

# Ni-NTA 6FF Prepacked Column

## 1 Contents

Cat. No.	Product Name	Package
HY-K0220-1 mL	Ni-NTA 6FF Prepacked Column	1 mL
HY-K0220-5 mL	Ni-NTA 6FF Prepacked Column	5 mL

## 2 Introduction

Ni-NTA His-Tag Purification Agarose, a 6% highly cross-linked agarose medium covalently coupled to a chelating agent that binds Ni<sup>2+</sup> by four coordination sites, enables high-yield, high-purity purification of polyhistidine-tagged proteins. Ni-NTA His-Tag Purification Agarose has low Ni<sup>2+</sup> leakage, high protein-binding capacity and stability, and is compatible with a wide range of chemicals and pH values. This makes Ni-NTA 6FF Prepacked Column ideal for high performance purification of polyhistidine-tagged proteins expressed in E.coli, yeast, insect and mammalian expression systems.

## 3 Characteristics

Composition	6% highly cross-linked agarose
Bead Diameter	45-165 µm
Binding Capacity	>40 mg 6× His-tagged Protein/mL Settled Resin
Maximum Pressure	0.3 MPa, 3 bar
Storage Solution	50% slurry in a 20% ethanol solution

## 4 General Protocol

### 1. Recommended Buffers

#### For native conditions

Lysis Buffer	1 L	50 mM NaH <sub>2</sub> PO <sub>4</sub> (7.80 g NaH <sub>2</sub> PO <sub>4</sub> • 2H <sub>2</sub> O); 300 mM NaCl (17.54 g NaCl); 10 mM imidazole (0.68 g imidazole); adjust pH to 8.0 with NaOH; filtered and sterilized using 0.22 µm or 0.45 µm filter
Wash Buffer	1 L	50 mM NaH <sub>2</sub> PO <sub>4</sub> (7.80 g NaH <sub>2</sub> PO <sub>4</sub> • 2H <sub>2</sub> O); 300 mM NaCl (17.54 g NaCl); 20 mM imidazole (1.36 g imidazole); adjust pH to 8.0 with NaOH; filtered and sterilized using 0.22 µm or 0.45 µm filter
Elution Buffer	1 L	50 mM NaH <sub>2</sub> PO <sub>4</sub> (7.80 g NaH <sub>2</sub> PO <sub>4</sub> • 2H <sub>2</sub> O); 300 mM NaCl (17.54 g NaCl); 250 mM imidazole (17.0 g imidazole); adjust pH to 8.0 with NaOH; filtered and sterilized using 0.22 µm or 0.45 µm filter

## For denaturing conditions

Lysis Buffer	1 L	8 M Urea (480.50 g Urea); 100 mM NaH <sub>2</sub> PO <sub>4</sub> (15.60 g NaH <sub>2</sub> PO <sub>4</sub> • 2H <sub>2</sub> O); 100 mM Tris • HCl (15.76 g Tris • HCl); adjust pH to 8.0 with HCl; filtered and sterilized using 0.22 µm or 0.45 µm filter
Wash Buffer	1 L	8 M Urea (480.50 g Urea); 100 mM NaH <sub>2</sub> PO <sub>4</sub> (15.60 g NaH <sub>2</sub> PO <sub>4</sub> • 2H <sub>2</sub> O); 100 mM Tris • HCl (15.76 g Tris • HCl); adjust pH to 6.3 with HCl; filtered and sterilized using 0.22 µm or 0.45 µm filter
Elution Buffer	1 L	8 M Urea (480.50 g Urea); 100 mM NaH <sub>2</sub> PO <sub>4</sub> (15.60 g NaH <sub>2</sub> PO <sub>4</sub> • 2H <sub>2</sub> O); 100 mM Tris • HCl (15.76 g Tris • HCl); adjust pH to 4.5 with HCl; filtered and sterilized using 0.22 µm or 0.45 µm filter

## 2. Sample preparation

### For protein expressed in E. coli or yeast cytoplasm

- (1) Harvest cells from culture medium by centrifugation at 7000 rpm for 15 minutes, remove and discard the supernatant.
- (2) Resuspend the cells in 1/10 volume of Lysis Buffer with appropriate amount of protease inhibitors added, which must have no effect on the binding capacity of agarose.
- (3) Sonicate the solution on ice. If the lysate is too viscous, add 10 µg/mL RNase A and 5 µg/mL DNase I and incubate on ice for 10-15 minutes.
- (4) Centrifuge the lysate at 10000 rpm for 20-30 minutes at 4°C. Transfer the supernatants to a new tube for further analysis or store at -20°C for future use.

### For soluble protein secreted by yeast, insect, or mammalian expression systems

- (1) Harvest cells from culture medium by centrifugation at 5000 rpm for 10 minutes, transfer the supernatant to a new tube. If the supernatant does not contain EDTA, histidine, or any other reducing agents, it can be applied to the column. Otherwise, perform the dialysis against 1× PBS at 4°C before applying it onto the column.
- (2) For large volume of supernatant, concentrate the proteins by ammonium sulphate precipitation, dialyze it against 1× PBS at 4°C before applying it onto the column.

