Amintra™ NI-NTA Resin

Metal Chelate Affinity Resin

expedeon

INDEX

ORDERING INFORMATION

PRODUCT	SIZE	CAT. NO.
Amintra™ Ni-NTA	10 ml	ANN0010
Amintra™ Ni-NTA	25 ml	ANN0025
Amintra™ Ni-NTA	100 ml	ANN0100
Amintra™ Ni-NTA	Custom Volume	ANNCUST

INTRODUCTION

Expedeon's Ni-NTA affinity resin 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 Expedon's Amintra™ Ni-NTA resin may be used in a wide range of structure and activity-based laboratory procedures.

Expedeon offers Amintra™ Resins in prepacked columns too, 1 and 5ml.

STORAGE

Store the Amintra™ Ni-NTA resin at 2-8˚C. 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% slurry in 20% ethanol. Amintra™ Ni-NTA resin is stable for up to 2 years at 2-8˚C from the date of manufacture. For expiry date please see product.

SPECIFICATION

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

CHEMICAL COMPATIBILITY

All resins are susceptible to oxidative agents. Avoid high temperatures. The resin are 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 20mM beta-mercaptoethanol can be used. See table for more detail.

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

REAGENTS

CHEMICAL COMPATIBILITY

	REAGENTS	COMMENTS
BUFFERS	• Sodium Phosphate	• Sodium Phospate buffer 50mM pH 7.4 is recommended
	• Sodium acetate	• Up to 100mM may be used. pH 4 is recommended
	• Tris-HCl, Tris-acetate, HEPES, MOPS	• Coordinate with metal ions, causing a decrease in binding capacity. Up to 100 mM may be used. pH 7.4 is recommended
DENATURING AGENTS	• Urea	• Solubilizes protein. Use 8M for purification under denaturing conditions.
	• Guanadine-HCl	• Solubilizes protein. Up to 6M can be used
ADDITIVES	• Imidazole	• Competes with the His-tag protein. • Reduces non-specific binding: 20 mM. • Up to 500 mM can be used
	• Glycerol	• Avoids hydrophobic interactions between proteins. • Up to 50% can be used
	• EDTA	• Coordinates with nickel, causing a decrease in capacity. Not recommended, but up to 1 mM in samples has been used successfully in some cases. • Generally, chelating agents should be used with caution and only in the sample, not in the buffers
	• Ethanol	• Avoids hydrophobic interactions between proteins but may precipitate proteins causing column clogging and low flow rates. • Up to 20% can be used
	• Sodium sulfate	• 100 mM can be used
	• Sodium chloride	• Up to 1.5M has been used
	• Citrate	• Up to 60mM in samples has been used
REDUCING AGENTS	• Reduced glutathione	• Can reduce Ni^{2+} ions at higher concentrations. Up to 30 mM samples has been used
	• b-mercaptoethanol	• Avoids formation of disulphide bonds. Can reduce Ni^{3+} ions at higher concentrations. Up to 20mM in samples has been used
	• DTE	• Can reduce Ni^{3+} ions at higher concentrations. Up to 10mM in samples has been used
	• DTT	• Can reduce Ni^{2+} ions at higher concentrations. Up to 0.5 - 1 mM in samples can be used
	• TCEP	• Breaks disulfide bonds. Up to 5 mM in samples can be used
	• SDS	• Avoids hydrophobic interactions between proteins. • Conditions with cations, causing a decrease in capacity. • Not recommended but up to 0.3% in samples
DETERGENTS	• Non ionic detergents (Tween, Triton, etc)	• Removes background proteins.
	• Anionic detergent (Cholate)	• Up to 2% can be used
	• Zwitterionic detergent (CHAPS)	• Up to 1% can be used

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^{2+}, Co^{2+}, Cu^{2+}, Zn^{2+} (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^{2+} is the most widely used metal ion as most IMAC tags seem to have very high affinity for immobilized Ni^{2+}. 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 pre-treatment 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.

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 posttranslational 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 Baculovirus-infected insect cells can be lysed by sonication at +4ºC 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 Proteoloc™, Basemuncher™ endonuclease 99% purity and 10 mM β-mercaptoethanol can also be added to the lysis buffer. Addition of β-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 βmercaptoethanol 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 Ni-NTA resin can be achieved by rapid dialysis, diafiltration using ultrafiltration concentrators, gel-filtration chromatography in the appropriate pre-equilibration 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 non-denaturing conditions during metal chelate chromatography.

