Fastback Ni IMAC Resin
Technical Datasheet

Metal chelate affinity chromatography is a rapid one-step purification, which removes most contaminants and can achieve purities close to homogeneity. Protein Ark's Fastback Nickel IMAC resin is designed for simple, rapid His-tagged recombinant protein purification from a cell lysate under native or denaturing conditions.

The loose metal chelate resin adds a layer of scalability to the Proteus IMAC purification kit portfolio which incorporates pre-packed Nickel agarose resin plugs in ready-to-use spin columns. The objective is to offer the researcher total protein purification solutions from the initial fractionation stage to the final polishing steps.

Specification:

Specificity:	Polyhistidine tag
Matrix:	Agarose
Coupled ligand:	Iminodiacetic acid (IDA)
Binding capacity:	5 - 10 mg/ml (6xHis-tagged recombinant protein/ml)
Bead size:	45-165 µm
Flow rate:	0.25-2 ml/min
Maximum pressure:	42 psi
Buffer compatibility:	Common aqueous buffers from pH 2-12
Chemical stability:	High
Endotoxin levels	Unknown
Solubility in water:	Insoluble
Shipping/delivery:	50% (v/v)resin suspension in 20% ethanol
Storage:	Equilibration buffer at 2-8°C (short-term) 20% ethanol at 2-8°C (long-term)

Chemical compatibility of the Fastback Ni IMAC Resin

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.

Storage conditions:

- Store the Ni IMAC 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 de-fined. It is transported in 20 % ethanol. Protein Ark's resin is stable for up to 2 years at 2-8°C from the date of manufacture. The expiry date is recorded clearly on the label of the bottle

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.

Ordering Information:

Product	Volume	Order Code
Fastback Ni IMAC Resin (10 ml)	10 ml	Fastback-Ni-10
Fastback Ni IMAC Resin (25 ml)	25 ml	Fastback-Ni-25
Fastback Ni IMAC Resin (100 ml)	100 ml	Fastback-Ni-100

Accessories	Quantity	Order Code
Proteus Metal Chelate Buffer Pack (250 ml 5 x PBS Buffer A, 150 ml 1 x Imidazole Buffer B)	1	PC-BMCP

Principles of IMAC chromatography:

Proteins are engineered with affinity tags attached to the 5' or 3' end of the target gene. Examples of such tags are hexahistidine (Mr 700-900) and an 8-residue peptide containing alternating histidines (Mr 900).

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+} (see 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 (K_d-10^{-13} at pH 8.0).

The most common matrix for IMAC purification is iminodiacetic acid (IDA).
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.

Application drivers for IMAC chromatography:

Screening expression clones for high levels of His-tagged proteins
Purification of recombinant proteins for raising antibodies
Purification of recombinant proteins for activity and/or structural studies

Affinity chromatography:

The essence of affinity chromatography utilizes the concept of bio-specificity, implying an interaction between a natural binding site and the natural ligand, whether it be enzyme-substrate, enzyme-inhibitor or His-tagged protein-metal ion interactions.

Often a researcher needs to choose the correct base matrix, select the optimal activation chemistry and couple a suitable ligand to develop an affinity column to purify the target protein. Protein Ark's resins remove the guesswork associated with optimizing the resin chemistry by offering ready-to-use resins that have satisfied stringent quality control to guarantee reproducible purification performance. Optimal buffer and elution conditions for the purification step of many His-tagged proteins

General considerations for selecting optimal binding conditions for the IMAC resin:

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.

Typically, a protease inhibitor cocktail, such as Boehringer "Complete EDTA-free", 5-50 µg/ml DNase I and 10 mM β-mercaptoethanol are 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 times for the binding, washing and elution steps to be increased. It is important that the sample is clarified to remove particulates that could clog the resin flow channels. It is good practice to filter the sample just prior to loading even if it has 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 Fastback-Ni 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.

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. Of equal importance is the ability to process the samples rapidly and, if the need arises, to be able to purify the target protein at 4°C.

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 can be adversely affected.

Choosing the correct buffer conditions for IMAC separations:

We recommend sodium phosphate buffers as buffers with secondary or tertiary amines e.g. Tris buffers can reduce the nickel ion. pH 7-8 works well for most immobilized Ni^{2+} applications and 0.15-0.5 M NaCl is added to the buffers in order to prevent non-specific ionic interactions and to stabilize some proteins in solution.

