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User's Manual and Instructions

Metal-chelating Sepharose

Catalog Number: L3241025

Introduction

Metal-chelating Sepharose consists of iminodiacetic acid groups coupled to Sepharose. It is supplied free of metal ions and has to be charged with a suitable metal ion before use. It is not always possible to predict which metal ion is most appropriate. The metal ions most frequently used are Ni2+, Cu2+, Zn2+. A single exposed histidine residue may result in adsorption of the protein on Cu2+ while two vicinal histidine residues are needed for adsorption on Zn2+. Ni2+ ions are often used for the purification of polyhistidine-tagged fusion protein. In some cases Cu2+, Co2+, and Ca2+ have been used with success.

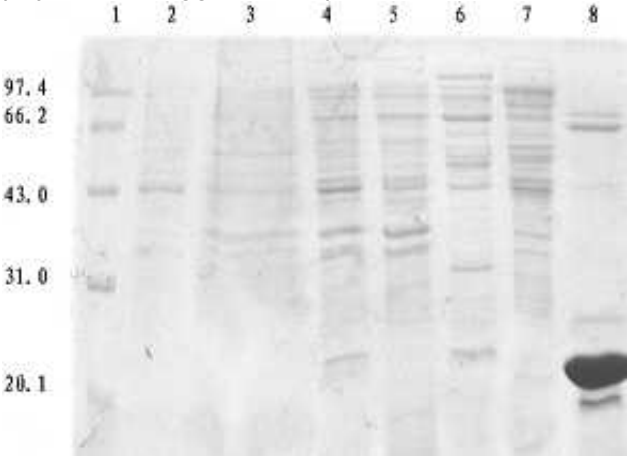

Fig. A His tagged recombinant protein purified from E. Coli lysate by Metal-chelating Sepharose
Lane 1: Marker;
Lane 2-4: H409 cell lysate with different concentration
Lane 5: Pass through fraction;
Lane 6-7: Wash fraction
Lane 8: Purified eluted target protein

Features

- Multiple ligands: Cu2+, Zn2+, Ni2+, and Co2+
- High Binding Capacity: 5-8 mg polyhistidine-tagged fusion protein per ml gel
- Fast flow rate: up to 300 cm/h

Applications

- Purification of peptide, nucleotide, and polyhistidine-tagged fusion protein

Description

The Metal-chelating Sepharose is an affinity chromatographic metrix with metal ions immobilized by the chelating effect to Sepharose 6B Fast Flow. The coupling technique is optimized to give a high binding capacity for peptide, nucleotide, and recombinant polyhistidine-tagged fusion protein. The metal ions is covalently coupled to the agarose with the ligand density of about 30-40 umol/ml

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Characteristics

The supporting matrix Sepharose 6B Fast Flow is 6% highly cross-linked agarose with high chemical and physical stabilities. It is particularly suitable for process scale applications where starting material volumes are large and flow rates are high.

The gel tolerates all commonly used aqueous buffers and denaturants, such as 6 M guanidine hydrochloride, 8 M urea, and chaotropic salts. The covalent coupling between the ligand and the metrix is very stable. The pH stability range in regular uses is pH 3-10. The characteristics of the product are summarized in Table 1.

Table 1. Metal-chelating Sepharose 6B FF characteristics

Bead structure	Highly cross-linked 6% agarose
Ligand	Cu^{2+}, Zn^{2+}, Ni^{2+}, Co^{2+}
Ligand density	30-40 µmol /ml
Binding capacity	5 8 mg recombinant polyhistidine-tagged fusion protein /ml gel
Mean partical size	80-200 µm
Max. flow rate	300 cm/h
Chemical stability	All commonly used buffers
pH stability Regular use leaning-in-place	 3-10 2-11
Temperature stability Regular use Storage	 +4–37 °C +4–8 °C
Storage buffer	20% ethanol

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Protocol

Buffer preparation
Water and chemicals used for buffer preparation should be of high purity. It is recommended to filter the buffers by passing them through a 0.45 µm filter before use.

Recommended buffers:
Binding buffer: 20mM sodium phosphate, 0.5 M NaCl, pH 7.4
Elution buffer: 20mM sodium phosphate, 0.5 M NaCl, 20mM~500mM imidazole
Note: To obtain highest purity the optimal concentration of imidazole during elution has to be determined. The optimal concentration can be determined by eluting the fusion protein with a stepwise gradient of imidazole from 20 mM to 500 mM and testing each fraction for the presence of fusion protein and impurities by SDS-PAGE.

Sample preparation
The sample should be adjusted to the composition of the binding buffer. This can be done by either diluting the sample with binding buffer or by buffer exchange. The sample should be filtered through a 0.45 µm filter or centrifuged before it is applied to the column. Chelating agents such as EDTA or EGTA should not be included in the sample.

Purification
a) Wash out the ethanol preservative with at least 5 column volumes of distilled water.
b) Equilibrate the column with 5-10 column volumes of binding buffer.
c) Apply the sample, using a syringe fitted to the luer adaptor or by pumping it onto the column.
d) Wash the column with 5-10 column volumes of binding buffer or until no material appears in the effluent.
e) Elute with 2–3 column volumes of elution buffer containing different concentration of imidazole respectively(e.g 20 mM 50 mM 100 mM 500 mM). Save the eluates in different tubes for analysis, e.g absorbance measurement at 280 nm, SDS-PAGE, ELISA, Western Blotting.
f) Wash with 5-10 column volumes of distilled water and 20% ethanol

Regeneration
Before the gel is immobilized with a new metal ion, the gel must be regenerated. To ensure that the gel is totally free from metal ions wash with 10 column volumes of 0.05M EDTA, 0.5 M NaCl. Remove residual EDTA by washing with 2-3 column volumes of 0.5 M NaCl.

Cleaning
Remove ionically bound proteins by washing the column with 0.5 column volumes of a 2 M NaCl solution, contact time 10-15 minutes, reversed flow direction. Remove precipitated proteins, hydrophobically bound proteins and lipoproteins by washing the column with 0.5 M NaOH solution at a linear flow rate of approximately 40 cm/h, contact time 1-2 hours, reversed flow direction.
In both cases, wash with at least 3 bed volumes of starting buffer.

Storage
Store the gel for longer periods of time in 20% ethanol.