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A Geno Technology, Inc. (USA) brand name

Nickel Chelating Resin Spin Columns

A Ni-IDA IMAC resin for 6X-His Tagged Protein Purification

(Cat. # 786-392, 786-393, 786-394)

INTRODUCTION

Immobilized Metal Ion Affinity Chromatography (IMAC), developed by Porath (1975), is based on the interaction of certain protein residues (histidines, cysteines, and to some extent tryptophans) with cations of transition metals. The Nickel Chelating Resin is specifically designed for the purification of recombinant proteins fused to the 6 x histidine (6XHis) tag.

The Nickel Chelating Resin is specifically designed for the purification of recombinant proteins fused to the 6 x histidine (6XHis) tag expressed in bacteria, insects, and mammalian cells. The resin is high affinity and selectivity for recombinant fusion proteins that are tagged with six tandem histidine residues.

The Nickel Chelating Resin can be used to purify 6X His tagged proteins under native and denaturing conditions. Proteins bound to the resin can be eluted with low pH buffer or competition with imidazole or histidine.

The Nickel Chelating Resin uses IDA (iminodiacetic acid) as its functional ligand. The tertiary amine and carboxylic acid side chains of IDA serve as the chelating ligands for di- or trivalent metal ions. The structure offers selective binding of recombinant His-tagged proteins when this resin is charged with transition metals. As a result, the desired proteins can often be purified close to homogeneity in a single step.

ITEM(S) SUPPLIED

Cat. #	Description	Total Column Volume	Size
786-392	Nickel Chelating Resin, 0.2ml Spin Column	1ml	25 columns
786-393	Nickel Chelating Resin, 1ml Spin Column	5ml	5 columns
786-394	Nickel Chelating Resin, 3ml Spin Column	10ml	5 columns

**Nickel Chelating Resin is supplied as a 50% slurry in 20% ethanol*

STORAGE CONDITIONS

It is shipped at ambient temperature. Upon arrival, store it refrigerated at 4°C, DO NOT FREEZE. This product is stable for 1 year at 4°C.

SPECIFICATIONS

- Ligand Density: 20-40µmoles Ni^{2+}/ ml resin
- Binding Capacity: >50mg/ml resin. *We have demonstrated binding of >100mg of a 50kDa 6X His tagged proteins to a ml of resin*
- Bead Structure: 6% cross-linked agarose

IMPORTANT INFORMATION

- The purity and yield of the recombinant fusion protein is dependent of the protein's conformation, solubility and expression levels. We recommend optimizing and performing small scale preparations to estimate expression and solubility levels.
- Avoid EDTA containing protease inhibitor cocktails, we recommend our Recom ProteaseArrest™ (Cat. # 786-376, 786-436) for inhibiting proteases during the purification of recombinant proteins.
- For recombinant proteins that are sequestered to inclusion bodies we recommend out IBS™ Buffer (Cat. # 786-183)

ADDITIONAL ITEMS REQUIRED

- Binding Buffer, see protocol for details
- Wash Buffer, see protocol for details
- Elution Buffer, see protocol for details
- Regeneration Buffer, see protocol for details

PREPARATION BEFORE USE

Sample preparation: Refer to manufacturer's protocols for optimal conditions for growth, induction and lysis of recombinant His-tagged clones. To avoid clogging of the resin filter the sample through a 0.45µm filter. The preferred buffers that improve binding affinity are 50mM acetate or 10-150mM phosphate buffers with pH 7-8, although this can fluctuate between pH 5.5-8.5. Avoid buffers with primary amines (Tris, Glycine) as these weaken binding affinity and can even strip metal ions. The buffer should be supplemented with 0.15-0.5M NaCl to suppress secondary ionic interactions and proteins/protein interactions.

