ChangZhou Smart-Lifesciences Biotechnology Co., Ltd.
Hotline Service: 0086-519-83820182 **E-mail:**sales@smart-lifesciences.com
West Taihu science and Technology Industrial Park,Wujin District,Changzhou City,Jiangsu Province,China

Co NTA Beads

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1. Product Description

Co NTA Beads is intended for preparative purification of histidine-tagged recombinant proteins from all prokaryotic and eukarotic expression systems. **Co NTA Beads** consists of 90μm beads of 4% agarose with an immobilized chelating group. The talon ligand is a tetra-dentate chelator charged with cobalt. **Co NTA Beads** offers enhanced selectivity for histidine-tagged proteins compared to nickel-charged medium. The characteristics of **Co NTA Beads** are summarized in Table 1.

Table 1. Characteristics of **Co NTA Beads**

Item	Description
Matrix Spherical	4% agarose
Precharged ion	Cobalt ion
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	2-8℃

Co NTA Beads is compatible with all commonly used aqueous buffers(see Table 2).

Table 2. Chemical compatibilities for **Co NTA Beads**

Reagent	Stability
Reductants	10 mM β-mercaptoethanol[1]
Denaturants	8 M urea
	6 M Gua-HCl
Detergent	< 1% Triton™ X-100
	1% NP-40
	1% CHAPS ,SDS, sarcosyl
Other additives	≤500 mM imidazole[2]at pH7.0 to 8.0 for elution
	30% ethanol[3]
	20% glycerol
	500 mM KCl
	1 M NaCl

Note: [1] Do not store the medium in buffers containing β-Mercaptoethanol.

[2] Imidazole at concentrations higher than 5-10 mM may cause lower yields of histidine-tagged proteins, because it competes with the histidine side chains (imidazole groups) for binding to the immobilized metal ions.

[3] Ethanol may precipitate proteins, causing low yields and column clogging.

Avoid using the following reagents

DTT (dithiothreitol), DTE (dithioerythritol) and TCEP (TRIS (2-carboxyethyl) phosphine). Protein binding capacity will decrease rapidly. EDTA (ethylenediaminetetraacetic acid) and EGTA (ethylene Glycolbis ([β-amino-ethyl ether]). These chelators will strip off the cobalt ions from the medium.

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. We recommend binding at neutral to slightly alkaline pH (pH 7 to 8) in the presence of 0.3 M to 0.5 M NaCl. Sodium phosphate buffers are often used. Tris-HCl can generally be used, but should be avoided in cases where the metal-protein affinity is very weak, since it may reduce binding strength. Avoid chelating agents such as EDTA or citrate in buffers. In general, imidazole is used for elution of histidine-tagged proteins. Below pH 4, metal ions will be stripped off the medium. Water and chemicals used for buffer preparation should be of high purity. It is recommended filtering the buffers by passing them through a 0.22 μm or 0.45 μm filter before use. **HisCap Co 6FF** can be used for the his-tagged protein purification under native conditions and denaturing conditions, which need different buffer. See table 3 and table 4.

Table 3. Recommended buffer for his-tagged protein purification under native conditions

Name	Volume	Ingredient
Lysis Buffer	1 L	50 mM NaH_2PO_4 (7.80 g $NaH_2PO_4 \cdot 2H_2O$) 300 mM NaCl (17.54 g NaCl) Adjust the buffer pH to 8.0 with NaOH solution
Wash Buffer	1 L	50 mM NaH_2PO_4 (7.80 g $NaH_2PO_4 \cdot 2H_2O$) 300 mM NaCl (17.54 g NaCl) 5 mM imidazole (0.34 g imidazole) Adjust the buffer pH to 8.0 with NaOH solution
Elution Buffer	1 L	50 mM NaH_2PO_4 (7.80 g $NaH_2PO_4 \cdot 2H_2O$) 300 mM NaCl (17.54 g NaCl) 250 mM imidazole (17.0 g imidazole) Adjust the buffer pH to 8.0 with NaOH solution

