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A Geno Technology, Inc. (USA) brand name

Copper Chelating Resin

(Cat. # 786-285)

INTRODUCTION

Immobilized Metal Ion Affinity Chromatography (IMAC), developed by Porath et al (1), is based on the interaction of certain protein residues (histidines, cysteines, and to some extent tryptophans) with cations of transition metals. The Copper Chelating Resin is specifically designed for the purification of proteins that associate with copper ions (2-3), including 6x histidine tagged proteins. The Copper Chelating Resin has stronger binding affinity than Nickel, Cobalt and Copper resins, and therefore is a good choice for initial studies when little is known about the protein of interest.

ITEM(S) SUPPLIED (Cat. # 786-285)

Description	Size
Copper Chelating Resin*	10ml

**Copper Chelating Resin is supplied as 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 Cu^{2+}/ ml resin
- Binding Capacity: >50mg/ml resin
- Bead Structure: 6% cross-linked agarose

ADDITIONAL ITEM(S) 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

1. Add an appropriate amount of Copper Chelating Resin to a suitable tube (suitable to hold 7 columns volumes (CV)). Pellet the resin by centrifugation at 500x*g* for 2-5 minutes and carefully decant the storage buffer.
2. Add 5CV of distilled water and mix end over end for 5 minutes. Pellet the resin by centrifugation at 500x*g* for 2-5 minutes and carefully decant the supernatant. Repeat step twice more.
3. Resuspend resin in 1CV suitable sample buffer (i.e. 50mM Na_2HPO_4, 0.3M NaCl pH8.0)) supplemented with 10mM imidazole.
4. Add sample to the Copper chelating resin and incubate with mechanical rotation for 15-20 minutes at room temperature.
5. Pellet the resin by centrifugation at 500x*g* for 2-5 minutes. Decant off the supernatant and save for analysis by SDS-PAGE.
6. Wash the resin with 5CV of binding buffer and mix with mechanical rotation for 5 minutes. Pellet the resin by centrifugation as in step 5. Decant and save the supernatant for further analysis. Repeat step twice more.
7. Add 2CV of elution buffer (50mM Na_2HPO_4, 0.3M NaCl, 0.25M Imidazole, pH 8.0) and mix with mechanical rotation for 5 minutes.
8. Pellet the resin by centrifugation at 500x*g* for 2-5 minutes. Carefully decant the supernatant and retain for further analysis. Repeat steps 7 and 8 four more times. Pool together the samples of interest.

COLUMN REGENERATION

1. Wash resin with 10 bed volumes of 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 4°C 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 Copper 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:*
   - *i. 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.*
   - *ii. 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.*
   - *iii. 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.*
   - *iv. 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 Copper 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 4ºC in 20% ethanol.*

REFERENCES

1. Porath, J. et al (1975) Metal chelate affinity chromatography, a new approach to protein fractionation. Nature. 258: 598-599
2. Kaur-Atwal, G et al (2008) Immobilized Metal Affinity Chromatography for the Analysis of Proteins and Peptides Curr. Anal. Chem. 4: 127-135
3. Smith, S.D. et al (2004) Using Immobilized Metal Affinity Chromatography, Two-Dimensional Electrophoresis and Mass Spectrometry to Identify Hepatocellular Proteins with Copper-Binding Ability J. Proteome Res. 3:834–840

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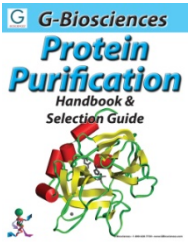

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