Data Sheet

6% CL-Cobalt ChroMatrix™

Purification of His-tagged Recombinant Proteins

Cat. No.: AC-203

Product	*Cat-No.*	*Amount*
6% CL-Cobalt **ChroMatrix™** Bulk material	AC-203-10	10 ml
	AC-203-50	50 ml
	AC-203-100	100 ml
	AC-203-500	500 ml

Introduction

6% CL-Cobalt ChroMatrix™ is a superior immobilized metal affinity chromatography (IMAC) resin that uses immobilized cobalt ions for purifying recombinant polyhistidine-tagged proteins with minimal non-specific contamination. The high surface area to volume ratio, fast flow rates and the notable mechanical and chemical stability of ChroMatrix™ facilitates rapid, high-yielding protein purification. The durable nature of this material means that it can be regenerated and reused several times without significant decreases in yield.

Product Use Limitations and Safety

6% CL-Cobalt ChroMatrix™ is developed, designed and sold for research purposes only. It is not to be used in human diagnostics or for drug purposes. All due care and attention should be exercised in the handling of this product. We recommend wearing a suitable lab coat, disposable gloves, and protective goggles.

Storage and Stability

Store 6% CL-Cobalt ChroMatrix™ at 4°C. If stored as recommended, Jena Bioscience guarantees optimal performance of the material for 12 months after date of delivery.

Product Characteristics

Bead size	45 – 170 µm
Support	6% cross linked agarose
pH stability	2 - 12
Binding capacity*	> 20 mg/ml
Max. Pressure	0.3 MPa, 42 psi

* Tested using His-tagged human Glutathione S-transferase π

Experimental Protocol

Sample preparation

- Harvest cells by centrifugation at 5,000 g x 10 min
- Remove supernatant and assist cell lysis by subjecting the cell pellet to one freeze-thaw cycle
- Resuspend cell pellet using 1/10 of the growth culture volume of ice cold Resuspension Buffer (100 mM NaCl, 50 mM Tris-HCl, pH 7.8)
- Add lysozyme (final conc. 1 mg/ml cell resuspension) and DNase (final conc. 50 µg/ml cell resuspension) and incubate at 4°C for 30 min
- Sonicate cells on ice
- After sonication, remove cell debris by centrifugation at 19,000 x g and 4°C for 45 min

Protein Purification

Equilibration of the 6% CL-Cobalt ChroMatrix™

- Gently resuspend 6% CL-Cobalt ChroMatrix™ and transfer resin slurry to an appropriate column
- Wash column with 10 column volumes of Equilibration Buffer (20 mM Tris-HCl, 100 mM NaCl, 20 mM imidazole, pH 8.0)

Column Loading

- Load cell lysate supernatant from above onto the 6% CL-Cobalt ChroMatrix™ column (flow rate 0.5 – 1 ml/min)

Alternatively pour supernatant into a 50 ml tube and add equilibrated 6% CL-Cobalt ChroMatrix™. Incubate rotating at 4°C for 1 h before transferring to an appropriate column.

Column Washing

- Wash 6% CL-Cobalt ChroMatrix™ column with Equilibration Buffer until the $A_{280\ nm}$ of column flow-through reaches 0.03 (flow rate 1 – 5 ml/min)

Column Elution

- Start elution of His-tagged protein by washing with five column volumes of Elution Buffer 1 (100 mM NaCl, 50 mM imidazole, 20 mM Tris-HCl, pH 8.0)
- Continue Elution by washing column with five column volumes of Elution Buffer 2 (100 mM NaCl, 200 mM imidazole, 20mM Tris-HCl, pH 8.0)
- Analyze eluted fractions by SDS-PAGE
- Pool fractions containing the protein of interest and remove imidazole by gel filtration using a G-25 column

Regeneration of Cobalt ChroMatrix™

- Wash Cobalt ChroMatrix™ with 10 bed volumes of 50 mM EDTA or alternatively leave the gel in 10 bed volumes of 50 mM EDTA overnight
- Wash ChroMatrix™ with a minimum of 20 bed volumes of 500 mM NaCl
- Wash ChroMatrix™ with 10 bed volumes of 100 mM sodium phosphate pH 7.0
- Let ChroMatrix™ stand in 10 bed volumes of 10 mM cobalt sulphate overnight
- Wash ChroMatrix™ with 10 bed volumes of 100 mM sodium phosphate pH 7.0
- Equilibrate the regenerated Cobalt ChroMatrix™ in your chosen column loading buffer

Note: Over multiple rounds of regeneration, the efficiency of the resin may decrease.

Storage

For long term storage, it is recommended storing the Cobalt ChroMatrix™ in 20 mM Tris-HCl (pH 7.5) and 20 % ethanol at 4°C.