

# ab270018

## Cobalt NTA Affinity Resin - Amintra

A product of Expedeon, an Abcam company

Applicable to Expedeon product codes ACO0010, ACO0025, ACO0100.

View ab270018

Cobalt NTA Affinity Resin - Amintra datasheet:

[www.abcam.com/ab270018](http://www.abcam.com/ab270018)

(use [www.abcam.cn/ab270018](http://www.abcam.cn/ab270018) for China, or [www.abcam.co.jp/ab270018](http://www.abcam.co.jp/ab270018) for Japan)

For simple, rapid His-tagged recombinant protein purification from a cell lysate under native or denaturing conditions.

This product is for research use only and is not intended for diagnostic use.

# Table of Contents

1. Overview	2
2. Materials Supplied and Storage	3
3. Materials Required, Not Supplied	3
4. Technical Considerations	4
5. Sample Preparation	10
6. Assay Procedure	11
7. Frequently Asked Questions	14
8. Troubleshooting	17

## 1. Overview

Cobalt NTA Affinity Resin - Amintra (ab270018) is designed for simple, rapid His-tagged recombinant protein purification from a cell lysate under native or denaturing conditions. Metal chelate affinity chromatography is a rapid one-step purification, which removes most contaminants and can achieve purities close to homogeneity.

The rapid purification protocols for affinity chromatography permit the recovery of high levels of pure recombinant protein in minutes. Large numbers of samples can be processed at the same time. Recombinant proteins purified using Cobalt NTA Affinity Resin - Amintra may be used in a wide range of structure and activity-based laboratory procedures.

Cobalt NTA Affinity Resin - Amintra consists of highly cross-linked 6% agarose with an immobilized chelating group. The ligand is a tetra-dentate chelator charged with cobalt.

Cobalt NTA Affinity Resin - Amintra can be used for preparative purification of histidine-tagged recombinant proteins from all prokaryotic and eukaryotic expression systems. Cobalt NTA Affinity Resin - Amintra is suitable also for purification of low expressing proteins. It binds polyhistidine-tagged proteins with high selectivity and exhibits a reduced affinity for host proteins. Cobalt NTA Affinity Resin - Amintra offers enhanced selectivity for histidine-tagged proteins compared to nickel-charged media. Cobalt NTA Affinity Resin - Amintra has low cobalt ion ( $\text{Co}^{2+}$ ) leakage.

## 2. Materials Supplied and Storage

Store kit at +4°C immediately on receipt. **Do not freeze or store the resin at room temperature. Freezing the suspension will damage the agarose beads.** The resin is pre-swollen and de-fined. It is formulated in 20% ethanol.

Item	Quantity			Storage temperature
Cobalt NTA Affinity Resin - Amintra	10 mL	25 mL	100 mL	+4°C

## 3. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Binding buffer (see Section 4.4)
- Wash buffer (see Section 4.4)
- Elution buffer (see Section 4.4)

## 4. Technical Considerations

### 4.1 Characterization of the Resin:

Supporting matrix	Highly cross-linked 6% agarose
Charged metal ion	Co <sup>2+</sup>
Bead size range	45-165 µm
Recommended working pH	Short term: pH 2.0-14.0 – Long term: pH 3.0-12.0
Typical binding capacity	> 20 mg 6x HIS-tagged recombinant protein/mL resin
Maximum pressure	0.3 MPa (3 bar)
Chemical stability	High
Solubility in water	Insoluble

### 4.2 Metal chelate chromatography:

IMAC technology was introduced by Porath et al (1975). The matrix is attached to chelating groups that immobilize transition metal ions such as Ni<sup>2+</sup>, Co<sup>2+</sup>, Cu<sup>2+</sup>, Zn<sup>2+</sup> (Porath and Olin, 1983; Porath, 1988; Sulkowski, 1989). Certain amino acids such as histidine, tryptophan, cysteine and tyrosine can act as electron donors on the surface of the protein and bind reversibly to the transition metal ion. In the vast majority of instances, 6x histidine tag is engineered at the N or C terminus of the protein (Kd-10-13 at pH 8.0).

