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A Geno Technology, Inc. (USA) brand name

Ni-NTA Resin

Nickel IMAC Resin for 6X His Tagged Protein Purification

(Cat. # 786-939, 786-940, 786-941, 786-942)

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 Ni-NTA 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 Ni-NTA 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 Ni-NTA resin uses nitrilotriacetic acid (NTA), a tetradenate chelating ligand, in a highly cross-linked 6% agarose matrix. The NTA binds Ni^{2+} ions by four coordination sites.

ITEM(S) SUPPLIED

Cat. #	Description	Size
786-939	Ni-NTA Resin*	10ml
786-940	Ni-NTA Resin*	100ml
786-941	Ni-NTA Resin*	500ml
786-942	Ni-NTA Resin*	2 x 500ml

**Ni-NTA 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 confirmation, 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)

ADDITONAL ITEMS REQUIRED

- Disposable columns
- Binding Buffer and Elution 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

1. Add an appropriate amount of Ni-NTA Resin to a suitable column (suitable to hold 7 columns volumes (CV)). Allow the storage buffer to flow through the column or centrifuge at 800xg for 1 minute. Discard the flow-through.
   NOTE: *If using tubes, as opposed to column, pellet the resin by centrifugation at 500xg for 2-5 minutes and carefully decant the storage buffer.*

2. Add 5CV of distilled water and resuspend the resin. Remove water as above

3. Wash the resin by resuspending in 1CV suitable binding buffer (i.e. 50mM Na_2HPO_4, 300mM NaCl pH8.0) supplemented with 10mM imidazole). Remove wash buffer as above and repeat this wash step once.
   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.*

4. Add sample to the Ni-NTA resin and incubate with mechanical rotation for 15-20 minutes at room temperature or 4°C for 60 minutes.

5. Collect the sample lysate by gravity flow or centrifuge at 800xg for 1 minute. Save the supernatant to analyze by SDS-PAGE
   NOTE: *If using tubes, as opposed to column, pellet the resin by centrifugation at 500xg for 2-5 minutes and carefully decant the storage buffer.*

6. Wash the resin with 5CV of wash buffer (i.e. 50mM Na_2HPO_4, 300mM NaCl pH8.0) supplemented with 20mM imidazole). Mix with mechanical rotation for 5 minutes and remove the wash buffer as before. Repeat step twice more.
   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.*

7. Add 2CV of elution buffer (50mM Na_2HPO_4, 300mM NaCl, 250mM Imidazole, pH 8) and mix with mechanical rotation for 5 minutes.

8. Collect the eluate as above and repeat the elution four more times. Examine the eluates by SDS-PAGE and pool together the samples of interest.
   NOTE: *To remove imidazole for downstream applications use gel filtration (G-Biosciences SpinOUT™ Desalting Columns) or dialysis (G-Biosciences Tube-O-DIALYZER™).*

PROTOCOL FOR DENATURING PROTEINS

1. Add an appropriate amount of Ni-NTA Resin to a suitable column (suitable to hold 7 columns volumes (CV)). Allow the storage buffer to flow through the column or centrifuge at 800xg for 1 minute. Discard the flow-through.
   NOTE: *If using tubes, as opposed to column, pellet the resin by centrifugation at 500xg for 2-5 minutes and carefully decant the storage buffer.*

2. Add 5CV of distilled water and resuspend the resin. Remove water as above

3. Wash the resin by resuspending in 1CV suitable binding buffer (i.e. 50mM Na_2HPO_4, 6M guanidine·HCl, 300mM NaCl pH8.0) supplemented with 10mM imidazole). Remove wash buffer as above and repeat this wash step once.
   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.

4. Add sample to the Ni-NTA resin and incubate with mechanical rotation for 15-20 minutes at room temperature or 4°C for 60 minutes.

5. Collect the sample lysate by gravity flow or centrifuge at 800xg for 1 minute. Save the supernatant to analyze by SDS-PAGE
   NOTE: *If using tubes, as opposed to column, pellet the resin by centrifugation at 500xg for 2-5 minutes and carefully decant the storage buffer.*

6. Wash the resin with 5CV of wash buffer (i.e. 50mM Na_2HPO_4, 6M guanidine·HCl, 300mM NaCl pH8.0) supplemented with 20mM imidazole). Mix with mechanical rotation for 5 minutes and remove the wash buffer as before. Repeat step twice more.
   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.

7. Add 2CV of elution buffer (50mM Na_2HPO_4, 6M guanidine·HCl, 300mM NaCl, 250mM Imidazole, pH 8) and mix with mechanical rotation for 5 minutes.
   NOTE: *8M urea can be used as an alternative to the 6M guanidine·HCl.*

8. Collect the eluate as above and repeat the elution four more times. Examine the eluates by SDS-PAGE and pool together the samples of interest.
   NOTE: *To remove imidazole for downstream applications use gel filtration (G-Biosciences SpinOUT™ Desalting Columns) or dialysis (G-Biosciences Tube-O-DIALYZER™). Samples containing6M guanidine·HCl can be cleaned with G-*

Biosciences PAGE-Perfect™ or must be dialyzed against a buffer containing 8M urea prior to SDS PAGE analysis.

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 Ni-NTA 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)

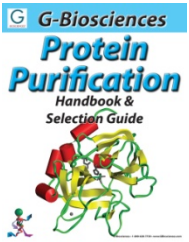
CHEMICAL COMPATIBILITIES

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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