Ni NTA Resin

His Capture Nickel Affinity Resin Users Manual

Revision 2

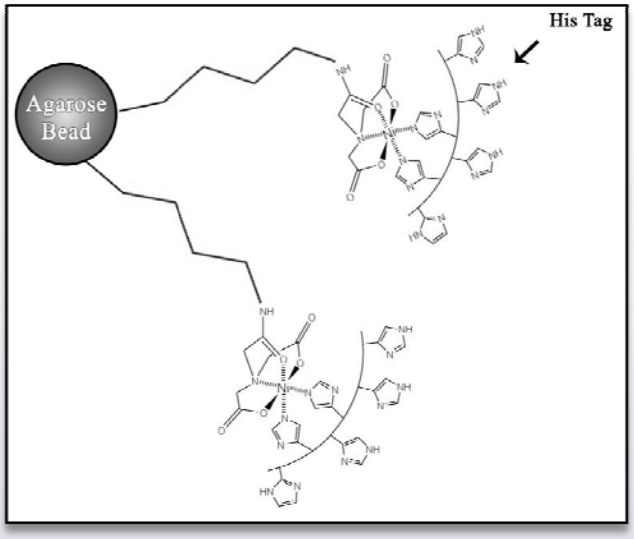
THIS MANUAL APPLIES TO THE FOLLOWING PRODUCTS:

3N2345 His Capture Nickel Affinity Resin, 5 mL

3N2325 His Capture Nickel Affinity Resin, 25 mL

3E3850 His Capture Equilibration Buffer, 50 ml

3E3865 His Capture Elution Buffer, 50 ml



SPECIFICATIONS

BEAD (Geometry, Size)	Spherical, Standard: ~ 50-170 µm
CROSS-LINKED	Yes
AGAROSE %	6% agarose
LIGAND	Nitrilotriacetic acid (NTA)
BINGING CAPACITY	>50 mg / ml gel*
ANTIMICROBIAL AGENT	30% Ethanol
STORAGE TEMPERATURE	4 - 8°

*Binding capacity will modify for each target protein.

About the Product

His Capture Nickel Affinity Resin is used for the purification of Histidine tagged proteins (His-tag). The resin consists of agarose beads with Nickel bound to the surface via chelation to NTA (nitrilotriacetic acid). The His-tag binds strongly to the nickel ions in a coordination complex that is highly selective for histidine oligomers. Once the protein is bound it is washed to free it of contaminating proteins, metabolites, etc. The tagged protein is then eluted from the resin using a high concentration of imidazole which displaces the histidine residues from the Nickel ions.

The binding capacity of this resin is approximately 70 mg of a 30 kDa GFP protein per ml of settled resin. The binding capacity will vary from protein to protein, however, and is also affected by the presence of competing ligands that may be present in the lysate or the buffer. Many commonly used buffer components are known to affect the performance of the resin. Please see the **Ni NTA Resin Buffer Compatibility Chart** below.

This product is supplied with both equilibration and elution buffers which are also available separately should additional buffer be required. The buffers are proprietary and contain surfactants that stabilize the protein and protect against denaturation while undergoing purification.

The resin can be used under both native and denaturing conditions which are described below.

NATIVE CONDITIONS
BATCH PURIFICATION

The resin is a suspension and settles rapidly out of solution so make sure to swirl the container to resuspend the particles before dispensing.

For 500 µl of *E. coli* cell lysate, use 50 µl of HIS-Capture Nickel Affinity Resin (Teknova Cat. No: 3N2345 or Cat. No: 3N2325).

1. **REMOVING THE STORAGE BUFFER**. Add 50 µl of HIS-Capture Nickel Affinity Resin (Teknova Cat. No: 3N2345 or Cat. No: 3N2325), equal to 100 µl suspension, to a micro centrifuge tube and centrifuge for 1 min at 4000 x g.
2. Carefully remove the supernatant and discard.
3. Add 500 µl (10x column volume) of Reagent Grade Water and mix well. Centrifuge 1min at 4000 x g and remove the supernatant carefully.
4. **EQUILIBRATING THE RESIN**. Add 100 µl of Equilibration Buffer (2x column volume) and mix well. Centrifuge for 1 min at 4000 x g and remove the supernatant carefully.
5. **LOADING TO PROTEIN ONTO RESIN.** Add 500 µl of clarified lysate and mix for 10 min, rocking or on a nutator.
6. Centrifuge the mixture at 4000 x g for 1 min and remove the supernatant (supernatant can be saved for analysis; at this point the His-protein binds to the resin).
7. **WASHING THE SAMPLE.** Wash the resin with 250 µl (5x column volume) equilibration buffer by mixing the buffer with the resin for 5 min rocking and centrifuge 1 min at 4000 x g. Discard the wash buffer (or save it for analysis).
8. Do a second wash with 250 µl (5x column volume) equilibration buffer by mixing the buffer with the resin for 5 min rocking and centrifuge 1 min at 4000 x g. Discard the wash buffer (or save it for analysis).
9. **ELUTING THE PROTEIN.** Elute the target protein with 50 µl (1x column volume) of Elution buffer: add the buffer and mix well. Incubate for 10 min in a nutator or other device to mix slowly. If none is available it is OK to manually agitate the sample every few minutes. Centrifuge 1 min at 4000 x g.
10. Carefully remove the supernatant which contains the protein of interest.

