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

ProteIndex™ Co-NTA Agarose 6 Fast Flow

Cat. No.	Package Size
11-0231-010	ProteIndex Co-NTA Agarose 6 Fast Flow, 10 mL settled resin
11-0231-050	ProteIndex Co-NTA Agarose 6 Fast Flow, 50 mL settled resin
11-0231-100	ProteIndex Co-NTA Agarose 6 Fast Flow, 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 6 Fast Flow consists of highly cross-linked 6% agarose as matrix support, on which a tetra-dentate chelator is covalently immobilized and pre-charged with the cobalt ion.

Co-NTA Agarose 6 Fast Flow can be used for both small scale and preparative scale purification of histidine-tagged recombinant proteins from all prokaryotic and eukaryotic expression systems. It binds polyhistidine-tagged proteins with high selectivity and lower nonspecificity for host proteins. Co-NTA Agarose 6 Fast Flow offers enhanced selectivity for histidine-tagged proteins compared to nickel-charged media. Co-NTA Agarose 6 Fast Flow is suitable for purifying proteins that are expressed at low levels or when Nickel is not the optimal choice of metal ion. Leakage of cobalt ion (Co^{2+}) of Marvelgent's Co-NTA Agarose 6 Fast Flow resins is low.

Table 1. Characteristics of Co-NTA Agarose 6 Fast Flow.

Matrix	Highly cross-linked 6% agarose supplied as 50% slurry
Binding capacity*	>20mg 6xHis-tagged protein/ml medium
Particle size	45 µm – 165 µm
Max Pressure	0.3 MPa, 3 bar
Precharged ion	Cobalt
pH stability	
Short term	pH 2-14
Long term	pH 3-12
Storage solution	1x PBS containing 20% ethanol
Storage temperature	2°C – 8°C

***The binding capacity for individual proteins may vary.

Co-NTA Agarose 6 Fast Flow is compatible with all commonly used aqueous buffers, denaturants such as 6 M Gua-HCl and 8 M

urea, and a wide range of other additives. The Compatible reagents are summarized in Table 2.

Table 2. Compatible reagents for Co-NTA Agarose 6 Fast Flow.

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 6 Fast Flow 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 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 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°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 8M 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 Packing Co-NTA Agarose 6 Fast Flow

1. Assemble the column (and packing reservoir if necessary).
2. Remove air from the end-piece and adapter by flushing with water. Make sure no air has been trapped under the column bed support. Close the column outlet leaving the bed support covered with water.
3. Resuspend the medium and pour the slurry into the column in a single continuous motion. Pouring the slurry down a glass rod held against the column wall will minimize the introduction of air bubbles.
4. If using a packing reservoir, immediately fill the remainder of the column and reservoir with water. 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.
5. Open the bottom outlet of the column and set the pump to run at the desired flow rate.
6. Maintain packing flow rate for at least 3 bed volumes after a constant bed height is reached. Mark the bed height on the column.
7. Stop the pump and close the column outlet.
8. If using a packing reservoir, disconnect the reservoir and fit the adapter to the column.
9. With the adapter inlet disconnected, push the adapter down into the column until it reaches the mark. Allow the packing solution to flush the adapter inlet. Lock the adapter in position.
10. Connect the column to a pump or a chromatography system and start equilibration. Re-adjust the adapter if necessary.

2.4 Sample Purification

1. Add 5 column volumes binding buffer to the column to equilibrate the beads.
2. Apply the sample to the column. Collect the flow-through for measuring the binding efficiency to the beads, i.e. by SDS-PAGE.
3. Wash the column with 10 column volumes wash buffer or until the absorbance of the effluent at 280 nm is stable.

4. Elute the target protein with elution buffer and collect the eluate.
5. Equilibrate the column with 5 column volumes of binding buffer, distilled water and 1XPBS containing 20% ethanol.

2.5 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 6 Fast Flow 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 6 Fast Flow according to the following procedure:

1. 0.2 M 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 $CoCl_2$, 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 and stored in an equal volume of 1x PBS containing 20% ethanol at 2-8°C.

4. Storage

Store Co-NTA Agarose 6 Fast Flow in 1XPBS containing 20% ethanol at 2-8°C.

5. Troubleshooting Guide

Problem	Probable cause	Solution
Back pressure exceeds 1 bar	Column is clogged	• Cleaning in place (Section 3). • Increase the centrifugation speed or filter the sample.
	Sample is too viscous	Increase sonication or add DNase I (5 µg/ml with 1 mM Mg^{2+}). Incubate on ice for 15 min.
	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 protein expression level by estimating the amount in the extract. 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 – 100 mM EDTA solution, at the same time you can obtain target protein.
Medium color is shallow.	Cobalt ion was stripped off.	Chelate cobalt ions according to the Section 3.
Protein precipitates during purification	Temperature is too low.	Perform the purification at room temperature.
	Aggregate formation.	Add solubilizing agents to the sample and buffers (eg 0.1% Triton X-100, Tween-20, ≤20% glycerol) to facilitate protein solubility.

6. Related Products

Product Name	Package Size	Cat. No.
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-NTA Agarose 6 Fast Flow		
	10 mL settled resin	11-0225-010
	50 mL settled resin	11-0225-050
	100 mL settled resin	11-0225-100
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™ Ni-IDA Agarose 6 Fast Flow		
	25 mL settled resin	11-0222-025
	250 mL settled resin	11-0222-250
ProteIndex™ Ni-NTA Magnetic Agarose		
	1 mL settled resin	11-0226-01
	5 mL settled resin	11-0226-05
ProteIndex™ Ni-IDA Magnetic Agarose		
	1 mL settled resin	11-0223-01
	5 mL settled resin	11-0223-05

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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