Ni-IDA Resin

Description:

NI-IDA resin is a valuable tool 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.

Components and Storage

Ni-IDA Resin	**5 mL**
Column & head and bottom plugs	**1 set**
Filter (polypropylene frit)	**2 pcs**

* Store kit at +4°C. Ni-IDA Resins are supplied as a suspension in 20% ethanol. The NI-IDA resins are stable at room temperature. All reagents are stable for up to 24 months under proper storage conditions. Do not freeze.

General Protocol at the native condition
(For soluble proteins)

If there is no peristaltic pump, To control flow rate of column, remove the bottom plug and attach a flexible tube to the outlet and press tube with a paper clip or infusion (serum set) controller.

1. Elimination of the preservative: Wash the resin with 5 – 10 column volumes of 0.15 M NaCl solution. It means 25-50 ml of 0.15 M solution for 5 ml of resin.

NOTE. Do not leave the column without buffer. It can result in formation of cracks in the resin bed and air bubble entrapment. Column should be repacked at this condition.

2. Equilibration of the resin: Equilibrate the beads with 5 – 10 column volumes of the binding buffer (0.15 M NaCl, 10% glycerol, 0.5% Triton X-100, 50 mM sodium phosphate buffer pH 7.8).

NOTE. The choice of buffer depends on the properties of the protein of interest. **Some proteins need more NaCl to expose their His-tag (between 0.3 to 0.5 M).**

3. Application of the sample: Once the resin has been washed and equilibrated, the protein sample can be applied to the column. The binding capacity is affected by the flow rate during sample application. The recommended flow rate is 0.25ml – 0.5 ml/ min. Sample should be prepared in the binding buffer. You can use it as your lysis buffer. Be sure of clarity of your sample by centrifuge or filtration.

4. Washing of resin with start buffer: Wash the beads with 5 column volumes of the binding buffer. This step will wash off non-bound proteins. The recommended flow rate is 0.5 ml/ min.

5. Washing of resin with wash buffer: Wash the beads with 5 – 10 column volumes of the wash buffer. This step will wash off any non-specific proteins bound to the beads and increase the purity of the final protein product. The recommended flow rate is 0.5 ml/ min.

NOTE. The most frequently used wash buffer contains sodium phosphate buffer pH 7.8 (50 mM), 0.15 NaCl with 20-30 mM Imidazole. The concentration of imidazole depends on the properties of the protein of interest.

6. **Elution of the pure protein:** Adding a competitive ligand (usually imidazole) will elute the retained protein from the column. In general, 0.5 M of imidazole is sufficient to elute the protein. Recommended elution buffer is 0.5 M imidazole, 0.15 M NaCl with 50 mM sodium phosphate buffer pH 7.8. The recommended flow rate is 0.5 ml/ min.

7. Collect the elution in 1.5 ml fractions and check the protein content by SDS-PAGE.

8. Pool the fractions containing protein of interest and dialyze against appropriate buffer.

General Protocol at the denaturing condition (for Insoluble proteins)

Purification steps are the same as the non denaturing condition, but denaturing agents such as 3-6 M guanidine chloride or 6 M urea should be added to the binding buffer (start buffer) and half of this amount to the wash and elution buffers. If the desire protein is very insoluble, it prevents reduction of denaturing agents of wash and elution buffers.

Regenerating Ni-IDA Resin

The Resin 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 Resin Regeneration Protocol

1. Strip the resin with 5 column volumes of phosphate buffer containing 0.15 M NaCl and 50mM of EDTA at pH 8.0.

2. Wash the resin with column volumes of 0.15 M NaCl once before reloading the resin with nickel metal.

3. Regenerate the resin with 2 column volumes of the 0.1M nickel metal solution (normally chlorides or sulphates are used). The recommended flow rate is 0.25ml/min. stop the flow for 15 min.

4. Wash the resin again with 5 column volumes of of 0.15 M NaCl before equilibrating the resin for use.