PROTOCOL FOR NATIVE PROTEINS

NOTE: *The total volume of the columns is indicated in the table above. For sample volumes larger than the column capacity, simply perform multiple applications and centrifugations until the entire sample has been applied. Ensure that the capacity of the column is not exceeded. The protocol below is for spin columns, however the columns can be used as gravity flow columns.*

1. Allow the columns to equilibrate to the appropriate purification temperature (4°C or room temperature).

2. Mix the protein sample with the Binding Buffer (i.e. 50mM Na_2HPO_4, 300mM NaCl pH8.0 supplemented with 10mM imidazole) so the total volume is equivalent to two resin bed volumes (RBV). 0.4, 2 and 6ml for the 0.2, 1 and 3ml columns respectively.
   NOTE: *The imidazole and sodium chloride is present to reduce non-specific*

interactions, however these may interfere with the binding of some 6X His tagged proteins. Optimize with 0-20mM imidazole and 100-500mM NaCl.

3. Remove the bottom cap from the column and transfer to an appropriate centrifuge tube. 0.2, 1 and 3ml columns use 2, 15 and 50ml centrifuge tube respectively.

4. Centrifuge at 700xg for 2 minutes and discard the storage buffer.

5. Add two RBV Binding Buffer to the columns and allow to enter the resin bed.

6. Centrifuge at 700xg for 2 minutes and discard the Binding Buffer.

7. Add the protein sample to the columns and allow to enter the resin bed. For maximal binding incubate with mechanical end over end rotation for 15-30 minutes at room temperature or 4°C.

8. Centrifuge at 700xg for 2 minutes and collect the flow-through. Save the supernatant to analyze by SDS-PAGE.

9. Wash the column with 2 RBV Wash Buffer (i.e. 50mM Na_2HPO_4, 300mM NaCl pH8.0 supplemented with 20mM imidazole).
   NOTE: *The imidazole and sodium chloride is present to reduce non-specific interactions, however these may interfere with the binding of some 6X His tagged proteins. Optimize with 10-50mM imidazole and 100-500mM NaCl.*

10. Centrifuge at 700xg for 2 minutes and collect the wash. Repeat the wash two more times, collecting fractions in different tubes.

11. Elute the bound protein by the addition of 1 RBV of Elution Buffer (50mM Na_2HPO_4, 300mM NaCl, 250mM Imidazole, pH 8).

12. Centrifuge at 700xg for 2 minutes and collect the elution. Repeat steps 11-12 two more times, collecting fractions in different tubes.

13. The elution of the protein can be monitored by measuring absorbance at 280nm or with a CB-X™ Protein Assay (Cat. # 786-12X). The fractions can also be analyzed by SDS-PAGE.

14. To remove the imidazole for downstream processing we recommend our SpinOUT™ GT-600 columns or our Tube-O-DIALYZER™ dialysis systems. PAGE-Perfect™ (Cat. # 786-123) can be used to remove guanidine from denatured samples prior to SDS-PAGE.

PROTOCOL FOR DENATURING PROTEINS

NOTE: *The total volume of the columns is indicated in the table above. For sample volumes larger than the column capacity, simply perform multiple applications and centrifugations until the entire sample has been applied. Ensure that the capacity of the column is not exceeded. The protocol below is for spin columns, however the columns can be used as gravity flow columns.*

1. Allow the columns to equilibrate to the appropriate purification temperature (4°C or room temperature).

2. Mix the protein sample with the Binding Buffer (i.e. 50mM Na_2HPO_4, 6M guanidine·HCl, 300mM NaCl pH8.0) supplemented with 10mM imidazole) so the total volume is equivalent to two resin bed volumes (RBV). 0.4, 2 and 6ml for the 0.2, 1 and 3ml columns respectively.
   NOTE: *The imidazole and sodium chloride is present to reduce non-specific interactions, however these may interfere with the binding of some 6X His tagged proteins. Optimize with 0-20mM imidazole and 100-500mM NaCl.*
   8M urea can be used as an alternative to the 6M guanidine·HCl.

3. Remove the bottom cap from the column and transfer to an appropriate centrifuge tube. 0.2, 1 and 3ml columns use 2, 15 and 50ml centrifuge tube respectively.

4. Centrifuge at 700xg for 2 minutes and discard the storage buffer.

5. Add two RBV Binding Buffer to the columns and allow to enter the resin bed.

6. Centrifuge at 700xg for 2 minutes and discard the Binding Buffer.

7. Add the protein sample to the columns and allow to enter the resin bed. For maximal binding incubate with mechanical end over end rotation for 15-30 minutes at room temperature or 4°C.

