Marvelgent biosciences

Store product at 2°C – 8°C. Do not freeze. The product is shipped at ambient temperature.

ProteIndex™ Co-NTA Agarose

Cat. No.	Package Size
11-0252-010	ProteIndex Co-NTA Agarose, 10 mL settled resin
11-0252-050	ProteIndex Co-NTA Agarose, 50 mL settled resin
11-0252-100	ProteIndex Co-NTA Agarose, 100 mL settled resin

Intended Use

This product is intended for research use only. Not intended for any animal or human therapeutic or diagnostic use.

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1. Product Description

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

Table 1. Characteristics of Co-NTA Agarose.

Matrix	4% agarose
Precharged ion	Cobalt ion
Capacity	>20 mg 6xHis-tagged protein/mL medium
Particle size	45 µm – 165 µm
Maxi pressure	0.1 MPa, 1 bar
Storage buffer	20% ethanol
Storage	2°C – 8°C

Co-NTA Agarose 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 Agarose.

Reducing agents	10 mM β-mercaptoethanol[1]
Denaturing agents	8 M urea, 6 M Gua-HCl
	<1% Triton™ X-100 (nonionic) 1% NP-40 (nonionic) 1% CHAPS (zwitterionic) SDS, sarcosyl

Other additives	≤500 mM imidazole[2] at pH7.0 ~ 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 Agarose 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. Operation

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.

Note:

- 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 from 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 purification

If the recombinant polyhistidine-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 7,000 rpm for 10 – 15 min at 4°C. Discard supernatant and determine weight of pellet. Resuspend pellet in 1:10 ratio (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 and 5 µg/ml DNase I. Sonicate the cell suspension/lysate on ice.

4. Centrifuge the homogenized lysate at 10,000 rpm for 20 min at 4°C 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°C. Save supernatant. If the supernatant without EDTA, histidine and reductant, it can be purified directly, otherwise it need dialysis to 1xPBS under 4°C.
2. If supernatant is of a large volume, it may require precipitation by adding ammonium sulfate and subsequent dialysis against 1xPBS under 4°C.

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°C. Discard supernatant and determine weight of pellet.
2. Resuspend pellet in 1:10 ratio (w/v) with Lysis Buffer. Sonicate the cell suspension/lysate on ice.
3. Centrifuge the homogenized sample at 10,000 rpm for 20min at 4°C to pellet the inclusion.
4. Resuspend pellet in 1:10 ratio (w/v) with denaturing binding Buffer (containing 8 M urea). Sonicate, as needed, to dissociate the pellet.
5. Analyze the concentration of the target protein and continue with purification protocols under denaturing conditions.

2.3 Purification Protocol

1. Mix the slurry by gently inverting the bottle several times to completely suspend the Co-NTA Agarose.
2. Close the column outlet leaving the net covered with packing buffer. Transfer the slurry to the column.
3. 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.

4. Apply the sample to the column. Collect the flow-through for measuring the binding efficiency to the beads, i.e. by SDS-PAGE.
5. Wash the column with 10 column volumes wash buffer or until the absorbance of the effluent at 280 nm is stable.
6. Elute the target protein with elution buffer and collect the eluate.
7. 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°C.

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 Agarose 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 may need to strip the metal ions and recharge the Co-NTA Agarose according to the following procedure:

1. Wash column with 10 column volumes of 0.2 M EDTA, pH7.0;
2. Rinse with 10 column volumes of distilled water;
3. Charge the medium with 100 mM $CoCl_2$, 10 column volumes;
4. Rinse with 5 column volumes of distilled water;
5. Rinse sequentially with 3 column volumes of 300 mM NaCl and 5 column volumes of distilled water;
6. After regeneration, the medium can be used immediately.

4. Storage

For long-term storage, Co-NTA Agarose should be stored in 20% ethanol at 2 - 8°C.

5. Trouble Shooting Guide

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.
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.

6. Related Products

Product Name	Package Size	Cat. No.
ProteIndex™ Co-NTA Agarose 6 Fast Flow		
	10 mL settled resin	11-0231-010
	50 mL settled resin	11-0231-050
	100 mL settled resin	11-0231-100
ProteIndex™ Chemical-Tolerant Ni-Penta™ Agarose		
	10 mL settled resin	11-0227-010
	50 mL settled resin	11-0227-050
	100 mL settled resin	11-0227-100
ProteIndex™ Chemical-Tolerant Ni-Penta™ Agarose 6FF		
	10 mL settled resin	11-0228-010
	50 mL settled resin	11-0228-050
	100 mL settled resin	11-0228-100
ProteIndex™ Ni-IDA Agarose		
	25 mL settled resin	11-0221-025
	250 mL settled resin	11-0221-250
ProteIndex™ Ni-NTA Agarose		
	10 mL settled resin	11-0224-010
	50 mL settled resin	11-0224-050
	100 mL settled resin	11-0224-100
ProteIndex™ SEC G-25 Cross-linked Dextran Resin, Coarse		
	100 g	11-0248-100
	500 g	11-0248-500
ProteIndex™ SEC G-25 Cross-linked Dextran Resin, Medium		
	100 g	11-0249-100
	500 g	11-0249-500

ProteIndex™ SEC G-25 Cross-linked Dextran Resin, Fine		
	100 g	11-0250-100
	500 g	11-0250-500
Empty Gravity Flow Columns		
	12 mL, pack of 50	11-0257-050
	3 mL, pack of 50	11-0258-050
	6 mL, pack of 50	11-0259-050
	30 mL, pack of 25	11-0260-025
	300 mL, pack of 10	11-0261-010

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