Non-ionic detergents 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 6xHis-tag much more accessible for interaction with the immobilized Ni^{2+} matrix.

Attempts can be made to renature the target protein by dialyzing it sequentially against binding buffers containing decreasing levels of urea or passing decreasing levels of urea in binding buffer over the washed protein bound to the Fastback-Ni resin and eluting the refolded protein with 300 mM imidazole (between pH 7 and pH 8). Alternatively, denatured proteins can be diluted into a large volume of buffer lacking denaturant. The dispersive effect dilutes out the denaturant resulting in the re-folding of the protein.

Eluting the protein from the IMAC resin:

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. Above all, the elution conditions must preserve the integrity and activity of the target protein. Most observed denaturation is caused by harsh elution conditions.

Binding kinetics of IMAC resin:

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. This IMAC resin chemistry has sufficiently rapid association kinetics between the protein molecule and the immobilized ligand to allow for optimal diffusional flow through the internal bead structure.

Fastback-Ni IMAC Resin:

IDA cross-linked agarose resin consists of iminodiacetic acid groups ligated by stable ether linkages via a flexible spacer arm. The resin is charged with Ni^{2+} to give a marine blue appearance.

Protocol for purifying His-tagged proteins:

Under NATIVE conditions:

These protocols refer to the purification of active folded proteins. As strength of binding will be determined by length and accessibility of the His-tag the metal ion, buffer composition and pH and elution conditions, some adjustments of the procedure in this instruction sheet is often required.

Ideally, the cell lysate should be made up in phosphate buffer pH 7.4-8.0 in the presence of NaCl (to reduce non-specific adsorption effects).

The following metal chelate buffers are proposed for your IMAC separation. The procedure to prepare these working buffer solutions from stock buffers is shown in

Recommended buffers for NATIVE IMAC purifications:

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.

NOTE: All buffers contain sodium azide as a preservative

Table 1: Showing how to prepare your metal chelate binding, wash and elution buffers.

Imidazole Concentration (mM)	Recommended usage	Buffer (ml) (5 x PBS Buffer)	Elution Buffer (ml) (1 M Imidazole)	Distilled Water (ml)	Total Volume (ml)
10	Binding buffer	20	1	79	100
20		20	2	78	100
30	Wash buffer	20	3	77	100
50		20	5	75	100
100		20	10	70	100
150		20	15	65	100
200		20	20	60	100
250		20	25	55	100
300	Elution buffer	20	30	50	100
350		20	35	45	100
400		20	40	40	100
450		20	45	35	100
500		20	50	30	100

NOTE: Always measure the pH of the working buffer solutions when they are prepared and adjust to pH 7.4 whenever necessary. It is not uncommon for buffers to precipitate or freeze partially in the cold during long term storage or when laboratory temperatures drop at night. The buffers should be warmed up and they can be used once all the precipitate has re-dissolved.

Under DENATURING conditions:

Recombinant proteins often form insoluble inclusion bodies when they are expressed at high levels. These proteins can be solubilized easily in the presence of denaturants such as 6-8 M urea or 6 M guanidine hydrochloride. Additionally, a researcher may be choose to purify their recombinant protein under denaturing conditions if they wish to use the purified denatured protein for raising antibodies. Two buffer configurations can be used under denaturing conditions. One buffer system employs imidazole to competitively elute the target protein under denaturing conditions and the other buffer system uses a more acidic pH to elute the target protein in the absence of imidazole. Choosing either buffer system will depend critically upon the nature of your target protein e.g. stability in acid environment.

Recommended buffers for DENATURING purifications:

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.

Note: 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 be checked and adjusted, if necessary, immediately before use.

Metal chelate buffer formulations supplied in the buffer pack (PC-BMCP)
(See Accessories in the 'Ordering Information' section):

Under denaturing conditions, you may choose to add urea to the buffers below. In this case, 480.48 g urea (Mr 60.06) should be added to a final 1 L buffer volume. Also note that the final pH of the buffers containing urea only should be adjusted immediately before use. All buffers contain 0.1 % sodium azide as preservative.