8. Centrifuge at 700xg for 2 minutes and collect the flow-through. Save the supernatant to analyze by SDS-PAGE.

9. Wash the column with 2 RBV Wash Buffer (i.e. 50mM Na_2HPO_4, 6M guanidine·HCl, 300mM NaCl pH8.0 supplemented with 20mM imidazole).
   NOTE: *The imidazole and sodium chloride is present to reduce non-specific interactions, however these may interfere with the binding of some 6X His tagged proteins. Optimize with 10-50mM imidazole and 100-500mM NaCl.*
   8M urea can be used as an alternative to the 6M guanidine·HCl.

10. Centrifuge at 700xg for 2 minutes and collect the wash. Repeat the wash two more times, collecting fractions in different tubes.

11. Elute the bound protein by the addition of 1 RBV of Elution Buffer (50mM Na_2HPO_4, 6M guanidine·HCl, 300mM NaCl, 250mM Imidazole, pH 8).
NOTE: *8M urea can be used as an alternative to the 6M guanidine·HCl.*

12. Centrifuge at 700xg for 2 minutes and collect the elution. Repeat steps 11-12 two more times, collecting fractions in different tubes.

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COLUMN REGENERATION

1. Wash resin with 10 bed volumes of 20mM MES buffer, pH 5.0

2. Wash resin with 10 bed volumes of distilled water.

3. Wash resin with 10 bed volumes of 20% ethanol.

4. Store resin at 4ºC in 20% ethanol.

COLUMN RECHARGING PROTOCOL

Column regeneration should be performed when a different protein is being isolated or when there is a significant loss in the yield of protein. If the Nickel Chelating Resin loses its blue color the column needs recharging.

1. Wash the resin with 5 column volumes of a solution 20mM sodium phosphate supplemented with 0.5M NaCl, 50mM EDTA at pH 7.0.

2. Wash with 5 column volumes of distilled water to remove EDTA.
NOTE: *If the loss in yield is suspected to be due to denatured proteins or lipids a more drastic regeneration protocol should be followed. After step 2:*

   A. *Elimination of ionic interactions: Wash in batch for approximately 20 minutes in a solution with 1.5M NaCl, follow with a wash with 10 column volumes of distilled water.*

   B. *Elimination of precipitated proteins. Wash in batch for at least 2 hours with a solution 1M NaOH, follow with a wash with 10 column volumes of distilled water.*

C. *Elimination of strong hydrophobic interactions: Resuspend the resin in batch with 30% isopropanol and wash for approximately 20 minutes, follow with a wash with 10 column volumes of distilled water.*

D. *Elimination of lipids: Wash in batch for 2 hours with a solution 0.5% of non-ionic detergent in 0.1 M acetic acid. Rinse away the detergent with approximately 10 column volumes of 70% ethanol, follow with a wash with 10 column volumes of distilled water.*

3. Add 5 volumes of 0.1M nickel sulfate hexahydrate.

4. Wash with 5 column volumes of distilled water.

5. Add 5 column volumes of the binding buffer. The column is now ready for use. ***NOTE:*** *If storing the column for a while store at 4°C in 20% ethanol.*

TROUBLESHOOTING

Issue	Possible Reason	Suggested Solution
Viscous sample	High levels of nucleic acids in lysate	Treat sample with nuclease. LongLife™ Nuclease, 786-039
	Too little lysis/ homogenization buffer used	Dilute sample with more buffer
Column becomes clogged after sample application	Sample poorly clarified before loading	Centrifuge the sample at higher speed or filter the sample
No protein found in elution	Low protein expression of target protein	Check protein expression levels.
		Apply larger volume
	Recombinant protein targeted to inclusion bodies or possible insufficient lysis	Increase intensity/ duration of lysis
		Use denaturing conditions (6M guanidine·HCl or 8M urea) is protein is insoluble
	Target protein in flow-through	Reduce imidazole concentration in binding and wash buffers.
		Check pH levels of sample and adjust to pH7-8
		Histidine tag may not be accessible. Use denaturing conditions or reclone with tag at opposite terminus
		Proteolytic cleavage during extraction has removed the tag, include protease inhibitors (Recom ProteaseARREST™, 786-436)
	Elution conditions are too mild	Elute with acidic pH or imidazole step-elution