Ni<sup>2+</sup> is the most widely used metal ion as most IMAC tags seem to have very high affinity for immobilized Ni<sup>2+</sup>. The simplicity of IMAC technology is extremely attractive as it lends itself to the bind, wash and elute mode of operation if the appropriate buffer formulations are selected. IMAC can often be used with samples without any pretreatment e.g. buffer exchange step. The use of metal chelate affinity is widespread for the selective adsorption of engineered recombinant proteins and has largely superseded non-affinity methods of chromatography for purifying recombinant proteins.

### 4.3 Improving binding conditions:

This resin exploits the hexahistidine sequence that permits efficient purification of the expressed protein from a broad host such as bacterial cells, Baculovirus vectors, mammalian cells or yeast. Baculovirus, mammalian cells and yeast expression vectors are often used to express eukaryotic proteins as they generate proteins with the similar post-translational modifications such as phosphorylations and glycosylations.

Lysis conditions, such as the nature of the lysis buffer, depend upon the type of expression vector. Mammalian or Baculovirusinfected insect cells can be lysed by sonication at +4°C with either freeze/thaw cycles or addition of up to 1% non-ionic detergents and cell lysis of *E.coli* is usually achieved by sonication on ice or homogenization either with or without lysozyme treatment.

The culture pellet is resuspended in lysis buffer at a pH close to pH 7.4-8.0 using a similar concentration of buffer, imidazole and NaCl to that of a pre-equilibration buffer used for metal chelate chromatography. Binding of His-tagged soluble proteins present in the cytoplasm or periplasm and insoluble aggregates in the presence of denaturants occurs close to physiological pH.

Protease inhibitor cocktails, such as Proteolock, BaseMuncher endonuclease and 10 mM  $\beta$ -mercaptoethanol can also be added to the lysis buffer. Addition of  $\beta$ -mercaptoethanol to the lysis buffer and the binding, wash and elution buffers are optional. Its inclusion depends upon whether the His-tagged protein elutes with contaminants as  $\beta$ -mercaptoethanol can reduce all disulphide bonds formed between the contaminating proteins and the target protein. Initially, the researcher should try to bind the His-tagged protein directly from the cleared lysate.

It is imperative that the lysate is completely clear as any particulate matter e.g. cell debris will partially foul the resin and cause additional back pressure and reduced flow rates. It is important that the sample is clarified to remove particulates that could clog the resin flow channels. It is good practise to filter just prior to loading even if they have been filtered several days before the chromatographic run.

If the binding efficiency is poor and the lysis buffer differs significantly from the pre-equilibration buffer, optimal binding of the His-tagged

protein to the CoHS resin can be achieved by rapid dialysis, diafiltration using ultrafiltration concentrators, gel-filtration chromatography in the appropriate preequilibration buffer or titration with a concentrated stock solution of pre-equilibration buffer.

Please note that the precise conditions for binding, washing and eluting your target protein may need to be optimized empirically as there are several factors such as accessibility of the His-tag which affect protein behaviour in nondenaturing conditions during metal chelate chromatography.

Aggregation/precipitation of proteins is common during storage and repeated freeze/thaw cycles.

Optimal conditions for binding the target molecule to a resin are critical for successful separation of the protein. If the binding conditions are not optimal with respect to pH, salt concentration, presence of metal ions or chelating agents, flow rates, residence time etc, purification is adversely affected.

#### **4.4 Buffer selection:**

##### **Native Proteins:**

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

Wash buffer: 50 mM sodium phosphate; pH 7.4, 300 mM NaCl, 5 mM imidazole.

Elution buffer: 50 mM sodium phosphate; pH 7.4, 300 mM NaCl, 150 mM imidazole.

##### **Denatured Proteins:**

Binding buffer: 50 mM sodium phosphate; pH 7.4, 300 mM NaCl, 6M Gua-HCl or 8 M Urea.

Wash buffer: 50 mM sodium phosphate; pH 7.4, 300 mM NaCl, 5 mM imidazole, 6M Gua-HCl or 8 M Urea.

Elution buffer: 50 mM sodium phosphate; pH 7.4, 300 mM NaCl, 150 mM imidazole, 6M Gua-HCl or 8 M Urea.

