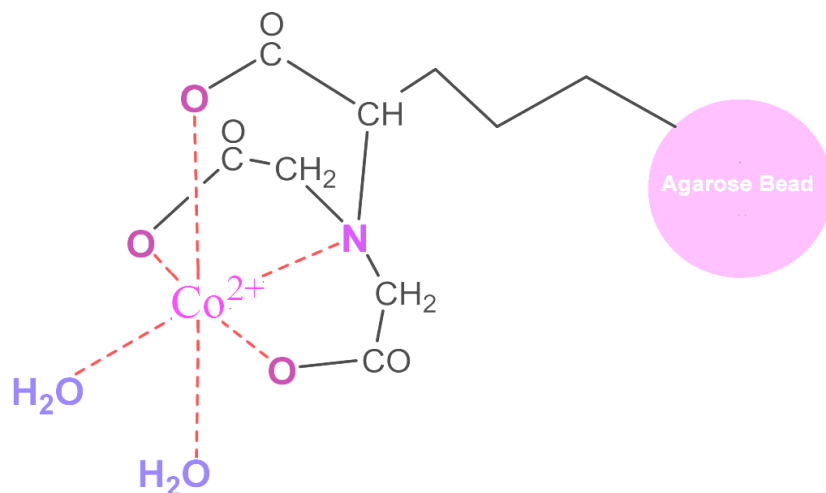


Product Name	Cat. No.	Pack Size
Co-NTA Agarose Beads	# BB-CA003A	10 ml Packed Bead Volume
	# BB-CA003B	25 ml Packed Bead Volume
	# BB-CA003C	50 ml Packed Bead Volume
	# BB-CA003D	100 ml Packed Bead Volume



Immobilized metal ion affinity chromatography (IMAC) is the most widely used purification technique. It is based on the interaction between certain protein residues (histidines, cysteines), with transition metal cations forming chelates. These transition metal ions bind to the agarose beads through a chemical reaction, giving the agarose an activation state.

Use resin, after loading with a divalent transition metal ion (Co^{2+}) is ideal for rapid purifications of His-tagged proteins. In comparison with other chelating resins such as iminodiacetic acid (IDA)-agarose, the NTA has

two sites (cis) available for the interaction with imidazole of His-tagged proteins, instead of the three in IDA, and so have lesser probability of nonspecific binding. NTA resins are usually more easily regenerated, allowing a better elution of the fused proteins bound with smaller concentrations of imidazole.

Though Ni-resin is being widely used for His-tag recombinant protein purification but in some cases Co-resins is preferable due to its higher specificity. Where purity is of prior importance over quantity, it is recommended to use Co-resins for better result. Product is supplied as 50% suspension of Co-NTA Agarose resin in 20% aqueous ethanol; the settled bead volume is measured in ml, when supplied.

- NextGen Bioproducts Co-NTA resins can withstand autoclaving at 120°C for 20 min without any significant loss of binding efficiency.

TECHNICAL SPECIFICATIONS

BEAD GEOMETRY & SIZE	: Spherical, Standard: ~ 50-150 μm diameters
CROSSLINKED	: Yes
AGAROSE %	: 4%
ACTIVATING GROUP	: Epoxy
MATRIX STABILITY	: Stable in all commonly used reagents
STORAGE SOLUTION	: 20% Ethanol
STORAGE TEMPERATURE	: 4°C to 8°C. DO NOT FREEZE.

For Research use only – Not for use in diagnostics procedures.

His-tagged protein purification using Co-NTA Agarose beads

Prepared bacterial cell pellet can be stored at -80°C till use. Wash the beads with water to remove preservative and equilibrate the beads in cell lysis buffer.

- Thaw cell pellet and resuspend in *cell lysis buffer* (minimum volume 5 ml).
- Lyse the cells using sonication/French press/any alternative methods and Centrifuge the lysate at 13,500 rpm for 30 min at 4°C to pellet the cellular debris. Collect supernatant and place on ice.
- Add 4 ml cleared lysate to the pre-equilibrated Co-NTA Agarose and mix gently by shaking (200 rpm, rotary shaker) for 60 min at 4°C.
- Load the lysate on Co-NTA Agarose mixture into a column with the bottom outlet capped.
- Remove bottom cap and collect the column flow-through. Save flow through for SDS-PAGE analysis (Use approximately 15 µl for gel analysis).
- Wash twice with 10 ml *cell lysis buffer* (may be increased if nonspecific is too high). Collect wash fractions for SDS-PAGE analysis (Use approximately 15 µl for gel analysis).
- Wash the beads with 30 ml *wash buffer* (may be increased if nonspecific is too high). Collect the wash fraction for SDS-PAGE analysis (Use approximately 15 µl for gel analysis).
- Elute the protein with 10 ml *elution buffer*. Collect the elute in four tubes and analyze by SDS-PAGE (Use approximately 15 µl for gel analysis).

Buffer composition

❑ Cell Lysis buffer:

25 mM Tris-HCl (pH- 8), 150 mM NaCl, 10 mM Imidazole (reduce to 0–5 mM, If tagged protein don't bind), Upto 10 mM βME (DTT not recommended), 0.05% Triton-X-100, 5% Glycerol

❑ Wash Buffer:

25 mM Tris-HCl (pH- 8), 150 mM NaCl, 20 mM Imidazole (reduce to 5–10 mM, If tagged protein washes out), Upto 10 mM β ME (DTT not recommended), 0.05% Triton-X-100, 5% Glycerol

❑ Elution buffer:

25 mM Tris-HCl (pH- 8), 150 mM NaCl, 250 mM Imidazole (can be increased upto 500 mM), upto 10 mM, βME (DTT not recommended), 5% Glycerol

Please note: This Cell Lysis Buffer is recommended for most applications. However, the choice of buffer depends on the particular protein properties. Use 7M urea or 6M Guanidine-HCl in Buffers during protein purification from E.coli under denaturing Conditions.

Regeneration Procedure for Co-NTA Agarose:

- Wash the resin with 10 column volumes of distilled water.
- Eliminate metal from the resin by washing with 10 column volumes of 25 mM Tris-HCl of pH-8 containing NaCl (1M) and EDTA (100 mM).
- Eliminate excess EDTA by washing with 10 column volumes of distilled water.
- Add 2 column volumes of 100 mM Cobalt ion aqueous solution (usually, chlorides or sulphates are used). Mix properly and incubate 20-30 min in room temperature.
- Wash the beads with 10 column volumes of distilled water. Store at 4°C in 20% ethanol.