GBond Ni-IDA Agarose Bead

 # GBI-01C 50 ml

Storage: 4^0C

Bead geometry & size	Spherical, ~ 50 - 150 µm diameter
Crosslinked	Yes
Bead agarose %	4%
Activating agent	Epichlorohydrin
Matrix	Stable in all commonly used reagents
Storage temperature	4^0C to 8^0C. **Don't Freeze**
Storage solution	20% Ethanol

Description

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.

Iminodiacetic acid (IDA)-crosslinked-agarose resin consists of IDA groups covalently immobilized on agarose beads by stable ether linkages via a spacer arm. IDA is a tridentate chelating agent forms hexagonal coordination complex with divalent transition metal cations (Ni^{2+}) along with two water molecules occupied in cis manner. This ready to use resin, after loading with a divalent transition metal ion (Ni^{2+}) is ideal for rapid purifications of His-tagged proteins.

In comparison with other chelating resins such as Nitrilotriacetic acid (NTA)-agarose, the IDA has three sites (cis) available for the interaction with imidazole of His-tagged proteins, instead of the two in NTA, thus generating greater yield of target protein.

Protocol

Notes before starting:
Prepare bacterial cell pellet. Cell pellet can be stored at -80°C till use. Wash the beads (Approx 1ml bed volume) with double distilled water (at least 5X column volume) and equilibrate the beads in lysis buffer.

- Thaw cell pellet for 15 min on ice and resuspend in **lysis buffer** (the cell lysate should be prepared at a 10-50 fold concentrated relative to the original culture volume.
- Add lysozyme (final conc.1mg/ml) and incubate for 30-60 min.
- Lyse the cells using sonication or any alternative methods.
- Centrifuge 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 Ni- IDA Agarose in a 15 ml falcon and mix gently by shaking (200 rpm, rotary shaker) for 60 min at 4°C.
- Load the lysate on Ni-IDA 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 15µl for gel analysis).
- Wash twice with 5- 10 CV of lysis buffer. Collect wash fractions for SDS-PAGE analysis (Use approximately 15µl for gel analysis).
- Wash the beads with 30 ml **Wash buffer** (Lysis buffer containing 20/30/40 mM Imidazole). Collect flow-through for SDS-PAGE analysis (Use approximately 15µl for gel analysis).
- Elute the protein with 10ml **Elution buffer**. Collect the eluate in four tubes and analyze by SDS-PAGE (Use approximately 15µl for gel analysis).

Buffer composition:

Cell Lysis buffer: 25mM Tris-HCl (pH- 8), 150mM NaCl, 10mM Imidazole (If tagged protein doesn't bind under these conditions, reduce imidazole to 1-5 mM), 1-5mM βME, 0.05% Triton-X-100, 5% Glycerol. **NOTE**: A light brown color may appear under this condition which will not affect the binding efficiency.

Wash Buffer: 25mM Tris-HCl (pH- 8), 150mM NaCl, 20-40 mM Imidazole, 5 mM βME, 0.05% Triton-X-100, 5% Glycerol.

Elution buffer: 25mM Tris-HCl (pH- 8), 150mM NaCl, 250-500mM mM Imidazole, 5 mM βME, 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.

NOTE: GBond Ni-IDA can be re-used instantly after regeneration of the beads. Regeneration Procedure for Ni-IDA Agarose:

- Wash the resin with 10 column volumes of distilled water.
- Eliminate metal from the resin by washing with 10 column volumes of 25mM Tris-HCl (pH-8), 1M NaCl, 100 mM EDTA Solution.
- 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.
- Wash the beads with 10 column volumes of distilled water. Store at 4°C.

NOTE: Use of DTT in any purification buffer is not recommended.

1.