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 from the medium.

Sodium phosphate buffers are recommended. Buffers with secondary or tertiary amines (e.g. Tris buffers) can be used but these buffers can reduce the cobalt ion which will adversely impact on the purification. Buffer pH between 7-8 is well suited for most immobilized  $\text{Co}^{2+}$  applications. NaCl (0.3M to 0.5M) can be added to the buffers to reduce non-specific ionic interactions and may also stabilize some proteins.

Chaotropic agents such as 8 M urea and 6 M guanidinium HCl do not interfere with metal chelate affinity separations. When a recombinant protein is expressed at high levels in *E.coli*, the protein elutes as insoluble aggregates called inclusion bodies. These denaturants completely unfold the target protein making the His-tag much more accessible for interaction with the immobilized  $\text{Co}^{2+}$  matrix.

#### **4.5 Protein elution:**

The most common elution conditions for IMAC separations involve the use of a competitive counter-ligand such as imidazole. This is the preferred elution method for purifications under native conditions. For purifications under denaturing conditions, elution is performed either using imidazole in the presence of denaturant such as 8 M urea or by a reduction in elution pH from pH 7.4 to pH 4.5. It is important to appreciate that a few proteins are acid-labile and they can lose their activity at very low pH values.

#### **4.6 Binding kinetics:**

The flow rate through an affinity chromatography support is important in achieving optimal separation. Flow rate through the column support is inextricably related to the efficiency of the separation; too fast a flow will cause the mobile phase to move past

the beads faster than the diffusion time necessary to reach the internal bead volume.

The resin chemistries used in Amintra CoHIS resin result in rapid association kinetics between the protein molecule and the immobilized ligand to allow for optimal diffusional flow through the internal bead structure.

#### 4.7 Chemical compatibility:

All resins are susceptible to oxidative agents. Avoid high temperatures. The resin is resistant to short exposure to organic solvents (e.g. 30% ethanol) and is stable in all aqueous buffers commonly used for metal chelate chromatography cleaning-in-place e.g. 1 M NaOH, 0.01 M HCl. IMAC resin is resistant to 6 M guanidine-HCl and 8 M urea.

Reducing agents can reduce the resin matrix adversely. Compatible reagents and concentrations are summarized in the following table:

Reducing agents	10 mM $\beta$ -mercaptoethanol
Denaturing agents	8 M urea 6 M Gua-HCl
Detergents	1% Triton X-100 (nonionic) 1% NP-40 (nonionic) 1% CHAPS (zwitterionic) SDS, sarcosyl
Other additives	$\leq$ 500 mM imidazole at pH 7.0 - 8.0 for elution 30% ethanol 20% glycerol 500 mM KCl 1.0 M NaCl 20 mM MES 50 mM Tris 50 mM HEPES 50 mM MOPS

#### ***$\Delta$ Notes:***

- *Use the resin immediately after equilibrating in buffers containing  $\beta$ -mercaptoethanol. Otherwise, the medium will change color. Do not store the resin in buffers containing  $\beta$ -mercaptoethanol.*
- *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.*
- *Ethanol may precipitate proteins, causing low yields and column clogging.*
- *Tris coordinates weakly with metal ions, causing a decrease in capacity.*
- *Avoid using the following reagents:*
  - *Reducing agents: DTT (dithiothreitol), DTE (dithioerythritol) and TCEP (TRIS (2-carboxyethyl) phosphine). In the presence of these reagents, protein binding capacity will decrease rapidly.*
  - *Chelators: EDTA (ethylenediaminetetraacetic acid) and EGTA (ethylene Glycolbis ([ $\beta$ -amino-ethyl ether])). These chelators will strip off the cobalt ions from the medium.*

## 5. Sample Preparation

### 5.1 Recombinant native protein expressed in *E.coli* or yeast:

- 5.1.1 Culture single colonies in LB medium. According to the instructions, add inducers for a period of time.
- 5.1.2 Harvest cells from an appropriate volume of bacterial culture by centrifugation at 7,000 rpm for 10-15 minutes at +4°C. Discard supernatant and determine weight of pellet. Resuspend pellet in 1:10 ratio (w/v) in lysis buffer and add lysozyme (0.2-0.4 mg/mL cell paste; if the host cell containing pLysS or pLysE, it can be without lysozyme) and PMSF (1 mM/mL cell paste).
- 5.1.3 If high concentration of cell suspension, it is recommended to add BaseMuncher endonuclease (ab270049). Sonicate the cell suspension/lysate on ice.
- 5.1.4 Centrifuge the homogenized lysate at 10,000 rpm for 20 minutes at +4°C to clarify sample. Save supernatant.

