Cu IDA Beads

Index

1. Product Description.....1
2. Purification Procedure.....1
3. Cleaning-in-Place.....2
4. Regeneration.....2
5. Troubleshooting.....3
6. Related Products.....3

1. Product Description

Cu IDA Beads can be used to purify 6xHis-tagged proteins expressed in series of expression systems, such as *E.coli*., yeast, insect cells and mammalian cells. **Cu IDA Beads** consists of 90μm beads of 4% agarose, to which iminodiacetic acid (IDA) has been coupled. The chelating group has then been charged with copper ions (Cu^{2+}). This form is relatively stable plane structure, which can be destroyed by other competitive small molecule. The structure of Cu-IDA has more sites to bind histidine-tagged proteins. Cu IDA Beads has the advantages of higher binding capacity and cost-effective. See table 1.

Table 1. Characteristics of Cu IDA Beads

Item	Description
Matrix Spherical	4% agarose
Static Binding Capacity	＞20mg 6×His-tagged protein/ml medium
Particle size	45-165μm
Maximum Pressure	0.1MPa, 1bar
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 7,000rpm(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 和 5μg/ml DNase I .Sonicate the cell suspension/lysate on ice.
4) Centrifuge the homogenized lysate at 10,000rpm(15,000×g) for 20min 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 5,000rpm(3,800×g) 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 1XPBS under 4℃ .

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

2.3 Column Purification

1) Mix the slurry by gently inverting the bottle several times to completely suspend the **Cu IDA Beads.** Close the column outlet leaving the net covered with packing buffer. Transfer the slurry to the column.

2) Allow the resin to settle down and the buffer to drain from the column. Add 5 column volumes Lysis Buffer onto the column to equilibrate the beads.

3) 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.

4) Wash the column with 10-15 column volumes Wash Buffer or until the absorbance of the effluent at 280 nm is stable.

5) Elute the sample with 5-10 column volumes Elution Buffer and collect the eluate.

6) Equilibrate the column with 3 column volumes of Lysis Buffer , 5 column volumes of distilled water and 5 column volumes of 1XPBS containing 20% ethanol. Finally store the resin with 1XPBS containing 20% ethanol at 4℃.

2.4 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 15-20min. 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.

4. Regenerating the medium

In general, **Cu IDA Beads** may be used a number of times 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 **Cu IDA Beads** as the following procedure.

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 $NiSO_4$, 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.

5. Troubleshooting

Problem	Probable cause	Solution
Back pressure exceeds 1 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 2mM.
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
Cu IDA Beads	SA041005	5 ml
	SA041025	25 ml
	SA041100	100 ml
	SA041500	500 ml
	SA04101L	1 L
	SA04110L	10 L