5 x PBS buffer (to prepare: 1 L 5 x binding buffer):

Add 4.2103 g NaH_2PO_4 (Mr 137.99, monohydrate), 58.8455 g Na_2HPO_4 (Mr 268.1, heptahydrate), 87.66 g NaCl (Mr 58.44), 1.0 g NaN_3 to 800 ml distilled water. Make up volume to 1 L with distilled water. Do not adjust the pH at 5 x. The pH of the concentrated stock solution will be lower than that of the diluted form.

1 x Imidazole buffer (to prepare 1 L 1 x binding buffer):

Add 5.52 g NaH_2PO_4 (Mr 137.99, monohydrate), 2.681 g Na_2HPO_4 (Mr 268.1, heptahydrate), 17.532 g NaCl (Mr 58.44), 1.0 g NaN_3, 68.08 g imidazole (Mr 68.08) to 750 ml distilled water. Use 6 N hydrochloric acid to adjust the pH to 7.4 at 25°C. Make up volume to 1 L with distilled water.

Gel Preparation:

As the gel is shipped in 20 % ethanol, decant off the ethanol and add distilled water to a final slurry concentration of approximately 50 %.

Resin packing:

Gently shake the bottle to form a gel slurry. It is often preferable to de-gas the resin slurry. Pour or pipette the resin slurry into a glass or plastic column with the column outlet slightly open. Add 5-10 column volumes of distilled water to wash the Ni-metal chelate resin and to ensure that the resin is packed well. Close the column outlet valve. The column is now ready for pre-equilibration with binding buffer.

PROTOCOL USING GRAVITY FLOW OR A PERISTALTIC PUMP:

Pre-equilibration:
Equilibrate the Ni IMAC column with 3-5 column volumes of binding buffer.

Sample loading:
Load an appropriate amount of 0.45 µm filtered cleared lysate on to the Ni column. Please note that the binding capacity of the resin is approximately 5-10 mg 6x His-tagged protein/ml sedimented resin. Collect the sample wash for further analyses.

Washing:
Wash the column with 5 x 3 column volumes of wash buffer. The washes should be collected for further analyses to ensure that all unbound protein is removed. As imidazole absorbs UV radiation at 280 nm, we recommend that the wash buffer (containing imidazole) is used as the reference solution for auto-zeroing the UV-Vis spectrophotometer.

Elution:
Elute the bound 6xHis-tagged protein into fresh tubes with a minimum 5 x 2 column volumes (e.g. 10-15 CV's) of elution buffer. The eluate should be collected for further analyses. Check the protein content of each eluted fraction before pooling them. Otherwise, you risk diluting a concentrated, purified sample.

DESALTING AND CONCENTRATING THE PURIFIED PROTEIN

Imidazole should be removed by diafiltration using ultrafiltration concentrators or rapid dialysis against an appropriate buffer for your downstream application. Otherwise, imidazole may strip the metal ion from a metalloprotein of interest or the target protein may irreversibly precipitate out of solution when stored at -20°C or -80°C.

REGENERATION OF THE Fastback Ni IMAC RESIN

Wash the column with 10 CV's of elution buffer followed by 10 CV's of binding buffer. Proceed to the pre-equilibration step of another bind-wash-elute cycle if the column is to be re-used immediately. After regeneration, the resin can also be stored in a screw capped bottle in 0.1 % sodium azide (made up in distilled water) at 2-8°C until further 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 metal chelate separation?*
   Many chromatographic procedures demand that the sample is pre-conditioned prior to loading. We recommend that all samples are filtered to at least 0.45 µm pore size. 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 β-mercaptoethanol to the lysis buffer?*
   Reducing agents can reduce the resin matrix and adversely affect binding of the 6xHis-tagged protein to the resin. Its inclusion depends upon whether the 6xHis-tagged protein elutes with contaminants as β-mercaptoethanol can reduce all disulphide bonds formed between the contaminating proteins and the target protein. Concentrations less than or equal to 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 with 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 Ni^{2+}. 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 5-10 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 Ni^{2+} salt. Reductants will cause the resin to turn brown and chelating agents will cause the resin to turn white. Ensure that all solutions are compatible with the Fastback-Ni resin.

8. *How can I re-charge the resin with $NiSO_4$?*
Wash the resin with min. 5 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 5 column volumes of binding buffer e.g. 1 x PBS buffer, pH 7.4 + 10 mM imidazole.