### 5.2 Recombinant native protein expressed in yeast, insect or mammalian cells:

- 5.2.1 Harvest the cells from an appropriate volume of culture by centrifugation at 5,000 rpm for 10-15 minutes at +4°C. Save supernatant. If the supernatant is without EDTA, histidine and reductant, it can be purified directly, otherwise it needs dialysis to 1X PBS under +4°C.
- 5.2.2 If supernatant is of a large volume, it may require precipitation by adding ammonium sulfate and subsequent dialysis against 1X PBS at +4°C.

### 5.3 Inclusion bodies from *E.coli*:

- 5.3.1 Harvest cells from an appropriate volume of bacterial culture by centrifugation at 7,000 rpm for 10-15 minutes at +4°C. Discard supernatant and determine weight of pellet.
- 5.3.2 Resuspend pellet in 1:10 ratio (w/v) with Lysis Buffer. Sonicate the cell suspension/lysate on ice.
- 5.3.3 Centrifuge the homogenized sample at 10,000 rpm for 20 minutes at +4°C to pellet the inclusions.

- 5.3.4 Resuspend pellet in 1:10 ratio (w/v) with denaturing binding Buffer (containing 8M urea). Sonicate, as needed, to dissociate the pellet.
- 5.3.5 Analyze the concentration of the target protein and continue with purification protocols under denaturing conditions.

## 6. Assay Procedure

### 6.1 Packing Cobalt NTA Affinity Resin - Amintra:

- 6.1.1 Assemble the column (and packing reservoir if necessary).
- 6.1.2 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.
- 6.1.3 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.
- 6.1.4 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.
- 6.1.5 Open the bottom outlet of the column and set the pump to run at the desired flow rate.
- 6.1.6 Maintain packing flow rate for at least 3 bed volumes after a constant bed height is reached. Mark the bed height on the column.
- 6.1.7 Stop the pump and close the column outlet.
- 6.1.8 If using a packing reservoir, disconnect the reservoir and fit the adapter to the column.
- 6.1.9 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.

- 6.1.10 Connect the column to a pump or a chromatography system and start equilibration. Re-adjust the adapter if necessary.

## 6.2 Sample Purification:

- 6.2.1 Add 5 column volumes binding buffer to the column to equilibrate the beads.
- 6.2.2 Apply the sample to the column. Collect the flow-through for measuring the binding efficiency to the beads, i.e. by SDS-PAGE. Expedeon recommends using our RunBlue range of precast gels along with our His-Tag Protein Expression Check lateral flow assay (ab270048).
- 6.2.3 Wash the column with 10 column volumes wash buffer or until the absorbance of the effluent at 280 nm is stable.
- 6.2.4 Elute the target protein with elution buffer and collect the eluate.
- 6.2.5 Equilibrate the column with 5 column volumes of binding buffer, distilled water and 1X PBS containing 20% ethanol.

## 6.3 Analysis:

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

**Δ Note:** *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.*

## 6.4 Regeneration:

In general, Cobalt NTA Affinity Resin - Amintra 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 Cobalt NTA Affinity Resin - Amintra as the following procedure:

- 6.4.1 0.2M acetic acid with 6 M Guanidine Hydrochloride, 2 column volumes.
- 6.4.2 Rinse with 5 column volumes of distilled water.
- 6.4.3 2% SDS, 3 column volumes.
- 6.4.4 Rinse with 5 column volumes of distilled water.
- 6.4.5 70% ethanol, 5 column volumes.
- 6.4.6 Rinse with 5 column volumes of distilled water.
- 6.4.7 100 mM EDTA (pH 8.0), 5 column volumes.
- 6.4.8 Rinse with 5 column volumes of distilled water.
- 6.4.9 100mM CoCl<sub>2</sub>, 5 column volumes.
- 6.4.10 Rinse with 5 column volumes of distilled water.

