Ni-NTA Resin Spin Plates

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

(Cat. # 786-994)

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. Although 6X His tagged proteins bind with a lower efficiency compared to nickel chelating resins there is a significant reduction in non-specific binding.

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.

The spin plate format are convenient, ready-to-use pre-dispensed filter plates for the rapid and efficient purification of polyhistidine tagged proteins from bacterial, mammalian and baculovirus infected cells. The plates are compatible with centrifugation and vacuum manifold systems for manual or automated purification. Ideal for fast, consistent well-to-well and plate-to-plate reproducibility for small scale high throughput purifications.

ITEM(S) SUPPLIED

Part. #	Description	Total Column Volume	Size
069N-A	Ni-NTA Resin Spin Plates	100µl resin/well	2 plates
111W-A	Wash/Collection Plates	-	4 plates

**Ni-NTA Resin is supplied as a 50% slurry in 20% ethanol*

STORAGE CONDITIONS

It is shipped at ambient temperature. Upon arrival, store 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: >1mg/well
- Bead Structure: 6% cross-linked agarose

IMPORTANT INFORMATION

- Plates are compatible with variable speed centrifuges with rotors and carriers capable of handling stacked plates. Use speed of 100-500xg with a maximum of 700xg.
- Ensure the spin plates are balanced throughout all centrifugations with a duplicate plate filled with an appropriate volume of water.
- The plates may be used with standard vacuum manifolds and is also dependent on sample properties and sample preparation. Use at a flow rate of 2-4psi (4-8 in Hg), which is equivalent to 1-2 props per second. To preserve the quality of the resin, avoid over-drying of the resin during vacuum application.
- 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.
- Each well has an estimated binding capacity of 1mg 6X His tagged protein. The maximum total protein (lysate) loading amount is 4mg and typical yields are 10-25% of total protein loaded. Do not exceed the capacity of the resin.
- 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.
- Avoid strong reducing agents, DTT or β-mercaptoethanol.
- For recombinant proteins that are sequestered to inclusion bodies we recommend our IBS™ Buffer (Cat. # 786-183)

ADDITIONAL ITEMS REQUIRED

- Variable speed centrifuge with rotor and carriers capable of handling stacked plates (4.5cm height) at 500xg or a vacuum manifold.
- Binding Buffer, see protocol for details
- Ultra pure water
- Wash Buffer, see protocol for details
- Elution Buffer, see protocol for details
- Regeneration Buffer, see protocol for details
- EDTA-free protease inhibitor cocktail, we recommend Recom ProteaseArrest™ (Cat. # 786-376, 786-436)
- Optional: Plate or orbital shaker
- Optional: Additional plates for collecting and storing fractions
- Optional: SpinOUT™ GT-600 Spin Plates (Cat. # 786-989, 786-990) for desalting and buffer exchange of samples
- Optional: Sealing tape for 96-well plates (Cat. # 786-422)

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 WITH CENTRIFUGATION

NOTE: *The total volume of the wells is <400µl/well. 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.*

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

2. Mix the protein sample 1:1 with the Binding Buffer (i.e. 50mM Na_2HPO_4, 300mM NaCl pH8.0 supplemented with 10mM imidazole) so the total volume is <400µl. ***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 seal from the plate(s) and place on the top of a wash/collection plate. Remove the top seal.

4. Place the plate assembly in the centrifuge with a 96 well carrier and centrifuge at 500xg for 3 minutes to remove the storage buffer. Discard the storage buffer.

5. Add 400µl ultra pure water to each well. Repeat centrifugation and discard the water.

6. Add 400µl Binding Buffer to each well and allow to enter the resin bed. Centrifuge as before and discard the Binding Buffer. Repeat this step once.

7. Add <400µl protein sample from step 2 to the columns and allow to enter the resin bed. For maximal binding incubate with moderate agitation on a plate shaker or orbital shaker for 15-30 minutes at room temperature or 4°C.

8. Centrifuge the plate assembly at 500xg for 3 minutes. Make sure the applied sample is drained from all wells. Discard the flow through or use additional collection plates to store the flow through for further analysis.

9. Place the plate back on the wash/collection plate and wash the resin with 400µl/well of 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 500xg for 3 minutes and discard the wash. Repeat the wash three more times. Additional wash steps may be required for some samples.