Protein precipitates	Temperature too low	Perform at room temperature
	Aggregate formation	Add solubilization agents, such as non-ionic detergents, glycerol or β-mercaptoethanol
Poor recovery of target protein	Binding capacity of column has been exceeded	Increase column size or reduce sample load
	Strong non-specific interactions of target protein on resin	Reduce interactions by including detergents, organic solvents or by increasing NaCl concentration
Poor protein purity	Contaminants in elute	Increase number of binding and wash steps and include 10-20mM imidazole in buffers
		Prolong wash steps containing imidazole
		Column too large, reduce amount of resin used
	Strongly bound contaminants elute	Reduce the amount of imidazole in the elution buffer
	Contaminants bind target protein through disulfide bounds	Include β-mercaptoethanol, avoid DTT
	Contaminants bind target protein through hydrophobic interactions	Add non-ionic detergents or alcohol
	Contaminants bind target protein through electrostatic interactions	Increase the concentration of NaCl
	Recombinant protein degraded	Include protease inhibitors (Recom ProteaseARREST™, 786-436)
	Contaminants have similar affinity to target protein	Explore additional chromatography step (Ion exchange, gel filtration)

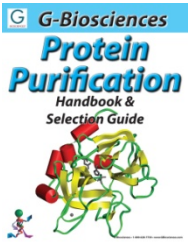
CHEMICAL COMPATIBILITIES

Reagent	Effect	Comments
BUFFER REAGENTS		
Tris, HEPES, MOPS	Buffers with secondary and tertiary amines will reduce metal ions	≤50mM secondary and tertiary amines
Sodium or potassium phosphate	No interference	50mM sodium or potassium phosphate are recommended
CHELATING REAGENTS		
EDTA, EGTA	Strip metal ions from resin	≤1mM has been used, but care must be taken >1mM causes significant reduction in binding capacity
REDUCING (SULFHYDRYL) REAGENTS		
B-mercaptoethanol	Reduces disulfide cross-linkages Can reduce metal ions	≤20mM
DTT, DTE, TCEP	Low concentrations will reduce metal ions	1mM maximum, but recommend β-mercaptoethanol
DETERGENTS		
Non-ionic detergents (Triton, Tween, NP-40, etc.)	Removes background proteins and nucleic acids	≤2%
Cationic detergents (CTAB)	Improves membrane and lipid associating proteins or hydrophobic proteins solubility	≤1%, be carefully of protein precipitation
Zwitterionic detergents (CHAPS, CHAPSO)	Solubilizes membrane proteins	≤1%
Anionic detergents (SDS, Sarkosyl)	Strips metal ions Selective solubilization membrane proteins	Not recommended
DENATURANTS		
Guanidine·HCl	Solubilize proteins	≤6M
Urea		≤8M
AMINO ACIDS		
Glycine, Glutamine, Arginine		Not recommended
Histidine	Binds resin and competes with 6X His tag histidines	Low (20mM) concentrations can block non specific binding and high (>100mM) concentrations will elute His tagged proteins.

OTHER ADDITIVES		
Sodium chloride (NaCl)	Reduces non-specific protein binding through ionic interactions	≤2M, at least 300mM NaCl should be included in buffers
Magnesium chloride ($MgCl_2$)	Required for purification of Ca^{2+} binding proteins	≤4M
Calcium chloride ($CaCl_2$)	Essential metal cofactor for nucleases	≤5mM
Glycerol	Prevents hydrophobic interactions between proteins	≤40%
Ethanol		≤20%
Imidazole	Binds resin and competes with 6X His tag proteins for metal ions	Low (<25mM) concentrations can block non specific binding and high (>100mM to ≤500mM) concentrations will elute His tagged proteins
Citrate	Carboxylic side chains may potentially act as chelation site for metal ions, causing metal leakage	≤60mM

RELATED PRODUCTS

Download our Protein Purification Handbook.



http://info.gbiosciences.com/complete-protein-purification-handbook/

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