

Version 2a Last updated 9 September 2020

ab270549

Ni-NTA Affinity Resin - Amintra

A product of Expedeon, an
Abcam company

Applicable to Expedeon product codes: ANN0010, ANN0025,
ANN0100

View ab270549

Ni-NTA Affinity Resin - Amintra datasheet:

www.abcam.com/ab270549

(use www.abcam.cn/ab270549 for China, or www.abcam.co.jp/ab270549 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	3
5. Sample Preparation	6
6. Assay Procedure	7
7. Frequently Asked Questions	15
8. Troubleshooting	17

1. Overview

Ni-NTA Affinity Resin - Amintra (ab270549) 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 provided in this handbook 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 Ni-NTA Affinity Resin - Amintra may be used in a wide range of structure and activity-based laboratory procedures.

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 posttranslational modifications such as phosphorylations and glycosylations.

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 defined. It is formulated as a 50% suspension in 20% ethanol.

Item	Quantity			Storage temperature
Ni-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 4.3)
- Wash buffer (see Section 4.3)
- Elution buffer (see Section 4.3)

4. Technical Considerations

4.1 Characteristics of the Resin:

Supporting matrix	Highly cross-linked 6% agarose
Charged metal ion	Ni ²⁺
Bead size range	45-165 µm
Recommended working pH	pH 2.0-12.0
Static binding capacity	>40 mg 6x His-tagged
Maximum pressure	0.3 MPa (3 bar)
Chemical stability	High
Solubility in water	Insoluble

4.2 Chemical compatibility

All resins are susceptible to oxidative agents. Avoid high temperatures. The resin is resistant to short exposure to organic solvents and are 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. Concentrations less than or equal to 20 mM beta-mercaptoethanol can be used. See table for more detail:

Buffers	Sodium phosphate (50 mM pH 7.4) Sodium acetate (\leq 100 mM, pH 4.0) Tris-HCl, Tris-acetate, HEPES, MOPS (\leq 100 mM, pH 7.4) – coordinate with metal ions
Denaturing agents	8 M urea Up to 6 M Gua-HCl
Detergents	Non-ionic (Tween, Triton) Anionic (Cholate) – up to 2% Zwitterionic (CHAPS) – up to 1%
Other additives	\leq 500 mM imidazole at pH 7.0 - 8.0 for elution; 20 mM to reduce non-specific binding \leq 20% ethanol – may precipitate proteins \leq 50% glycerol \leq 1 mM EDTA has been used, but NOT recommended; coordinates with nickel, causing decrease in capacity \leq 100 mM Sodium sulfate \leq 1.5 M Sodium chloride \leq 60 mM Citrate
Reducing agents	\leq 30 mM Reduced glutathione \leq 20 mM β -Mercaptoethanol \leq 10 mM DTE in samples 0.5 – 1.0 mM DTT in samples \leq 5 mM TCEP SDS – not recommended, but \leq 0.3% in samples

***Δ Note:** Reducing agents can affect the resin adversely.*

4.3 Recommended buffers

Generally, the concentration of imidazole in Lysis Buffer and Wash Buffer should be low, while it is high in Elution Buffer.

Water and chemicals used for buffer preparation should be of high purity. It is recommended to filter all buffers before use by passing through a 0.22 µm or 0.45 µm filter.

This resin can be used for His-tagged protein purification under both native and denaturing conditions, with different buffers:

Native Proteins:

- Binding buffer: 50 mM NaH₂PO₄-H₂O,; pH 8.0, 300 mM NaCl, 10 mM imidazole.
- Wash buffer: 50 mM NaH₂PO₄-H₂O,; pH 8.0, 300 mM NaCl, 20 mM imidazole.
- Elution buffer: 50 mM NaH₂PO₄-H₂O,; pH 8.0, 300 mM NaCl, 250 mM imidazole.

Denatured Proteins:

- Binding buffer: 100 mM NaH₂PO₄-H₂O,, 100 mM Tris-HCl, 8 M Urea; pH 8.0.
- Wash buffer: 100 mM NaH₂PO₄-H₂O,, 100 mM Tris-HCl, 8 M Urea; pH 6.3.
- Elution buffer: 100 mM NaH₂PO₄-H₂O,, 100 mM Tris-HCl, 8 M Urea; pH 4.5.

Sodium phosphate buffers are recommended. Buffers with secondary or tertiary amines (e.g. Tris buffers) can be used be these buffers can reduce the nickel ion which will adversely impact on the purification. Buffer pH between 7-8 is well suited for most immobilized Ni²⁺ applications. NaCl (0.15-0.5 M) 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 Ni²⁺ matrix.

4.4 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™ Ni-NTA 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.

