Ni-Pentadentate Agarose Kit for Purification of 6x His-tagged Proteins

Cat. #: REA005

Features:

-One-step purification from crude lysate to >99% pure protein
-High binding affinity, high capacity, and high specificity
-Purification under native or denaturing conditions
-No leaching of Ni ions
-Ready-to-use for any scale of purification

Kit components:

Ni-pentadentate Agarose resin, lysis buffer, wash buffer, binding buffer, and elution buffer

Binding capacity: 20-30 mg/ml (300–500 nmol @ ~60 kDa)
Support: Sepharose CL-6B
Bead structure: Cross-linked, 6% agarose
Bead size: 50–160 μm
Form: 50% suspension in 25% ethanol, precharged with Ni^{2+}

Principle:

The Ni-pentadentate Protein Purification System is based on the remarkable selectivity of patent pending resin for proteins containing an affinity tag of six consecutive histidine residues — the 6xHis tag. This technology allows one-step purification of almost any His-tagged protein from any expression system under native or denaturing conditions. Ni-pentadentate, which has five chelation sites for nickel ions, binds nickel more tightly than metal-chelating purification systems that only have three sites available for interaction with metal ions. The extra chelation site prevents nickel-ion leaching and results in a greater binding capacity and protein preparations with higher purity than those obtained using other metal-chelating purification systems. The Ni-pentadentate system can be used to purify His-tagged proteins from any expression system including baculovirus, mammalian cells, yeast, and bacteria.

Procedure:

The purification of 6xHis-tagged proteins consists of 4 steps: cell lysis, binding, washing, and elution. Purification of recombinant proteins using the Ni-pentadentate system does not depend on the 3-dimensional structure of the protein or 6xHis tag. This allows one-step protein purification under either native or denaturing conditions, from dilute solutions and crude lysates. Strong denaturants and detergents can be used for efficient solubilization and purification of receptors, membrane proteins, and proteins that form inclusion bodies. Reagents that allow efficient removal of nonspecifically binding contaminants can be included in wash buffers. Purified proteins are eluted under mild conditions by adding 100–250 mM imidazole as competitor or by a reduction in pH

Tel. (650)-964-2589, Fax. (650)964-2519 info@antageneinc.com, 1391 Old Middlefield Way, Suite K Mountain View, CA 94043

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1) Express and harvest cells. Resuspend cells in Binding Buffer. Lyse cells.
2) Prepare resin (~20- 25 ml?) in column - wet column and frit, transfer resin slurry to column. Wash with water (3 volume), Charge Buffer (5 volumn), Binding Buffer (3 volume).
3) Load column with cell lysate, at a rate of ~10 column volumn per hour.
4) Wash column (10 X volumn) with Binding Buffer
5) Wash column (6 X volumn) with Wash Buffer
6) Elute protein (6 X volume) with Elute Buffer (strip buffer can also be used to elute). Alternatively elute first with lower concentration of imidazole at 0.25 M.
7) To store column, wash with Strip Buffer (3 X volumn) and stored in Strip Buffer at 4 °C.

1X Buffer

reagents	**Binding Buffer**	**Wash Buffer**	**Elute Buffer**	**Strip Buffer**	**Charge Buffer**
Tris-HCl pH7.9	20 mM	20 mM	20 mM	20 mM	
Imidazole	1 mM	10 mM	250 mM		
NaCl	0.5 M	0.5 M	0.5 M	0.5 M	
EDTA				100 mM	
NiSO4					50 mM

Purification in Denaturing Condition (protein in inclusion body)
1) Same as before.
2) Centrifuge to collect inclusion body. Decant supernatant, resuspend in Binding Buffer (sonicate if necessary), centrifuged. Repeat until all trapped proteins are released.
3) Decant supernatant, resuspend in Binding Buffer + 6 M guanidine or Urea. Incubate on ice for 1 hour to dissolve.
4) Centrifuged at 39,000 g for 20 min. Filter supernatant (0.45 micron membrane).
5) Load onto column. Purification same as before, except that all buffer contain denaturant, and lower imidazole concentration in Wash Buffer (20 mM) and Elute Buffer (~300mM) (dilute Wash and Elute Buffer with Binding Buffer). Alternatively, you can wash and elute the protein without imidazole at low pH (wash at pH 6.5, elute at pH5.9 or pH4.5 if the protein failed to elute at the hight pH)

Regeneration of Column
- when flow rate is slow and column resin doesn't turn blue-green when charged
1) Wash with 2 vol 6 M guanidine-HCl, then 3 vol water.
2) Wash with 1 vol 2% SDS.
3) Wash with 1 vol each of 25%, 50% and 75% ethanol, then 5 vol of 100% ethanol, followed by 1 vol each of 75%, 50% and 25% ethanol.
4) Wash with 1 vol water, then 5 volume 100 mM EDTA (pH 8.0).
5) Wash with 3 vol water, then 3 vol 20% ethanol.
6) Store at 4 °C.

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Note:
1) Do not use ßME, DTT or EDTA in buffer.
2) Higher amount of imidazole may be used in the wash buffer (100 mM) but some protein may be eluted.
3) You can also elute the protein with salt concentration gradient or using lower pH.
4) It's sometimes useful to introduce a couple of glycines between your protein and the His-tag which allow the his-tag to be fully exposed for effective binding to resin (if your vector doesn't have a short linker sequence between the HIs-tag and your protein that is).

Reagents compatible with the Ni-NTA–6xHis interaction*
• 6 M guanidine HCl • 50% glycerol
• 8 M urea • 20% ethanol
• 2% Triton X-100 • 2 M NaCl
• 2% Tween 20 • 4 M MgCl2
• 1% CHAPS • 5 mM CaCl2
• 20 mM β-ME • ≤20 mM imidazole
* The reagents listed have been successfully used in concentrations up to those given.

Applications:
The Ni-pentadentate Protein Purification System provides reliable, one-step purification of proteins suitable for any application, including:
-Structural and functional investigations
-Crystallization for determination of three-dimensional structure
-Assays involving protein–protein and protein–DNA interactions
-Immunization to produce antibodies

Ni-pentadentate matrices can also be used to bind 6xHis-tagged proteins as immobilized affinity ligands to:
-Study molecular interactions with nucleic acids and binding proteins
-Purify antibodies
-Isolate nontagged, interacting subunits or nucleic acids
-Investigate ligand–receptor interactions

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