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A Geno Technology, Inc. (USA) brand name

# Co-NTA Resin

## Cobalt IMAC Resin for 6X His Tagged Protein Purification

## (Cat. # 786-932, 786-933, 786-934, 786-935)

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

## ITEM(S) SUPPLIED

| Cat. # | Description | Size |
|---|---|---|
| 786-932 | Co-NTA Resin* | 10ml |
| 786-933 | Co-NTA Resin* | 100ml |
| 786-934 | Co-NTA Resin* | 500ml |
| 786-935 | Co-NTA Resin* | 2 x 500ml |

**Co-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 $Co^{2+}$/ 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 Co-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 Co-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 Co-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 Co-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 Co-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 cobalt(II) sulfate heptahydrate.
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) |

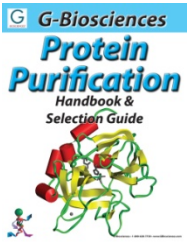
## CHEMICAL COMPATIBILITIES

| 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<br>>1mM causes significant reduction in binding capacity |
| **REDUCING (SULFHYDRYL) REAGENTS** | | |
| B-mercaptoethanol | Reduces disulfide cross-linkages<br>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<br>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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