Table 4. Recommended buffer for his-tagged protein purification under denaturing conditions, pH elution

Name	Volume	Ingredient
Lysis Buffer	1 L	8 M Urea (480.50 g Urea) 50 mM NaH_2PO_4 (7.80 g $NaH_2PO_4 \cdot 2H_2O$) 300 mM NaCl (17.54 g NaCl) Adjust the buffer pH to 8.0 with NaOH solution
Wash Buffer	1 L	8 M Urea (480.50 g Urea) 50 mM NaH_2PO_4 (7.80 g $NaH_2PO_4 \cdot 2H_2O$) 300 mM NaCl (17.54 g NaCl) 5 mM imidazole (0.34 g imidazole) Adjust the buffer pH to 8.0 with NaOH solution
Elution Buffer	1 L	8 M Urea (480.50 g Urea) 50 mM NaH_2PO_4 (7.80 g $NaH_2PO_4 \cdot 2H_2O$) 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 Recombinant native 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 (0.2-0.4 mg/ml cell paste, if the host cell containing pLysS or pLysE, it can be without lysozyme) and PMSF (1 mM/ml cell paste).

3) If the concentration of cell suspension is higher, it is recommended to add 10μg/ml RNase A and 5μg/ml DNase I. Sonicate the cell suspension/lysate on ice.

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

2.2.2 Native protein expressed in yeast, insect or mammalian cells

1) Harvest the cells from an appropriate volume of culture by centrifugation at 5,000 rpm 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 1×PBS under 4℃ .

2) for a large volume of supernatant, it need precipitation by adding ammonium sulfate and dialysis to 1×PBS under 4℃.

2.2.3 Inclusion bodies from E.coli

1) Harvest cells from an appropriate volume of bacterial culture by centrifugation at 7,000 rpm 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 10,000 rpm or 20 min at 4℃ to pellet the inclusion.

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

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

2.3 Column Purification

1) Mix the slurry by gently inverting the bottle several times to completely suspend the **Co NTA 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 pre-treated 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 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 distilled water and 1XPBS containing 20% ethanol. Finally store the resin with 1XPBS containing 20% ethanol at 4℃.

2.5 Analysis

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

3. Cleaning-in-Place

The 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 cleaning steps.

- **Remove the strong hydrophobic binding protein, lipoprotein and lipid**

Wash the column using 5-10 column volumes of 30% isopropanol contacting for 15-20 min. Or you can choose the 2CV of 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 10CV of distilled water.

- **Remove the proteins combined with ion interacting**

Wash the column with 1.5 M NaCl solution contacting for 10-15min. Finally wash the column with 10 column volumes of distilled water.

4. Regeneration

In general, **Co NTA Beads 6FF** 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 **Co NTA Beads 6FF** 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 Mg^{2+}. 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 binding buffer sample and wash buffer. Increase buffer pH.
	Elution conditions are too mild.	Increase imidazole concentration in elution buffer. Or decrease buffer pH.
		Strip cobalt ion by using 10-100mM EDTA solution, at the same time you can obtain target protein.
	Protein degradation or purification cause the his-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 cobalt ions was stripped.	Chelate cobalt ions according to the part 4.
Protein precipitates during purification	Temperature is too high	Perform the purification at room temperature.
	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
Co NTA Beads	SA037005	5 ml
	SA037025	25 ml
	SA037100	100 ml
	SA037500	500 ml
	SA03701L	1 L
	SA03710L	10 L
Co NTA Beads 6FF	SA038005	5 ml
	SA038025	25 ml
	SA038100	100 ml
	SA038500	500 ml
	SA03801L	1 L
	SA03810L	10 L
HisCap Co 6FF	SA038C11	1×1 ml
	SA038C51	5×1 ml
	SA038C15	1×5 ml
	SA038C55	5×5 ml
	SA038CS	3×1 ml+1×5 ml
Co Smart Beads 6FF	SA062005	5 ml
	SA062025	25 ml
	SA062100	100 ml
	SA062500	500 ml
	SA06201L	1 L
	SA06210L	10 L