Co NTA Beads 6FF

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1. Product Description

Co NTA Beads 6FF is intended for preparative purification of histidine-tagged recombinant proteins from all prokaryotic and eukaryotic expression systems. **Co NTA Beads 6FF** consists of highly cross-linked 6% agarose with an immobilized chelating group. The talon ligand is a tetra-dentate chelator charged with cobalt. **Co NTA Beads 6FF** offers enhanced selectivity for histidine-tagged proteins compared to nickel-charged medium. The characteristics of **Co NTA Beads 6FF** are summarized in Table 1.

Table 1. Characteristics of **Co NTA Beads 6FF**

Item	Description
Matrix Spherical	Highly cross-linked 6% agarose
Precharged ion	Cobalt ion
Static Binding Capacity	＞20mg 6×His-tagged protein/ml medium
Particle size	45-16μm
Maximum Pressure	0.3MPa, 3bar
Storage Solution	1×PBS containing 20% ethanol
Storage Temperature	4-30℃

Co NTA Beads 6FF is compatible with all commonly used aqueous buffers, denaturants such as 6 M Gua-HCl and 8 M urea, and a range of other additives (see Table 2).

Table 2. Chemical compatibilities for **Co NTA Beads 6FF**

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 1.0 M NaCl 20mM MES 50 mM Tris[4] 50 mM HEPES 50 mM MOPS

Note:[1] Use **Co NTA Beads 6FF** immediately after equilibrating with buffers containing β-Mercaptoethanol. Otherwise, the medium will change color. 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.

[4] Tris coordinates weakly with metal ions, causing a decrease in capacity.

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

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.

Native protein purification

Binding buffer: 50 mM sodium phosphate, 300 mM NaCl, pH 7.4

Wash buffer: 50 mM sodium phosphate, 300 mM NaCl, 5-20 mM imidazole, pH 7.4

Elution buffer: 50 mM sodium phosphate, 300 mM NaCl, 250 mM imidazole, pH 7.4

Denaturing protein purification

If the recombinant histidine-tagged protein is expressed as inclusion bodies, include 6 M Guanidine-HCl (Gua-HCl) or 8 M urea in all buffers and sample to promote protein unfolding. On-column refolding of the denatured protein may be possible, but depends on the protein.

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 (0.2-0.4mg/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 10,000rpm(15,000×g) for 20min 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 5,000rpm(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℃.

2.2.3 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.

2.3 Packing Columns

Co NTA 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 **Co NTA 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, **Co NTA 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. 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.

4. 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 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 3.
Protein precipitates during purification	Temperature is too low	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.

5. 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