Aggregation/precipitation of proteins is common during storage and repeated freeze/thaw cycles. Expedeon's NVoy technology is designed to stabilize your proteins in solution and enhance chromatographic purification.

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.

Buffer Selection

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 (0.15M – 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 Ni^{2+} matrix.

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. Expedeon's Nvoy technology can be used to enhance protein stability in under acid or other denaturing conditions.

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.

BUFFERS PREPARATION

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 0.22µm or 0.45 µm filter.

Expedeon's Ni-NTA Resins can be used for His-tagged protein purification under both native and denaturing conditions, which may require different buffers. Please see information below:

Native conditions

Binding buffer: 50 mM NaH_2PO_4-H_2O, 300mM NaCl, 10 mM imidazole. Adjust the buffer pH to 8.0 using NaOH solution.
Wash buffer: 50 mM NaH_2PO_4-H_2O, 300mM NaCl, 20 mM imidazole. Adjust the buffer pH to 8.0 using NaOH solution.
Elution buffer: 50 mM NaH_2PO_4-H_2O, 300mM NaCl, 250 mM imidazole. Adjust the buffer pH to 8.0 using NaOH solution.

Denatured conditions

Binding buffer: 100 mM NaH_2PO_4-H_2O, 100mM Tris-HCl, 8 M Urea. Adjust the buffer pH to 8.0 using HCl solution.
Wash buffer: 100 mM NaH_2PO_4-H_2O, 100mM Tris-HCl, 8 M Urea. Adjust the buffer pH to 6.3 using HCl solution.
Elution buffer: 100 mM NaH_2PO_4-H_2O, 100mM Tris-HCl, 8 M Urea. Adjust the buffer pH to 4.5 using HCl solution.

	NATIVE CONDITIONS			DENATURED CONDITIONS		
	Binding Buffer	Wash Buffer	Elution Buffer	Binding Buffer	Wash Buffer	Elution Buffer
NaH_2PO_4-H_2O	50 mM (7.80 g)	50 mM (7.80 g)	50 mM (7.80 g)	100 mM (15.60 g)	100 mM (15.60 g)	100 mM (15.60 g)
NaCl	300 mM (17.54 g)	300 mM (17.54 g)	300 mM (17.54 g)			
Imidazole	10 mM (0.68 g)	20 mM (1.36 g)	250 mM (17.0 g)			
Urea				8 M (480.50 g)	8 M (480.50 g)	8 M (480.50 g)
Tris-HCl				100 mM (15.76 g)	100 mM (15.76 g)	100 mM (15.76 g)

PROTOCOL

1. Gravity purification of His-tagged proteins under native conditions

1.1 Elimination of the Preservative

Determine the quantity of Amintra™ Ni-NTA needed for your purification: 2ml of Amintra™ Ni-NTA per ml of gel volume required.

Gently shake the bottle of Amintra™ Ni-NTA 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.

Note: Binding buffer: 50 mM NaH_2PO_4, 300 mM NaCl, 10 mM imidazole. Adjust the buffer pH to 8 with NaOH solution.

1.2 Application of the Sample

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 minutes 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.

1.3 Elution of the Pure Protein

Close column outlet with the cap. Wash with Wash Buffer using 10 to 15 bed volumes of the Amintra™ Ni-NTA suspension used.

Add 1 bed volume of Elution Buffer to the column. Close column inlet with the cap and mix thoroughly for 10 minutes 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 Expedeons' HisTag Check&Go! (Cat # 4003-0030) or Precast Gels before pooling your fractions (Not provided with the resin).

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).

Notes:
Wash Buffer: 50 mM NaH2PO4, 300 mM NaCl, 20 mM imidazole. Adjust the buffer pH to 8 with NaOH solution.
Elution buffer: 50 mM NaH2PO4, 300 mM NaCl, 250 mM imidazole. Adjust the buffer pH to 8 with NaOH solution.

1.4 Regeneration & Storage

In general, Amintra™ Ni-NTA beads 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 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:

1. 0.2M acetic acid with 6 M Guanidine Hydrochloride, 2 column volumes;
2. Rinse with 5 column volumes of distilled water

Store Amintra™ Ni-NTA Beads for longer periods of time in an equal volume of 1X PBS containing 20% ethanol at 2-8°C.

2. Batch purification of His-tagged proteins under native conditions

The following procedure is adapted for the purification of Histagged protein in batch and under native conditions.

2.1 Elimination of the Preservative

Determine the quantity of Amintra™ Ni-NTA needed for your purification following the recommendations below:

Option A: Gently shake the bottle of Amintra™ Ni-NTA to achieve a homogenous suspension. Immediately pipette the suspension (2 ml of the Amintra™ Ni-NTA 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.