## **6.5 Resin storage:**

After regeneration, the medium can be used immediately. Otherwise, it needs to be suspended and stored in an equal volume of 1X PBS containing 20% ethanol at 2 – 8°C.

## 7. Frequently Asked Questions

### 7.1 What is the shelf-life of Metal Chelate resin?

The resin is guaranteed for 2 years after the date of manufacture provided they are stored at 2-8°C.

### 7.2 Do I need to filter the buffers prepared in my laboratory?

It is good laboratory practice to filter all buffers.

### 7.3 How should I prepare my sample for the Amintra CoHIS resin?

Many chromatographic procedures demand that the sample is pre-conditioned prior to loading. We recommend that all samples are filtered. High viscosity is mostly attributed to contaminating DNA or RNA. The intrinsic viscosity of a lysate can be reduced by adding appropriate amounts of BaseMuncher endonuclease (ab270049).

### 7.4 Should I add β-mercaptoethanol to the lysis buffer?

Reducing agents can reduce the resin matrix and adversely affect binding of the His-tagged protein to the resin. Its inclusion depends upon whether the His-tagged protein elutes with contaminants as β-mercaptoethanol can reduce all disulphide bonds formed between the contaminating proteins and the target protein. We recommend 0.5 mM TCEP. Concentration less than 10 mM β-mercaptoethanol can be used with the IMAC resin. Do not use strong reducing agents such as DTT or DTE as these tend to reduce the metal ion, which will lower the binding efficiency of the IMAC column.

### 7.5 How can I regenerate the metal chelate resin?

We recommend washing the resin with elution buffer and then re-equilibrate the resin binding buffer. Proceed to the preequilibration step if resin is to be re-used immediately. After regeneration, the resin can also be stored in 1x PBS containing 20% ethanol at 2-8°C until further use.

### 7.6 What can I do if the resin has changed colour?

The pink colour is attributed to the Co<sup>2+</sup> salt. Reductants (e.g. DTT) will cause the resin to turn discolour and chelating agents (e.g.

EDTA) will cause the resin to turn white. Ensure that all solutions are compatible with the Cobalt NTA Affinity Resin - Amintra.

### **7.7 How can I re-charge the resin with CoSO<sub>4</sub>?**

Wash the resin with 3 column volumes of distilled water followed by 1 column volume of 0.1 M CoSO<sub>4</sub> solution (made up in distilled water). Wash off any unbound CoSO<sub>4</sub> with 5-10 column volumes of distilled water and equilibrate the resin with 1 x PBS buffer, pH 7.4.

### **7.8 Should I be concerned if the resin partially dried out during the chromatographic steps?**

The resin is robust. Partially dried resin rehydrates rapidly. There are no adverse effects upon the performance of the resin.

### **7.9 Should I remove imidazole after the final elution step?**

Imidazole is best removed after elution if the protein is going to be stored. Otherwise, the protein may precipitate out of solution at -20 or -80°C.

### **7.10 Can I load purified protein immediately on to an SDS-gel?**

Proteins purified under native conditions can be loaded on to an SDS-polyacrylamide gel. Those proteins purified under denaturing conditions in 8 M urea can also be loaded directly on to a denaturing SDS-polyacrylamide gel. Proteins purified in the presence of 6 M guanidine HCl should be buffer exchanged in buffers lacking the denaturant prior to a denaturing SDS-PAGE.

### **7.11 Do I need to remove the His-tag from the recombinant protein after purification?**

Normally, a protease cleavage site e.g. TEV or 3C-Express Proteases are engineered between the His-tag and the target protein. The target protein can then be re-purified using Cobalt NTA Affinity Resin - Amintra in order to purify undigested His-tagged protein. For most applications, it is not necessary to remove the His-tag. However, it is often desirable to remove the His-tag if X-ray crystallography or NMR is to be used to determine the structure of the target protein.

