Enzymax LLC Product Sheet

Tel: 859-219-8482 Web: www.enzymax.net

Ni60-NTA resin for purification of 6x His -tagged Proteins

Cat. #: Ni60-NTA (10, 25, 50, 100, 500ml)

Description:

Ni60-NTA resin is used for purification of histidine-tagges proteins. The binding of His-tagged protein is based on the interaction between polyhistidine tag and immobilized Ni ions. Ni60-NTA resin contains chelating ligand nitrilotriacetic acid (NTA) which is immobilized on 6% cross-link agarose resin. This non-charged and hydrophilic linkage of the NTA chelating ligand to the agarose ensures specific binding of his-tagged fusion protein to the resin, which allows for one-step purification of nearly any His-tagged protein from any expression system under native or denaturing conditions.

Features:

-One-step purification from crude lysate
-High binding affinity, high capacity, and high specificity
-Purification under native or denaturing conditions
-No leaching of Ni ions
-Precharged with Ni^{2+} and ready-to-use for any scale of purification

Binding capacity: 60 mg/ml
Support: Sepharose CL-6B
Bead structure: Cross-linked, 6% agarose
Bead size: 50–160 µm
Form: 50% suspension in 25% ethanol, precharged with Ni2+

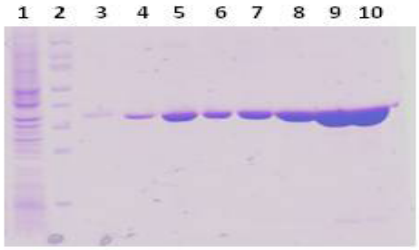
Columns suitable for Ni60-NTA resin:

0.5 ml and 2.0 ml Syringe columns (EZSC111, EZSC112, 25/pack @$45 and $59)

2.0ml Syringe Column for Affinity Resins
(Ni-, GST , Protein A & G and other resins)

Screw Cap Spin Column (EZC116, 50/pack @$79)

Sample of purification:



Purification of AIMF1 with Enzymax Ni60-NTA resin
Lane1: Whole cell extract
Lane 2: Protein Marker
Lane 3-10: Eluted fractions 1-8 with Imidazole gradient

Procedure: Based on 250 ml of *E.coli* Culture

Purification in Native Condition (soluble protein)

There are 4 steps in this procedure: Cell lysis → Binding → Washing → Elution.

1) Express and harvest cells. Resuspend cells in 10 ml Binding Buffer and add Lysozyme to the final concentration of 1 mg/ml. Stir the solution on ice for 30 min.
2) Sonicate the suspension on ice for 10 x15 second bursts with a 15 second cooling period between each bust.
   Note: Add 5 µg/ml DNase I (15 min on ice) if the lysate is viscous.
3) Centrifuge the lysate at 10,000 x g for 30 min at 4 °C.
   Note: centrifuge for 2nd time or filter through 0.45 µm membrane if the lysate is not clear. This will prevent clogging the column.
4) Prepare resin in column (**you can use gravity column, syringe column, or screw cap spin column**) - Shake the bottle and transfer 4 ml resin slurry to the column (2 ml Ni resin). Wash with 10 ml water and then 10 ml Binding Buffer.
5) Load the lysate to column and collect the flow-through.
4) Wash the column with 12 ml of Binding Buffer.
5) Wash the column with 12 ml of Wash Buffer.
6) Elute protein with 10 ml of Elute Buffer and collect 0.5-1 ml for each fraction.
6) Use 5-10 µl of protein to run SDS PAGE gel. Store samples at -80°C.