5. Sample Preparation

Lysis conditions, such as the nature of the lysis buffer, depend upon the type of expression vector. Mammalian or Baculovirus-infected 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.

Δ Note: *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 practice to filter just prior to loading even if they have been filtered several days before the chromatographic run.*

Δ Note: Protease inhibitor cocktails may be added to buffers (but EDTA should be avoided, see Section 4.2).

Δ Note: BaseMuncher Endonuclease (ab270049) may be added to the lysis solution.

6. Assay Procedure

- Equilibrate all materials and prepared reagents to desired purification temperature (4°C or RT) just prior to use and gently agitate.

6.1 Gravity purification of His-tagged proteins under native conditions:

6.1.1 Equilibration:

- Determine the quantity of Ni-NTA Affinity Resin - Amintra needed for your purification: 2mL Ni-NTA Affinity Resin - Amintra per mL of gel volume required.
- Gently shake the bottle of resin to achieve a homogeneous suspension and immediately pipette the suspension to an appropriate column.
- Remove first the upper and then the lower cap of the column to allow sedimentation of the gel and decant the supernatant by gravity flow.
- Equilibrate with Binding Buffer using 5 to 10 bed volumes of resin. Allow buffer to drain from the column

Δ Note: Binding buffer: 50 mM NaH₂PO₄, 300 mM NaCl, 10 mM imidazole. Adjust the buffer pH to 8 with NaOH solution.

6.1.2 Sample application:

- Prepare sample by mixing protein extract with an equal volume of binding buffer.
- Add the sample containing the His-tagged protein to be purified through the top of the column, keeping sample and

resin mixing in contact for 30-60 mins before removing the bottom cap.

- Remove the lower cap of the column. Collect the flow-through in a tube and if desired, re-apply the flow-through once to maximize binding.

6.1.3 Protein elution:

- Close column outlet with the cap. Wash with Wash Buffer using 10 to 15 bed volumes of the of Ni-NTA Affinity Resin - Amintra used.
- Add 1 bed volume of Elution Buffer to the column. Close column inlet with the cap and mix thoroughly for 10 mins at room temperature. Sediment the gel, remove the end cap and collect the eluate in a new tube and store on ice.
- Repeat this step 4 to 6 more times and check your eluates using His-Tag Protein Expression Check Kit (ab270048) or Precast Gels before pooling your fractions.

***Δ Note:** It is possible that a significant amount of His-tagged protein may remain bound to the resin. Conditions (volumes, times, temperatures) used for elution may vary among His-tag proteins. Eluates should be monitored (Bradford protein assay, SDS-PAGE or measure the absorbance at 280 nm to determine the yield of the eluted His-fused protein).*

6.1.4 Regeneration:

Ni-NTA Affinity Resin - Amintra may be used a number of times before it becomes necessary to recharge it with metal ions. When the back pressure is too high or the capacity significantly lower, it is needed to strip the metal ions and recharge the Ni-NTA Beads according to the following procedure.

Wash the column with one of the following solutions:

- 0.2 M acetic acid with 6 M Guanidine Hydrochloride, 2 column volumes;
- Rinse with 5 column volumes of distilled water

Store Ni-NTA Affinity Resin - Amintra for longer periods of time in an equal volume of 1X PBS containing 20% ethanol at 4°C.

6.2 Batch purification of His-tagged proteins under native conditions

6.2.1 Elimination of the Preservative:

- Determine the quantity of Ni-NTA Affinity Resin - Amintra needed for your purification following the recommendations below:
- Option A: Gently shake the bottle of Ni-NTA Affinity Resin - Amintra to achieve a homogenous suspension. Immediately pipette the suspension (2 mL of the Ni-NTA Affinity Resin - Amintra suspension per mL of gel volume required) to an appropriate tube. Sediment the gel by centrifugation at 500 x g for 5 minutes and carefully decant the supernatant and discard it.
- Option B: Manually shake the bottle of resin to obtain a homogenous suspension of beads and preservative. Invert the bottle of resin a few times and then filter the resin and put it in a container.

6.2.2 Equilibration of the Resin:

- Add 10 bed volumes of binding buffer to equilibrate the gel by mixing it thoroughly to achieve a homogenous suspension. Sediment the gel by centrifugation at 500 x g for 5 minutes and carefully decant the supernatant and discard it.

Δ Note: Binding buffer: 50 mM NaH₂PO₄, 300 mM NaCl, 10 mM imidazole, pH 8.0

- Binding buffer: The choice of buffer depends on the particular properties of the protein. The buffer used most frequently is phosphate (50 mM is recommended). The pH of binding buffers generally leads to neutrality (7.0-8.0).

Δ Note: In some cases to increase the selectivity of the binding of target protein it is necessary to add to the binding buffer a small concentration of imidazole (1040 mM). It is important to use high purity imidazole to avoid affecting the O.D. 280nm. It is important to avoid the presence of agents like EDTA or citrate at all times.