9. *How can I ensure that levels of contaminants in the final eluate remain low?*
We recommend 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 dry 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?*
You should always remove imidazole if the protein is going to be stored. Otherwise, the protein may precipitate out of solution at -20 or -80°C.

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. Pro-Chem offers centrifugal UF concentrators for this application.

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 by passing it through the Fastback-Ni 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.

14. *Under what circumstances should 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 Ni^{2+}, binding capacity may be reduced after repeated use.

Troubleshooting Assistance:

Bubbles or cracks appear in the resin bed

- The resin has been stored at a cool temperature and then rapidly warmed up. The 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 it has been used more than once and stored in the absence of a bacteriostat, microbial growth 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 denaturing conditions!
- 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 6xHis-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 6xHis-tagged protein. The target protein may pass through into the sample wash if the capacity of the resin bed 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 bacterium, *E.coli*.
- 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 bed. 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 metal chelate 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 add suitable detergents or organic solvents.
- 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 6xHis-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 6xHis-tag may have altered. If the 6xHis-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.

Glossary:

affinity chromatography - chromatographic separation based on a specific interaction between an immobilized ligand and a binding site on a macromolecule.

baculovirus – a virus vector for expression of recombinant proteins in insect cells.

bed volume - the total volume occupied by the chromatographic packed bed. It is also referred to as the column volume or CV.

chaotropic agent - a molecule which interferes with hydro-phobic interactions by disrupting the ordered structure of water molecules. Examples include urea and guanidine.

chelating agent – a compound such as EDTA or EGTA that is able to combine with a metal ion to form a structure with one or more rings.

cleared lysate – the soluble cell extract after the cell debris and other particulates have been removed by centrifugation.

expression vector – a cloning vector intended for the foreign gene to be expressed in the host organism.

french pressure cell – a device that uses high shear forces to rupture microbial cells. The suspension is poured into a chamber, which is closed at one end by a needle valve and at the other end by a piston. Pressures of up to 16,000 lb/in2 are applied by a hydraulic press against a closed needle valve. When the desired pressure is attained, the needle valve is fractionally opened to marginally relieve the pressure. The cells subsequently expand and rupture, thereby releasing the cellular components through the fractionally open valve.

freeze-thawing – a method that is sometimes used to break open cells by successive periods of slow freezing and thawing. Ice crystals are generated during the freezing stage, which disrupt the cells when they melt during thawing. The method, however, is slow and releases a limited amount of subcellular components.

his - a 3 letter symbol for L-histidine

his-tag – a permanent affinity tag engineered into the expression vector upstream or downstream of the gene of interest to facilitate the purification of the recombinant protein. The His-tag doesn't normally have any effect upon the protein structure or function, it comprises 6x Histidine residues (Hexahistidine) and has a molecular weight of 0.7-0.9 kDa

immobilized - bound to a surface, usually through covalent linkages.

inclusion bodies – quite a lot of proteins form insoluble crystalline aggregates known as inclusion bodies when they are expressed at high levels inside bacteria. The proteins can be solubilized using denaturants such as 8 M urea or 6 M guanidine hydrochloride.

ion exchange chromatography - chromatographic separation based on different charge properties of macromolecules.

isoelectric point - the pH at which the protein has no net charge.

lysozyme – an enzyme than hydrolyzes β-1,4-linkages between N-acetylmuramic acid and 2-acetamido-2-deoxy-D-glucone in peptidoglycan heteroploymers of prokaryotic cell walls. An example is egg white lysozyme and this enzyme is used to disrupt cells in order to liberate expressed proteins. 1 mg/ml lysozyme is normally added to *E.coli* cells in lysis buffer and incubated for 30 min to aid cell disruption. The pH optimum for lysozyme is pH 9.2 (Davies et al 1969).

metal chelate affinity chromatography – a form of affinity chromatography where a suitable chelator such as iminodiacetic acid is cross-linked via long stable hydrophilic spacer arm to a matrix such as agarose. The resin is then saturated with an appropriate metal ion, which then has a high affinity for peptidic metal chelates such as poly His-tags.

recombinant protein – a protein coded for by a cloned gene which has often been modified *to increase the expression of that protein or to alter the properties of the protein.*

sonication – this technique uses ultrasonic energy to generate high transient pressures that are believed to disrupt the cells.

truncate - terminate prematurely or to shorten by cutting.

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