11. About half of the protein will come off in the first elution volume so it is generally desirable to repeat steps 9 and 10 two or even three more times and then either pool all of the elution volumes together or run a small sample from each fraction (3-5 µl) on an SDS-PAGE gel and inspect them for purity and concentration

Note: The Equilibration Buffer contains 10 mM imidazole. To reduce background you can increase the concentration to 20 mM. The Elution buffer contains 250 mM imidazole.

DENATURING CONDITIONS
BATCH PURIFICATION

The protocol for His-tag purification under denaturing conditions is similar to the one for purification under native conditions. The buffers provided in this kit are for native conditions only so you will need to make denaturing buffers that contain 8 M urea as the denaturant. The recipes are provided below.

The resin is a suspension and settles rapidly out of solution so be sure to swirl the container to resuspend the particles before dispensing.

For 500 µl of *E. coli* cell lysate, use 50 µl of HIS-Capture Nickel Affinity Resin (Teknova Cat. No: 3N2345 or Cat. No: 3N2325).

1. **REMOVING THE STORAGE BUFFER**. Add 50 µl of HIS-Capture Nickel Affinity Resin (Teknova Cat. No: 3N2345 or Cat. No: 3N2325), equal to 100 µl suspension, to a microcentrifuge tube and centrifuge for 1 min at 4000 x g.
2. Carefully remove the supernatant and discard.
3. Add 500 µl (10x column volumn) of Reagent Grade Water and mix well. Centrifuge for 1 min at 4000 x g and remove the supernatant carefully.
4. **EQUILIBRATING THE RESIN**. Add 100 µl of Denaturing Equilibration Buffer (2x column volume) and mix well. Centrifuge for 1 min at 4000 x g and remove the supernatant carefully.
5. **LOADING TO PROTEIN ONTO RESIN.** Add 500 µl of clarified lysate that has been prepared using a denaturing lysis buffer. An example of a Denaturing Lysis Buffer is shown below.
6. Centrifuge the mixture at 4000 x g for 1 min and remove the supernatant (supernatant can be saved for analysis; at this point the His-protein is bound to the resin).
7. **WASHING THE SAMPLE.** Wash the resin with 250 l (5x column volume) Denaturing Equilibration Buffer by mixing the buffer with the resin for 5 min rocking and centrifuge 1 min at 4000 x g. Discard the wash buffer (or save it for analysis).
8. Do a second wash with 250 l (5x column volume) Denaturing Equilibration Buffer by mixing the buffer with the resin for 5 min rocking and centrifuge 1 min at 4000 x g. Discard the wash buffer (or save it for analysis).
9. **ELUTING THE PROTEIN.** Elute the target protein with 50 l (1x column volume) of Denaturing Elution Buffer: add the buffer and mix well. Incubate for 10 min in a nutator or other device to mix slowly. If none is available it is OK to manually agitate the sample every few minutes. Centrifuge for 1 min at 4000 x g.
10. Carefully remove the supernatant which contains the protein of interest.
11. About half of the protein will come off in the first elution volume so it is generally desirable to repeat steps 9 and 10 two or even three more times and then either pool all of the elution volumes together or run a small amount (10 l) on an SDS-PAGE gel to inspect them for purity and concentration.

BUFFERS FOR DENATURING CONDITIONS

Denaturing Lysis Buffer

100 mM NaH_2PO_4

10 mM Tris base

6 M guanidine hydrochloride

10 mM imidazole

Adjust to pH 8

Denaturing Elution Buffer

50 mM NaH_2PO_4

300 mM NaCl

8 M urea

250 mM imidazole

Adjust to pH 8

Denaturing Equilibration Buffer

100 mM NaH_2PO_4

150 mM NaCl

8 M urea

20 mM imidazole

Adjust to pH 8

Ni NTA Resin Buffer Compatibility Chart

Buffer	Maximum concentration
Tris	50 mM
HEPES	50 mM
Phosphate	50 mM
MES	50 mM
MOPS	50 mM
Glycerol	50 %
Urea	8 M
Guanidine	6 M
Triton X100	< 2 %
SDS	< 0.3 %
CHAPS	< 2 %
Tween	< 2 %
NaCl	0.3-2 M
EDTA	AVOID!
DTT, DTE	20 mM
Glutathione	20 mM
Mercaptoethanol	20 mM