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Ni-IDA Agarose Beads for His-tagged Protein Purification

Store at 2-8°C

Cat. No.	Description	Quantity
G250	Ni-IDA Agarose Resin	5ml
G251	Ni-IDA Agarose Resin	25ml
G253	Ni-IDA Agarose Resin	100ml

Product Description

Affinity chromatography is a method of separating biochemical mixtures based on highly specific molecular interactions. The Ni-IDA agarose is developed for the purification of proteins with an affinity tag of six consecutive histidine residues. This histidine-metal ion interaction makes one-step purification possible for proteins from any expression system, under native or denaturing conditions. The His-tag sequence binds to Ni^{2+} cations, which are immobilized onto a solid support using iminodiacetic acid groups (IDA). Impurities are removed and the purified His-tagged proteins can be eluted by imidazole or a reduced pH solution.

Ni-IDA Agarose Resins are supplied as a suspension in 20% ethanol and can be used in batch or column purifications. Ni-IDA Agarose Columns are in ready-to-use format for purification of histidine-tagged proteins by gravity flow.

Technical Specifications for Ni-IDA Agarose Beads

Beads (Geometry, Size)	Spherical, Standard: ~50-150 µm
Agarose %	6% Agarose
Binding/ Loading Capacity	20 - 40 µmol Ni^{2+}/ml gel
Column Material	Polypropylene and polyethylene frit
Storage Temperature	2 - 8 °C

Shipping and Storage

Upon arrival, the Ni-IDA Agarose Resin/ Column should be stored at 2–8°C. Do not freeze. For long term storage after use, it is recommended to leave the Ni-IDA Agarose Resin/ Column in 20% ethanol or other preservatives.

General Protocol

1. Elimination of the preservative: Wash the beads with 5 – 10 bead volumes/ column volumes of distilled water.
2. Equilibration of the resin: Equilibrate the beads with 5 – 10 bead volumes/ column volumes of the binding buffer.

   NOTE: The most frequently used buffers are acetate (50mM) or phosphate (10- 150 mM) with 0.15- 0.5 M of NaCl at neutral pH; however, the choice of buffer depends on the properties of the protein of interest.
3. Application of the sample: Once the resin has been washed and equilibrated, the protein sample can be applied to the beads/ column. In batch purification, it is recommended to incubate the beads/ sample mixture on a rotating device for at least 1 hour. In column purification, the binding capacity is affected by the flow rate during sample application. The recommended flow rate is 0.5ml - 1.0ml/ min.
4. Washing of resin: Wash the beads with 5 – 10 bead volumes/ column volumes of the binding buffer. This step will wash off any non-specific proteins bound to the beads and increase the purity of the final protein product.
5. Elution of the pure protein: Adding a competitive ligand (usually imidazole) will elute the retained protein from the beads/ column. In general, 0.5 M of imidazole is sufficient to elute the protein. Other competitive ligands include histidine and ammonium chloride.

Regenerating Ni-IDA Agarose

The agarose beads and column can be re-used for successive cycles; however, the binding capacity may decrease over time. The loss of the binding capacity may be due to retained proteins. To return the beads to the original state, it is necessary to completely replace the nickel metal bound to the resin.

Ni-IDA Agarose Regeneration Protocol

1. Strip the resin with 5 bead/ column volumes of phosphate buffer containing 50mM of EDTA at pH 7.0.
2. Wash the resin with 5 bead/ column volumes of distilled water once before reloading the resin with nickel metal.
3. Regenerate the resin with 5 bead/ column volumes of the 0.1M nickel metal solution (normally chlorides or sulphates are used).
4. Wash the resin again with 5 bead/ column volumes of distilled water before equilibrating the resin for use.

Recommendations for Optimal Results

- Avoid the use of high concentration reducing agents with the nickel resin/column as this will affect the binding capacity of the resin/column.
- Employ mild detergents such as up to 2% Triton X-100 or Tween-20 will help prevent non-specific binding to the resin/column. This addition will not affect the binding of proteins to the Ni-IDA resin/column.
- Ni-IDA agarose beads are fragile, therefore gentle handling is important to ensure the optimal result.