6.2.3 Application of the Sample:

- Once the resin is equilibrated, the sample containing the fused protein for purification is applied. In some cases a slight increase of contact time may facilitate the binding.

Δ Note: Binding capacity can be affected by several factors, such as sample concentration, binding buffer or the flow rate during sample application.

- Once the resin is equilibrated, add the clarified E. coli lysate or protein extract. Mix the suspension gently for 30-60 min at room temperature. Centrifuge the suspension at 500 x g for 5 minutes to sediment the resin. Carefully decant the supernatant and discard it.

6.2.4 Washing of the Resin:

- Wash the gel by adding 10 mL bed volumes of wash buffer. Invert to mix and centrifuge the suspension at 500 x g for 5 minutes to sediment the resin. Carefully decant the supernatant and discard it.
- Repeat the washing step twice (total wash 3 x 10 bed volumes of binding buffer).

Δ Note: Washing buffer: 50 mM NaH_2PO_4 , 300 mM NaCl, 20 mM imidazole, pH 8.0.

6.2.5 Elution:

- Add 1 bed volume of elution buffer to the gel. Mix thoroughly for 10 min at room temperature. Sediment the gel by centrifugation at 500 x g for 5 minutes and carefully decant or pipette the supernatant in a new tube and store on ice.

Repeat the elution step twice or more and pool the fractions containing the purified protein.

Δ Note: *Elution buffer: 50 mM NaH₂PO₄, 300 mM Cl, 250 mM imidazole pH 8.0. It is possible that a significant amount of His-tagged protein may remain bound to the resin. Conditions (volumes, times, temperatures) used for elution may vary among His-tag proteins. Eluates should be monitored (Bradford protein assay, SDS-PAGE or measure the absorbance at 280 nm to determine the yield of the eluted His-fused protein).*

Δ Note: *For proper storage it is recommended to remove the imidazole by ultrafiltration or dialysis.*

6.2.6 Regeneration:

During the life of the resin, the binding capacity may reduce and regeneration may be required.

Protein Removal:

- Solubilize and desorb contaminants: It is necessary to wash the resin with 0.5 M NaOH (30 min)
- Remove the NaOH solution by washing with 10 bed volumes of distilled water.
- If you are using the resin directly, wash with 10 bed volumes of 50 mM NaH₂PO₄, 300 mM NaCl, 10 mM imidazole,
- pH 8.0.
- For storage, wash with 2 volumes of 30% ethanol, resuspend the resin in 30% ethanol and store at 4-8°C.

Resin Recharge:

- wash the resin with 10 column volumes of distilled water.
- wash with 10 column volumes of 100 mM EDTA, pH 8.0
- in order to eliminate the residual EDTA before reloading the resin with the metal, the column should be washed
- with 10 column volumes of distilled water.

- wash with 2 column volumes of 100 mM metal ion aqueous solution (normally chlorides or sulphates are used).
- wash with 10 column volumes of distilled water.
- 10 column volumes of the binding buffer (50 mM NaH₂PO₄, 300 mM NaCl, 10 mM imidazole, pH 8.0).
- Note: If the resin is not going to be used for a while it is recommended to replace the last step by the addition of a preservative.

6.3 Spin Purification of His-tagged proteins under native conditions:

6.3.1 Equilibration:

- Gently shake the bottle of Ni-NTA Affinity Resin - Amintra to achieve a homogenous suspension. Remove first the upper inlet cap and immediately pipette 100 µL of the original suspension to the empty spin column. Remove the lower outlet cap and put the spin column in a collecting tube. Centrifuge at 500 x *g* for 30 secs.

Δ Note: 100 µL of the original 50% suspension corresponds to 50 µL of gel.

- Equilibrate the spin column with 500 µl of binding buffer. Mix manually, centrifuge at 500 x *g* for 30 seconds and discard flow through.

Δ Note: Binding buffer: 50 mM NaH₂ PO₄, 300 mM NaCl, 10 mM imidazole, pH 8.0.

6.3.2 Sample application:

- Close spin column outlet with cap. Add the sample containing the His-tagged protein to be purified through the top of the spin column, keeping sample and resin in contact 30 to 60 minutes before removing the bottom cap. Mix manually inverting the spin column. Remove bottom cap and place spin column in a collecting tube. Centrifuge at 500 x *g* for 30 secs and discard the flow through.

***Δ Note:** In some cases, a slight increase of contact time may facilitate binding.*

***Δ Note:** Binding capacity can be affected by several factors such as sample concentration.*

6.3.3 Washing:

- Add 500 µL washing buffer through the top to eliminate all the proteins that have not been retained in the column. Mix manually inverting the spin column. Centrifuge at 500 x *g* for 30 secs and discard the flow through. Repeat the washing step twice and discard flow through between washing steps.

