

Amintra

Affinty Resins

NiHIS

Metal Chelate Affinity Resin
Technical Data and Instruction Manual



expedeon
PROTEIN SOLUTIONS
Innovators of Protein Technologies

info@expedeon.com

Expedeon Protein Solutions
Innovators of Protein Technologies
www.expedeon.com

TABLE OF CONTENTS

Introduction	4
Storage.....	4
Specifications	4
Chemical compatibility.....	4
Disclaimer	5
Metal Chelate chromatography	6
Recommended Buffers.....	8
Questions and Answers	9
Troubleshooting assistant	11
Ordering Information	12
Contact Information.....	12

Introduction

Expedeon's Ni-charged 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 Expedeon's Amintra NiHIS resin may be used in a wide range of structure and activity-based laboratory procedures.

Storage

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

Specifications

Supporting matrix:	Covalently coupled to agarose resin
Charged metal ion:	Ni ²⁺
Bead size range:	45-165 µm
Recommended working pH:	pH 2.0-12.0
Typical binding capacity:	~10 mg His-tagged recombinant protein/ml resin
Linear Flow rate	Upto 300 cm/h (5cm diameter column, pressure 1 bar)
Maximum Flow rate	20-40 ml/min
Optimum Flow Rate	1-10 ml/min
Maximum pressure	0.1MPa (1 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 (e.g. 30 % ethanol) 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 10mM beta-mercaptoethanol can be used. Strong reducing agents such as DTT and DTE are not compatible as these reduce the metal ion resulting in lower binding capacity.

Disclaimer

This product is for research use only and is not intended for use in clinical diagnosis. No claims beyond replacement of unacceptable material or refund of purchase price shall be allowed.

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 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 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.

Protease inhibitor cocktails, such as Boehringer "Complete EDTA-free", 5-50 µg/ml DNase I 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 β-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 NiHIS 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 but 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 Ni²⁺ matrix. Following purification these protein can then be refolded with Expedeon's Refold SK or Refold Master kits.

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 NiHIS 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.

Recommended Buffers

Native proteins

Binding buffer:	50 mM sodium phosphate buffer pH 7.4, 300 mM NaCl, 10 mM imidazole.
Wash buffer:	50 mM sodium phosphate buffer pH 7.4, 300 mM NaCl, 30 mM imidazole.
Elution buffer:	50 mM sodium phosphate buffer pH 7.4, 300 mM NaCl, 300 mM imidazole.

Denatured proteins

Imidazole Elution:

Binding buffer:	50 mM sodium phosphate buffer pH 7.4, 300 mM NaCl, 10 mM imidazole, 6-8 M Urea.
Wash buffer:	50 mM sodium phosphate buffer pH 7.4, 300 mM NaCl, 30 mM imidazole, 6-8 M Urea.
Elution buffer:	50 mM sodium phosphate buffer pH 7.4, 300 mM NaCl, 300 mM imidazole, 6-8 M Urea.

Acid Elution:

Binding buffer:	50 mM sodium phosphate buffer pH 7.4, 300 mM NaCl, 6-8 M Urea.
Wash buffer:	50 mM sodium phosphate buffer pH 6.0, 300 mM NaCl, 6-8 M Urea.
Elution buffer:	50 mM sodium phosphate buffer pH 4.0, 300 mM NaCl, 6-8 M Urea.

Please note if you work from phosphate stock solutions that the addition of urea will cause the pH to drop. Titrate the buffer with NaOH to bring the pH back to pH 7.4. The pH of those buffers containing urea should always be checked and adjusted, if necessary, immediately before use.

Questions and Answers

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 Amintra NiHIS 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 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 *b*-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 0.5mM 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.

5. How can I regenerate the metal chelate resin?

We recommend that you wash the resin with elution buffer and then re-equilibrate the resin binding buffer. Proceed to the pre-equilibration step if resin is to be re-used immediately. After regeneration, the resin can also be stored in a screw-capped bottle containing 0.1 % sodium azide (made up in distilled water) at 2-8 °C until further use.

6. Can I immobilize the metal chelate resin with a different metal ion?

It is possible to charge the resin with a different metal ion. Ensure that the resin is stripped of Ni²⁺. This is achieved by successive washing with 1-2 column volumes of (i) 0.2 M EDTA, 0.5 M NaCl (ii) 0.2 M NaOH (iii) distilled water and finally (iv) 0.1 M metal salt. Then wash the column with at least 5 column volumes of distilled water to remove free metal ion.

7. 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 Amintra NiHIS resin.

8. How can I re-charge the resin with NiSO₄?

Wash the resin with 3 column volumes of distilled water followed by 1 column volume of 0.1 M NiSO₄ solution (made up in distilled water). Wash off any unbound NiSO₄ with 5-10 column volumes of distilled water and equilibrate the resin with 1 x PBS buffer, pH 7.4.

9. 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.

10. 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.

11. 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. Alternatively you can use a Stabil-PAC kit to enhance protein stability in imidazole solutions.

12. 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.

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

Normally, a protease cleavage site e.g. Factor Xa Protease is engineered between the His-tag and the target protein. The target protein can then be re-purified using Amintra NiHIS 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.

14. 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.

Troubleshooting assistant

Bubbles or cracks appear in the resin bed

- The resin has been stored at a cool temperature and then rapidly warmed up. Amintra NiHIS 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 2-8 °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.

Ordering Information

Cat #	Description
ANI0005	Amintra NiHIS - 5ml
ANI0025	Amintra NiHIS - 25ml
ANI0100	Amintra NiHIS- 100ml

Contact Information

Expedeon Protein Solutions

Unit 1A, Button End

Harston,

Cambridgeshire.

CB22 7GX

United Kingdom

Phone: +44 (0)1223 873364

Fax: +44 (0)1223 873371

E-mail: info@expedeon.com

Web : www.expedeon.com