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A Geno Technology, Inc. (USA) brand name

Zinc Chelating Resin

(Cat. # 786-287)

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INTRODUCTION

Immobilized Metal Ion Affinity Chromatography (IMAC), developed by Porath (1), is based on the interaction of certain protein residues (histidines, cysteines, and to some extent tryptophans) with cations of transition metals. The Zinc Chelating Resin is specifically designed for the purification of proteins that associate with Zinc ions (2-3), including 6x histidine tagged proteins.

ITEM(S) SUPPLIED (Cat. # 786-287)

Description	Size
Zinc Chelating Resin*	10ml

*Zinc Chelating Resin is supplied 20ml 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 Zn^{2+}/ ml resin
- Binding Capacity: >20mg/ml resin
- Bead Structure: 6% cross-linked agarose

ADDITIONAL 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

1. Add an appropriate amount of Zinc 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 Zinc 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.

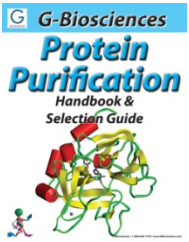
1. Wash the resin with 5 column volumes of a solution 20mM sodium phosphate supplemented with 0.5M NaCl, 50mM EDTA at pH 7.0.
2. Wash with 5 column volumes of distilled water to remove EDTA.
   NOTE: If the loss in yield is suspected to be due to denatured proteins or lipids a more drastic regeneration 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 Zinc 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.

REFERENCES

1. Porath, J. et al (1975) Metal chelate affinity chromatography, a new approach to protein fractionation. Nature. 258: 598-599
2. Mehle, A. et al (2006) A Zinc-binding Region in Vif Binds Cul5 and Determines Cullin Selection. J. Biol. Chem. 281: 17259-17265
3. Lopéz, C. et al (2004) Cysteine mediated multimerization of a recombinant dengue E fragment fused to the P64k protein following immobilized metal ion affinity chromatography. Protein Expr Purif. 34: 176-182

RELATED PRODUCTS

Download our Protein Purification Handbook



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

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