## 7.12 Can I re-use the resin?

The resin can be re-used. Re-use does depend on the properties of your target protein. You may observe that flow rates slow down in successive bind-wash-elute cycles as more samples are progressively loaded on to the columns. In addition, if the resin is not re-charged with  $\text{Co}^{2+}$ , binding capacity may be reduced.

## **8. Troubleshooting**

### **8.1 Bubbles or cracks appear in the resin bed**

The resin has been stored at a cool temperature and then rapidly warmed up. Amintra CoHIS resin should be warmed slowly to room temperature before use.

### **8.2 The sample does not flow easily through the resin**

- The resin is clogged with particulates. Pre-filter the sample just before loading it on to the metal chelate resin.
- If the resin is not stored at 2-8°C, or they have been used more than once and stored in the absence of a bacteriostat, microbial growth in the column may restrict flow through the resin.

### **8.3 No elution of the target protein is observed from the resin**

- The elution conditions are too mild to desorb the target protein. Use a higher concentration of imidazole or lower the elution pH further!
- Ensure that the resin is blue in appearance. Otherwise the expressed protein will not bind effectively to the resin.
- Ensure that there are no chelators or reductants in the sample which will interfere with binding of the target protein to the resin.
- The protein may have precipitated in the column.
- The cell disruption method may have liberated proteolytic activities. Purify the protein under denaturing conditions if you do not need to purify an active protein.

### **8.4 The recovery of target protein is low**

- The His-tag may be inaccessible. Either move the affinity tag to the other end of the protein or perform the purification under denaturing conditions.
- Ensure that the resin bed volume is proportionate to the level of expressed His-tagged protein. The target protein may pass through into the sample wash if the capacity of the resin plug is insufficient for the level of expressed protein.

- Confirm levels of target protein by immunoassay. This will help determine if your cell disruption methods have been successful.
- The target protein may contain hydrophobic stretches which could have been toxic to the host.
- Ensure that the protein is not insoluble i.e. exists in inclusion bodies and resides in the pellet. Solubilize the insoluble protein using 8 M urea or 6 M guanidine hydrochloride.
- Add further protease inhibitors to the buffers as the full length protein may have been degraded by hydrolytic enzymes. Alternatively, reduce the time of expression, lower the temperature at which the protein is exposed or use special E.coli strains devoid of proteases.

### **8.5 Poor resolution of the target protein**

- The sample volume or concentration may be too large for the capacity of the resin plug. In this case, reduce the sample load or sample volume.
- The sample may also need to be filtered carefully.

### **8.6 The target protein elutes at an unexpected position**

- There may be an ionic interaction between the protein and the resin. You should maintain the ionic strength above 0.1 M.
- There may be hydrophobic interactions between the sample and the resin. In this instance, reduce the salt concentration.
- Co-purification of contaminants may occur if both the expressed protein and the contaminant have similar affinities for the matrix. In this case, a further chromatographic method such as gel filtration or ion exchange chromatography is recommended.

### **8.7 The elution profile cannot be reproduced**

- The nature of the sample may have altered and so it may be important to prepare a fresh sample. The His-tag may have been removed by proteases. Work at 2-8°C and add a protease inhibitor cocktail to the lysis buffer.
- Accessibility of the His-tag may have altered. If the His-tag becomes buried in the protein, the binding capacity of any

metal chelate resin for this target protein will be significantly reduced under native conditions. In this instance, the purification needs to be performed under denaturing conditions.

- The sample load may be different from the original sample load. It is advisable to keep all these parameters constant.
- Proteins or lipids may have precipitated in the resin bed. Use elution conditions, which stabilize the sample.
- The buffer pH and ionic strength are incorrect and new buffers will need to be prepared.

# Technical Support

Copyright © 2020 Abcam. All Rights Reserved. The Abcam logo is a registered trademark. All information / detail is correct at time of going to print.

For all technical or commercial enquiries please go to:

[www.abcam.com/contactus](http://www.abcam.com/contactus)

[www.abcam.cn/contactus](http://www.abcam.cn/contactus) (China)

[www.abcam.co.jp/contactus](http://www.abcam.co.jp/contactus) (Japan)