### For protein of inclusion bodies (denaturing conditions)

- (1) Harvest cells from culture medium by centrifugation at 7000 rpm for 15 minutes, remove and discard the supernatant.
- (2) Resuspend the cells in 1/10 w/v volume of Lysis Buffer, sonicate the solution on ice.
- (3) Transfer the sonicated solution to a new tube, centrifuge at 10000 rpm for 20-30 minutes at 4°C. Remove and discard the supernatant.
- (4) Repeat step (2) & step (3) again.
- (5) Resuspend the inclusion bodies in 1/10 w/v of Lysis Buffer (with 8 M Urea contained), then it could be applied onto the column.

## 3. Purification

- (1) Fill the pump with ultrapure water and connect the Ni-NTA 6FF Prepacked Column to the chromatographic system.
  - (2) Wash the column of agarose with 3-5× bed volumes of ultrapure water.
  - (3) Equilibrate the column of agarose with 5× bed volumes of Lysis Buffer.
  - (4) Apply appropriate-sized sample to the column. If the sample contains insoluble matter, centrifuge or filter (0.22 µm or 0.45 µm filter) before use.
- Note: Binding capacity is flow rate- and protein-dependent. Higher flow rates will decrease production time, but may result in losing a small portion of the target protein.
- (5) Collect and save the flow-through for analysis.
  - (6) Wash the column with approximately 10-15× bed volumes of Wash Buffer or until the absorbance is stable. If desired, save supernatant for downstream analysis.
  - (7) Elute with approximately 5× bed volumes of Elution Buffer, collect fractions. With specific elution of protein with variant binding intensity, a range scale of Elution Buffer at different concentration is the optimal option.
  - (8) Analyze the target protein by SDS-PAGE, along with fractions collected from different steps if necessary.

## 4. Cleaning-in Place, CIP

- (1) To remove precipitated or denatured proteins and hydrophobic substances, wash the column with 5-10× bed volumes of 30% Isopropanol for 15-20 minutes of contact time followed by 10× bed volumes of ultrapure water washed.
- (2) Alternatively, wash the column with 2× bed volumes of acidic or basic solution with 1% nonionic detergent for 10-15 minutes of contact time followed by 5× bed volumes of 70% ethanol, with 10× bed volumes of ultrapure water washed finally.
- (3) To remove ionic binding proteins: wash the column with 5-10× bed volumes of 1.5 M NaCl for 10-15 minutes of contact time followed by 10× bed volumes of ultrapure water washed.
- (4) Store agarose in 20% ethanol at 2-8°C.

## 5. Regeneration

MCE Ni-NTA His-tag Purification Agarose can be used multiple times without protein yield or purity affected. If a decrease in performance is observed, the following regeneration procedures can be followed.

- (1) 2× bed volumes of 0.2 M acetic acid (with 6 M GuHCl contained).
- (2) 5× bed volumes of ultrapure water.
- (3) 3× bed volumes of 2% SDS.
- (4) 5× bed volumes of ultrapure water.
- (5) 5× bed volumes of anhydrous ethanol.
- (6) 5× bed volumes of ultrapure water.
- (7) 5× bed volumes of 100 mM EDTA (pH 8.0).
- (8) 5× bed volumes of ultrapure water.
- (9) 5× bed volumes of 100 mM NiSO<sub>4</sub>.
- (10) 10× bed volumes of ultrapure water.
- (11) Store agarose in 20% ethanol at 2-8°C.

## 5 Storage

Store at 4°C, and is stable for at least 2 years.

## 6 Precautions

1. Do not dry or freeze the agarose beads.
2. This product is for R&D use only, not for drug, household, or other uses.
3. For your safety and health, please wear a lab coat and disposable gloves to operate.

## 7 Troubleshooting

Problem	Possible Cause	Solution
Highly backpressure	Lysate was highly particulate	Centrifuge or filter (0.22 μm or 0.45 μm) before use. Sonicate the solution on ice; add 10 μg/ml RNase A and 5 μg/mL DNase I and incubate on ice for 10-15 minutes.
	Lysate was too viscous	Remove organic solvent or protein stabilizing reagent (glycerol); decrease flow rate.
Low protein yield	Poor expression of protein	Optimize expression conditions.
	His-tagged protein formed inclusion bodies	Alter growth conditions to minimize inclusion body formation; perform the purification for inclusion bodies with denaturing conditions.
	Not enough sample was loaded	Reapply the flow-through to the column.
	Column washing was too extensive	Reduce imidazole concentration in Wash Buffer.
	Protein degradation	Perform all procedure steps on 4°C; add some protease inhibitor.
Poor protein purity	Insufficient washing	Increase duration of wash.
	Existence of other His-tagged proteins in lysate	Modify imidazole concentration and pH of the Lysis Buffer or Wash Buffer.
	Nonspecific binding	Increase the salt or detergent concentration, or add ethanol or glycerol to decrease nonspecific interaction.
	Column was too dirty	Perform the "Clean-in Place" or/and "Regeneration".