GenScript Corporation
www.genscript.com
Tel: 1-732-885-9188
Fax: 1-732-210-0262
order@genscriptcorp.com

High-Affinity Ni-IDA Resin

Cat. No.	Size
L00223	20 ml

Description:

High-Affinity Ni-IDA Resin is an effective way for purification of recombinant proteins fused with a 6×Histidine tag. The product consists of the tridentate chelating agent, iminodiacetic acid (IDA), covalently coupled to 4% cross-linked agarose beads.

Contents:

Every 20 ml Ni-IDA Resin contains 50% slurry.

Application:

- Purification of polyhistidine-tagged proteins under native conditions.
- Purification of polyhistidine-tagged proteins from E. coli under denaturing conditions.

Storage:

Store at 4°C to 8°C.

Features:

- Widely used system
- Simple purification procedure under either native or denaturing conditions.
- Direct purification from crude bacterial lysates.
- Best purity of protein.
- pH stability of 3-13 (short term 2-14).
- Binding capacity: >10 mg 6×His-tagged protein/ml (CV).
- Resin can be regenerated for multible uses.
- Extremely cost effective.
- Purification of polyhistidine-tagged proteins.
- Under native conditions.

Related Products:

Cat. No.	Product	Size
L00206	GST Resin	20 ml
L00209	Protein G Resin	10 ml
L00210	Protein A Resin	10 ml

Protocol

I. Purification of polyhistidine-tagged proteins under native conditions

Before use, prepare the following two solutions:

Lysis-Equilibration-Wash buffer (LEW buffer, 1 liter):

- 50 mM NaH_2PO_4
- 300 mM NaCl
- Adjust pH to 8.0 using NaOH

Elution buffer (1 liter):

- 50 mM NaH_2PO_4
- 300 mM NaCl
- 250 mM imidazole
- Adjust pH to 8.0 using NaOH

1) Column preparation - slurry packing

Make a suspension of ~50 % (w/v) slurry of High-Affinity Ni-IDA Resin in 1×PBS with 20% ethanol by general shaking. Do not use a magnetic stirrer to avoid generating fine particles through excessive physical force. Slowly pour the suspension into the column. Avoid introducing air bubbles. Allow the resin to settle.

2) Column equilibration

Equilibrate the column with 4 bed volumes of LEW buffer or until A_{280} is stable.

3) Binding

Apply the cleared sample containing 6×His-taged protein to the column.

4) Washing

Wash the column with 8 bed volumes of LEW buffer or until A_{280} is stable.

5) Elution

Elute the polyhistidine-tagged protein with 5-10 bed volumes of Elution buffer

II. Purification of polyhistidine-tagged proteins from E. coli under denaturing conditions

We recommend this protocol if expression leads to the formation of inclusion bodies. Cells are disrupted under native conditions using lysozyme together with sonication. After centrifugation the polyhistidine-tagged protein is extracted and solubilized from the pellet by using a denaturant (8 M urea). The extract obtained is clarified by centrifugation and applied to High-Affinity Ni-IDA Resin under denaturing conditions. Purification of polyhistidine-tagged proteins underdenaturing conditions is similar to purification under native conditions except that the cell extract and buffers loaded on the column contain 8 M urea.