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.

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).

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.

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.

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.

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).

Note: Washing buffer: 50 mM NaH^2PO^4, 300 mM NaCl, 20 mM imidazole, pH 8.0.

2.5 Elution of the Pure Protein

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.

Note: Elution buffer: 50 mM NaH^2PO^4, 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).

For proper storage it is recommended to remove the imidazole by ultrafiltration or dialysis.

2.6 Regeneration & Storage

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

Protein Removal:

A Solubilize and desorb contaminants: It is necessary to wash the resin with 0.5 m NaOH (30 min)
B Remove the NaOH solution by washing with 10 bed volumes of distilled water.
C If you are using the resin directly, wash with 10 bed volumes of 50 mM NaH^2PO^4, 300 mM NaCl, 10 mM imidazole, pH 8.0.
D 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 NaH^2PO^4, 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.

3. Spin purification of His-tagged proteins under native conditions

3.1 Elimination of the Preservative

Gently shake the bottle of Amintra™ Ni-NTA 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 seconds.

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

3.2 Equilibration of the Spin Column

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

Note: Binding buffer: 50 mM NaH^2PO^4, 300 mM NaCl, 10 mM imidazole, pH 8.0.

3.3 Application of the Sample

Close spin column outlet with cap. Add the sample containing the Histagged protein to be purified (see recommendations in section 1.1) through the top of the spin column, keeping sample and resin in contact 30-60 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 seconds and discard the flow through.

Note: In some cases a slight increase of contact time may facilitate binding. Note: Binding capacity can be affected by several factors such as sample concentration.

3.4 Washing of the Spin Column

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 seconds and discard the flow through. Repeat the washing step twice and discard flow through between washing steps.

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

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

3.5 Elution of the Pure Protein

Close spin column outlet with cap. Add 500 µl of elution buffer and close the lid. Mix thoroughly for 10 minutes 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.

Note: Elution buffer: 50 mM NaH^2PO^4, 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).

3.6 Regeneration & Storage

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

Protein Removal:

A solubilize and desorb contaminants: It is necessary to wash the resin with 0.5 m NaOH (30 min)

B remove the NaOH solution by washing with 10 bed volumes of distilled water.

C 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.

D 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 NaH^2PO^4, 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.

FREQUENTLY ASKED QUESTIONS

Q. What is the shelf-life of Metal Chelate resin?
A. The resin is guaranteed for 2 years after the date of manufacture provided they are stored at 2-8˚C.

Q. Do I need to filter the buffers prepared in my laboratory?
A. It is good laboratory practice to filter all buffers.

Q. How should I prepare my sample for the Amintra™ Ni-NTA resin?
A. 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.

Q. Should I add b-mercaptoethanol to the lysis buffer?
A. 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 0.5mM TCEP. Concentration less than 20 mM β-mercaptoethanol can be used with the IMAC resin. Please see compatibility table for more detail

Q. Can I immobilize the metal chelate resin with a different 7. What can I do if the resin has changed colour?
A. 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 Amintra™ Ni-NTA resin.

Q. How can I ensure that levels of contaminants in the final eluate remain low?
A. Ensure that the binding buffer contains minimum 10 mM imidazole and the wash buffer contains minimum 20-30 mM imidazole.

Q. Should I be concerned if the resin partially dried out during the chromatographic steps?
A. The resin is robust. Partially dried resin rehydrates rapidly. There are no adverse effects upon the performance of the resin.

Q. Should I remove imidazole after the final elution step?
A. 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 -80ºC. Alternatively you can use a Stabil-PAC kit to enhance protein stability in imidazole solutions.

Q. Can I load purified protein immediately on to an SDS-gel?
A. 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.

Q. Do I need to remove the His-tag from the recombinant protein after purification?
A. Normally, a protease cleavage site is engineered between the His-tag and the target protein. The target protein can then be re-purified using Amintra™ Ni-NTA resin 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. When protein precipitation is observed during cleavage Expedeon's Stabil-PAC (# STP) can be used to maintain protein solubility.

Q. Can I re-use the resin?
A. 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.

TROUBLESHOOTING

Bubbles or cracks appear in the resin bed

- The resin has been stored at a cool temperature and then rapidly warmed up. Amintra™ Ni-NTA resin 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 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.

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. Use StabilPAC (# STP) to 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 exposed 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 and use Stabil-PAC (# STP) to reduce non-specific binding.
- 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 2-8ºC 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.

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