-100mM EDTA solution, at the same time you can obtain target protein.
	Protein degradation or purification cause the histidine tag to be removed.	Operate at 4°C. Add protease inhibitors.
		Make a new construct with his-tag attached to other terminus.
His-tagged protein is not pure	Wash is not enough	Increase the volume of Wash Buffer.
	Association between the his tagged protein and protein contaminant.	Optimize the wash condition by adjusting the pH and imidazole concentration.
		Add an additional chromatography step, that is ion exchange, hydrophobic interaction or size exclusion.

The color of medium becomes shallow.	The nickel ions was stripped.	Chelate nickel ions according to the part 4.
The medium are brown.	The buffer contains DTT.	Use Ni NTA Beads or Ni NTA Beads FF when the reductant concentration bellow 2 mM.
Protein precipitates during purification	Temperature is too low	Perform the purification at room time.
	Aggregate formation	Add solubilization agents to the samples and buffers, for example 0.1% Triton X-100 , Tween-20 and ≤20% glycerol to maintain protein solubility.

6. Related Products

Product	Cat. No.	Size
Ni IDA Beads	SA003005	5 ml
	SA003025	25 ml
	SA003100	100 ml
	SA003500	500 ml
	SA00301L	1 L
	SA00310L	10 L
Ni IDA Beads 6FF	SA052005	5 ml
	SA052025	25 ml
	SA052100	100 ml
	SA052500	500 ml
	SA05201L	1 L
	SA05210L	10 L