11. Place the spin plate on a new collection plate. Add 200µl Elution Buffer (50mM Na_2HPO_4, 300mM NaCl, 250mM Imidazole, pH 8) and incubate with gentle agitation for 1 minute.

12. Centrifuge at 500xg for 3 minutes and collect the eluate. Repeat the elution step once for maximum concentration or twice for maximum yield. The elution volume can be varied to achieve maximum concentration. Use additional plates for collecting and storing fractions.

13. Seal collection plate top with sealing tape and store at 4°C.

14. 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.

15. To remove the imidazole for downstream processing we recommend our SpinOUT™ GT-600 Spin Plates (Cat. # 786-989, 786-990) for desalting and buffer exchange of samples.

PROTOCOL WITH VACUUM MANIFOLD

NOTE: *The total volume of the wells is <400µl/well. For sample volumes larger than the column capacity, simply perform multiple applications and vacuum applications until the entire sample has been applied. Ensure that the capacity of the column is not exceeded.*

1. Adjust the vacuum to obtain a flow rate of 4-8 inches Hg (2-4psi), the equivalent of 1-2 drops/second.

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

3. Mix the protein sample 1:1 with the Binding Buffer (i.e. 50mM Na_2HPO_4, 300mM NaCl pH8.0 supplemented with 10mM imidazole) so the total volume is <400µl.
   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. Remove the bottom seal from the plate(s) and then the top seal and place onto the top of the vacuum manifold. Apply vacuum to remove storage solution. Let the solution drain or collect in a wash/collection plate. Discard the storage buffer.

5. Add 400µl ultra pure water to each well. Apply vacuum and let drain or collect in the wash/collection plate and discard.

6. Add 400µl Binding Buffer to each well and allow to enter the resin bed. Apply vacuum and let drain or collect in the wash/collection plate and discard. Repeat this step once.

7. Add <400µl protein sample from step 2 to the columns and allow to enter the resin bed. For maximal binding incubate with moderate agitation on a plate shaker or orbital shaker for 15-30 minutes at room temperature or 4°C.
   NOTE: *Detergent lysis solutions may foam and result in cross contamination. Lowering the flow rate can help alleviate this.*

8. Apply vacuum and let drain or collect in the wash/collection plate. Make sure the applied sample is drained from all wells. Discard the flow through or use additional collection plates to store the flow through for further analysis.

9. Place the plate back over the wash/collection plate on the vacuum manifold and wash the resin with 400µl/well of 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. Apply vacuum and let drain or collect in the wash/collection plate and discard. Repeat the wash three more times. Additional wash steps may be required for some samples.

11. Place the spin plate on a new collection plate. Add 200µl Elution Buffer (50mM Na_2HPO_4, 300mM NaCl, 250mM Imidazole, pH 8) and incubate with gentle agitation for 1 minute.

12. Place the plate and new wash/collection plate on the vacuum manifold. Apply vacuum and collect the eluate in the wash/collection plate. Repeat the elution step once for maximum concentration or twice for maximum yield. The elution volume can be varied to achieve maximum concentration. Use additional plates for collecting and storing fractions.

13. Seal collection plate top with sealing tape and store at 4°C.

14. 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.

15. To remove the imidazole for downstream processing we recommend our SpinOUT™ GT-600 Spin Plates (Cat. # 786-989, 786-990) for desalting and buffer exchange of samples.

PROTOCOL FOR DENATURED PROTEINS

For purification of denatured proteins, use the following buffers with the above procedures:

- Binding Buffer (i.e. 50mM Na_2HPO_4, 6M guanidine·HCl, 300mM NaCl pH8.0) supplemented with 10mM 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 0-20mM imidazole and 100-500mM NaCl. 8M urea can be used as an alternative to the 6M guanidine·HCl.*
- 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.*
- 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.*

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	Increase intensity/ duration of lysis
		Use denaturing conditions (6M

	bodies or possible insufficient lysis	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)

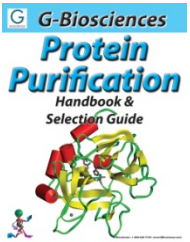
CHEMICAL COMPATIBILITIES

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.



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