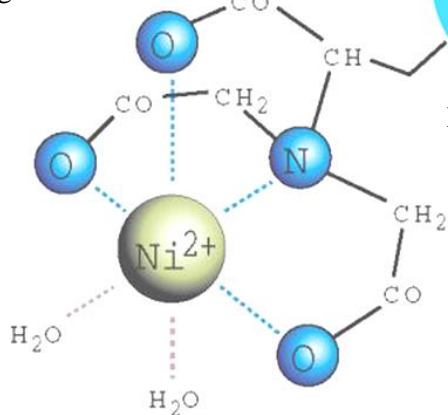


Product Name	Cat. No.	Pack Size
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**Ni-NTA Agarose Beads**

# BB-NA002A	5 ml Packed Bead Volume
# BB-NA002B	10 ml Packed Bead Volume
# BB-NA002C	25 ml Packed Bead Volume
# BB-NA002D	50 ml Packed Bead Volume
# BB-NA002E	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.



Nitrilotriacetic acid (NTA)-crosslinked-agarose resin consists of NTA groups covalently immobilized on agarose beads by stable ether linkages via a spacer arm.

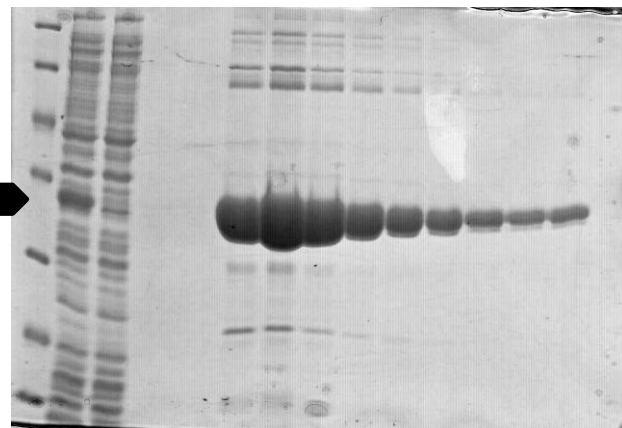
NTA is a tetradentate chelating agent forms octahedral coordination complex with divalent transition metal cations ( $\text{Ni}^{2+}$ ) along with two water molecules occupied in cis manner. This ready to use resin, after loading with a divalent transition metal ion ( $\text{Ni}^{2+}$ ) is ideal for 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.

Product is supplied as 50% suspension of Ni-NTA Agarose resin in 20% aqueous ethanol; the settled bead volume was measured in ml when supplied.

- Under optimum condition, 1.0 ml of Ni-NTA Agarose Bead can purify up to 50 mg of His-tagged protein.
- Ni-NTA Agarose Bead can withstand autoclaving at 120°C for 20 min without any significant loss of binding efficiency.

**TECHNICAL SPECIFICATIONS**

BEAD GEOMETRY & SIZE:	Spherical, ~ 50 - 150 $\mu\text{m}$ diameter
CROSSLINKED	: Yes
BEAD AGAROSE %	: 4%
ACTIVATING GROUP	: Epoxy
MATRIX STABILITY	: Stable in all commonly used reagents
STORAGE SOLUTION	: 20% Ethanol
STORAGE TEMPERATURE:	4°C to 8°C. <b>DO NOT FREEZE</b>



Purification of Reg  $\beta$  protein using 1 ml of Ni-NTA agarose bead

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



## His-tagged protein purification using Ni-NTA Agarose beads

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Prepared bacterial cell pellet can be stored at  $-80^{\circ}\text{C}$  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  $4^{\circ}\text{C}$  to pellet the cellular debris. Collect supernatant and place on ice.
- Add 4 ml cleared lysate to the pre-equilibrated Ni-NTA Agarose in a 15 ml falcon and mix gently by shaking (200 rpm, rotary shaker) for 60 min at  $4^{\circ}\text{C}$ .
- Load the lysate on Ni-NTA Agarose mixture into a gravity 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  $\mu\text{l}$  for gel analysis).
- Wash twice with 10 ml lysis buffer (may be increased if nonspecific is too high). Collect wash fractions for SDS-PAGE analysis (use approximately 15  $\mu\text{l}$  for gel analysis).
- Wash the beads with 30 ml *wash buffer* (may be increased if nonspecific is too high). Collect flow-through for SDS-PAGE analysis (use approximately 15  $\mu\text{l}$  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  $\mu\text{l}$  for gel analysis).

### Buffer composition

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#### Cell Lysis buffer:

25 mM Tris-HCl (pH- 8), 150 mM NaCl, 10 mM Imidazole (reduce to 0–5 mM, If tagged protein passes through), Upto 10 mM  $\beta$ ME (**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  $\beta$  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,  $\beta$ ME (**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. Phosphate buffer may also be used instead of Tris buffer but keep the reducing agent concentration not more than 10 mM. Also note that beads may become reddish in this condition, however purification profile remains unchanged.

### Regeneration of Ni-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 Nickel 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  $4^{\circ}\text{C}$  in 20% ethanol.