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Co-NTA XPure Agarose Resin

User's Guide

1. DESCRIPTION

Co-NTA XPure Agarose Resin is intended for preparative purification of histidine-tagged recombinant proteins from all prokaryotic and eukarotic expression systems. **Co-NTA XPure Agarose Resin** 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 XPure Agarose Resin** offers enhanced selectivity for histidine-tagged proteins compared to nickel-charged medium. The characteristics of **Co-NTA XPure Agarose Resin** are summarized in Table 1.

Table 1. **Characteristics of Co-NTA XPure Agarose Resin**

Item	Description
Matrix	Highly cross-linked 6% agarose
Precharged ion	Cobalt
Static binding capacity	>20mg 6XHis-tagged protein/ml medium
Particle size（μm）	45–165
Maximum Pressure	0.3 MPa, 3 bar
Storage solution	1X PBS containing 20% ethanol
Storage	2°C-8°C

Co-NTA XPure Agarose Resin 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 XPure Agarose Resin**

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 XPure Agarose Resin** 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).

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

for binding to the immobilized metal ions.

[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 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 mM imidazole, pH 7.4

Elution buffer: 50 mM sodium phosphate, 300 mM NaCl, 150 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 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 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 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 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 for 20min at 4℃ to pellet the inclusion.

4. Resuspend pellet in 1:10 ration (w/v) with denaturing binding 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 Column Purification

1. Mix the slurry by gently inverting the bottle several times to completely suspend the **Co-NTA XPure Agarose Resin**. 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 binding buffer to the column to equilibrate the beads.

3. Apply the sample to the column. Collect the flow-through for measuring the binding efficiency to the beads, i.e. by SDS-PAGE.

4. Wash the column with 10 column volumes wash buffer or until the absorbance of the effluent at 280 nm is stable.

5. Elute the target protein with elution buffer and collect the eluate.

6. Equilibrate the column with 5 column volumes of binding buffer, distilled water and 1XPBS containing 20% ethanol. Finally store the beads 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.

Tips: 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.

3. REGENERATION

In general, **Co-NTA XPure Agarose Resin** may be used a number of times before it becomes necessary to recharge them with metal ions. When the back pressure is too high or the capacity significantly lower, it need to strip the metal ions and recharge the **Co-NTA XPure Agarose Resin** as the following procedure.

Wash the column with one of the following solutions.

1) 0.2M acetic acid with 6 M Guanidine Hydrochloride, 2 column volumes；
2) Rinse with 5 column volumes of distilled water；
3) 2% SDS, 3 column volumes；
4) Rinse with 5 column volumes of distilled water；
5) 70% ethanol ,5 column volumes；
6) Rinse with 5 column volumes of distilled water；
7) 100 mM EDTA (pH 8.0), 5 column volumes；
8) Rinse with 5 column volumes of distilled water；
9) 100mM $NiSO_4$, 5 column volumes；
10) Rinse with 5 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 1 bar	Column is clogged	Cleaning in place
		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 lysis buffer sample and wash buffer. 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 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 nickel ions was stripped.	Chelate nickel 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.