***Δ Note:** Washing buffer: 50 mM NaH₂PO₄, 300 mM NaCl, 20 mM imidazole, pH 8.0.*

***Δ Note:** Wash the column with the binding buffer until the O.D. 280 nm of the eluent reached the baseline level.*

6.3.4 Protein elution:

- Close spin column outlet with cap. Add 500 µL of elution buffer and close the lid. Mix thoroughly for 10 mins at room temperature. Centrifuge the gel, remove the end cap and collect the eluate in a new tube and store on ice. Repeat the elution step twice and pool the collected eluates.

***Δ Note:** Elution buffer: 50 mM NaH₂PO₄, 300 mM NaCl, 250 mM imidazole pH 8.0. It is possible that a significant amount of His-tagged protein may remain bound to the resin. Conditions (volumes, times, temperatures) used for elution may vary among His-tag proteins. Eluates should be monitored (Bradford protein assay, SDS-PAGE or measure the absorbance at 280 nm to determine the yield of the eluted His-fused protein).*

6.3.5 Regeneration:

During the life of the resin, the binding capacity may reduce and regeneration may be required.

Protein removal:

- Solubilize and desorb contaminants: It is necessary to wash the resin with 0.5 M NaOH (30 mins).
- Remove the NaOH solution by washing with 10 bed volumes of distilled water.
- If you are using the resin directly, wash with 10 bed volumes of 50 mM NaH₂PO₄, 300 mM NaCl, 10 mM imidazole, pH 8.0.
- For storage, wash with 2 volumes of 30% ethanol, resuspend the resin in 30% ethanol and store at 4-8°C.

Resin recharge:

- Wash the resin with 10 column volumes of distilled water, then wash with 10 column volumes of 100 mM EDTA, pH 8.0.
- In order to eliminate the residual EDTA before reloading the resin with the metal, the column should be washed with 10 column volumes of distilled water.
- Wash with 2 column volumes of 100 mM metal ion aqueous solution (normally chlorides or sulphates are used).
- Wash with 10 column volumes of distilled water.
- Wash with 10 column volumes of the binding buffer (50 mM NaH₂PO₄, 300 mM NaCl, 10 mM imidazole, pH 8.0).

Δ Note: *If the resin is not going to be used for a while it is recommended to replace the last step by the addition of a preservative.*

6.4 **Analysis:**

- 6.4.1 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.*

7. Frequently Asked Questions

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.

2. Do I need to filter the buffers prepared in my laboratory?

It is good laboratory practice to filter all buffers.

3. How should I prepare my sample for the Ni-NTA Affinity Resin - Amintra?

Many chromatographic procedures demand that the sample is preconditioned 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 either drawing it through a syringe needle several times or by adding appropriate amounts of DNase and/or RNase (5-10 µg/mL) to the lysis buffer and incubating the mix on ice for 15 mins.

4. Should I add beta-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 beta-mercaptoethanol can reduce all disulphide bonds formed between the contaminating proteins and the target protein. We recommend 0.5 mM TCEP. Concentration less than 20 mM beta-mercaptoethanol can be used with the IMAC resin. Please see compatibility table for more detail

5. What can I do if the resin has changed colour?

The blue colour is attributed to the Ni²⁺ salt. Reductants (e.g. DTT) will cause the resin to turn brown and chelating agents (e.g. EDTA) will cause the resin to turn white. Ensure that all solutions are compatible with the Ni-NTA Affinity Resin - Amintra.

6. How can I ensure that levels of contaminants in the final eluate remain low?

Ensure that the binding buffer contains minimum 10 mM imidazole and the wash buffer contains minimum 20-30 mM imidazole.

7. 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.

8. 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.

9. 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 6-8 M urea can also be loaded directly on to a denaturing SDS-polyacrylamide gel. Proteins purified in the presence of 4-6 M guanidine HCl should be buffer exchanged in buffers lacking the denaturant prior to a denaturing SDS-PAGE.

10. Do I need to remove the His-tag from the recombinant protein after purification?

Normally, a protease cleavage site is engineered between the His-tag and the target protein. The target protein can then be re-purified using Ni-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.

11. 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 Ni²⁺, binding capacity may be reduced.

8. Troubleshooting

Bubbles or cracks appear in the resin bed

- The resin has been stored at a cool temperature and then rapidly warmed up. Ni-NTA Affinity Resin - Amintra should be warmed slowly to room temperature before use.

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 4°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.

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. Enhance protein solubility
- 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.

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 6-8 M urea or 4-6 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 expressed or use special E.coli strains devoid of proteases.

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.

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.

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

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)