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Cobalt Chelating Resin

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 Cobalt Chelating Resin is specifically designed for the purification of proteins that associate with Cobalt ions, including 6x histidine tagged proteins. Although 6 X His tagged proteins bind with a slightly lower efficiency compared to Nickel Chelating Resin there is a significant reduction in non-specific binding.

ITEM(S) SUPPLIED Cat # 786-286

Description	Size
Cobalt Chelating Resin*	12.5ml

*Cobalt Chelating Resin is supplied 25ml 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 Co²⁺/ ml resin

Binding Capacity: >50mg/ml

Bead Structure: 6% cross-linked agarose

ITEMS NEEDED BUT NOT SUPPLIED

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 are 50mM acetate or 10-150mM phosphate buffers with pH 7-8, although this can fluctuate between pH 5.5-8.5. The buffer should be supplemented with 0.15-0.5M NaCl.

PROTOCOL

- 1) Add an appropriate amount of Cobalt Chelating Resin to a suitable tube (suitable to hold 7 columns volumes (CV)). Pellet the resin by centrifugation at 500xg 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 500xg for 2-5 minutes and carefully decant the supernatant. Repeat step twice more.
- 3) Resuspend resin in 1CV suitable sample buffer (i.e. 20mM Na₂HPO₄, 0.5M NaCl pH 7.4)) supplemented with 10mM imidazole.
- 4) Add sample to the Cobalt chelating resin and incubate with mechanical rotation for 15-20 minutes at room temperature.
- 5) Pellet the resin by centrifugation at 500xg for 2-5 minutes. Decant off the supernatant and save for analysis by SDS-PAGE.



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- 6) Wash the resin with 5CV of binding buffer and mix with mechanical rotation for 5 minutes. Repellet 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 (20mM Na₂HPO₄, 0.5M NaCl, 0.5M Imidazole, pH 7.4) and mix with mechanical rotation for 5 minutes.
- 8) Pellet the resin by centrifugation at 500xg 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 Cobalt 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 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.

REFERENCES

1. Porath, J. et al (1975) Metal chelate affinity chromatography, a new approach to protein fractionation. Nature. 258: 598-599

RELATED PRODUCTS

1. **Calmodulin Resin (Cat.# 786-282):** Calmodulin on agarose beads for the purification of CBP (calcium binding protein) tagged proteins
2. **HOOK™ Agarose Coupling Kit (Sulfhydryl reactive) (Cat.# 786-064):** For the coupling of peptides and proteins to agarose through their sulfhydryl groups.
3. **HOOK™ Agarose (Amine reactive) (Cat.# 786-066):** Agarose for the coupling of peptides and proteins via their primary amine residues.
4. **Empty columns (Cat.# 786-169):** Empty columns for the generation of small affinity columns.

NOTE: For other related products, visit our web site at www.GBiosciences.com or contact us.