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Purification in Denaturing Condition (protein in inclusion body)

1. Follow steps 1-3 above.
2. Discard the supernatant after centrifuge. Wash the pellet (inclusion body) 3 times with Binding Buffer (sonicate the sample if it is viscous).
3. Resuspend the inclusion body in Binding Buffer + 6 M guanidine or 8M Urea. Incubate on ice for 1 hour.
4. Centrifuge the lysate at 10,000 x g for 30 min at 4 °C.
   Note: centrifuge for 2nd time or filter through 0.45 µm membrane if the lysate is not clear. This will prevent clogging the column.
5. Prepare resin in column (**you can use gravity column, syringe column, or screw cap spin column**) - Shake the bottle and transfer 4 ml resin slurry to the column (2 ml Ni resin). Wash with 10ml water and then 10 ml Binding Buffer.
6. Load the sample onto column. Collect the flow-through.
7. Wash the column with 12 ml of Binding Buffer + 6 M guanidine or 8 M Urea.
8. Wash the column with 12 ml of Wash Buffer+ 6 M guanidine or 8 M Urea.
9. Elute the protein with 10ml Elute Buffer+ 6 M guanidine or 8 M Urea. Collect 0.5-1 ml for each fraction.
10. Use 5-10 µl of protein to run SDS PAGE gel. Store the samples at -80°C.

Regeneration of Column

1) Wash with 2 column volumes of 6 M guanidine-HCl, then 3 column volumes of water.
2) Wash with 1 column volume of 2% SDS.
4) Wash with 3 column volumes of water, then 5 column volumes of 100 mM EDTA (pH 8.0).
5) Wash with 3 column volumes of water.
6) Charge the column with 100 mM NiSO4. Wash column with water.
7) Store column in 20% ethanol at 4 °C.

Note:

1) Do not use DTT or EDTA in buffer.
2) Higher amount of imidazole may be used in the wash buffer (30-100 mM) to remove non-specifically bound proteins.
3) You can also elute the protein using low pH.

Reagents compatible with the Ni-NTA–6xHis interaction*

- 6 M guanidine HCl
- 8 M urea
- 50% glycerol
- 20% ethanol
- 2% Triton X-100
- 2% Tween 20
- 5 mM CaCl2
- ≤20 mM imidazole
- 4 M MgCl2
- 2 M NaCl
- 1% CHAPS
- 20 mM β-ME

* The reagents listed have been successfully used in concentrations up to those given.

Price for Ni60-NTA:

Catalog Number	Size	Price
Ni60-NTA-10	10 ml	$79
Ni60-NTA-25	25 ml	$159
Ni60-NTA-50	50 ml	$299
Ni60-NTA-100	100 ml	$569
Ni60-NTA-500	500 ml	$1999

Buffers:

1xBinding Buffer, 1 L:
50 mM NaH_2PO_4 7.8 g NaH2PO4x2H2O (MW=156.01g/mol)
300 mM NaCl 17.5 g NaCl (MW=58.44 g/mol)
Adjust pH to 8.0 using NaOH

1x Wash Buffer (1 L): 1x Binding Buffer+10~60 mM Imidazole

1x Elution Buffer (1 L):
50 mM NaH_2PO_4 7.8g NaH2PO4x2H2O (MW=156.01g/mol)
300 mM NaCl 17.5 g NaCl (MW=58.44g/mol)
250 mM imidazole 17.0g imidazole (MW=68.08g/mol)
Adjust pH to 8.0 using NaOH

Binding buffer + 8 M Urea (1L):
50 mM NaH_2PO_4 7.8g NaH2PO4x2H2O (MW=156.01g/mol)
300 mM NaCl 17.5 g NaCl (MW=58.44g/mol)
8 M Urea 480.5 g (MW=60.06g/mol)
Adjust pH to 8.0 using NaOH

Wash Buffer + 8 M Urea (1L): 1x Binding Buffer+10~60 mM Imidazole +8 M Urea

Elution Buffer+ 8 M Urea (1 L):
50 mM NaH_2PO_4 7.8g NaH2PO4x2H2O (MW=156.01g/mol)
300 mM NaCl 17.5 g NaCl (MW=58.44g/mol)
250 mM imidazole 17.0g imidazole (MW=68.08g/mol)
8 M Urea 480.5 g (MW=60.06g/mol)
Adjust pH to 8.0 using NaOH