MCE USA
Tel: 609-228-6898
Email: tech@MedChemExpress.com

# Ni-NTA His-Tag Purification Agarose

## 1 Contents

| Components | HY-K0210-5 mL | HY-K0210-10 mL | HY-K0210-50 mL | HY-K0210-250 mL |
|---|---|---|---|---|
| Ni-NTA His-Tag Purification Agarose (Settled Resin) | 5 mL | 10 mL | 50 mL | 50 mL × 5 |

## 2 Introduction

MCE Ni-NTA His-Tag Purification Agarose, a 6% highly cross-linked agarose reagent, enables high-yield, high-purity purification of polyhistidine-tagged proteins. The affinity chromatography purification reagent consists of nitrilotriacetic acid (NTA) chelator-activated agarose beads that subsequently charged with divalent nickel ($Ni^{2+}$) ions by four coordination sites.

MCE Ni-NTA His-Tag Purification Agarose has low $Ni^{2+}$ leakage, high protein-binding capacity and stability, and is compatible with a wide range of chemicals and pH values, making it 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 |
| Binding Capacity | >40 mg 6 × His-tagged Protein/mL agarose |
| Diameter | 45-165 μm |
| 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 | 1L | 50 mM $NaH_2PO_4$ (7.80 g $NaH_2PO_4 \cdot 2H_2O$); 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 | 1L | 50 mM $NaH_2PO_4$ (7.80 g $NaH_2PO_4 \cdot 2H_2O$); 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 | 1L | 50 mM $NaH_2PO_4$ (7.80 g $NaH_2PO_4 \cdot 2H_2O$); 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 | 1L | 8 M Urea (480.50 g Urea); 100 mM $NaH_2PO_4$ (15.60 g $NaH_2PO_4 \cdot 2H_2O$); 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 | 1L | 8 M Urea (480.50 g Urea); 100 mM $NaH_2PO_4$ (15.60 g $NaH_2PO_4 \cdot 2H_2O$); 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 | 1L | 8 M Urea (480.50 g Urea); 100 mM $NaH_2PO_4$ (15.60 g $NaH_2PO_4 \cdot 2H_2O$); 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 4ºC. Transfer the supernatants to a new tube for further analysis or store at -20ºC 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 4ºC 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 4ºC 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 4ºC. 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. Load of Ni-NTA His-Tag Purification Agarose:**

(1) Mix the slurry by gently inverting the bottle several times to completely suspend the agarose.

(2) Transfer an appropriate-sized slurry to the column and allow the agarose to settle down and the storage buffer to drain from the column.

(3) Equilibrate the column with 3× bed volumes of ultrapure water.

**4. Procedure:**

(1) Equilibrate the column of agarose with 5× bed volumes of Lysis Buffer.

(2) 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.

(3) Collect and save the flow-through for analysis.

(4) 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.

(5) 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.

(6) Analyze the target protein by SDS-PAGE, along with fractions collected from different steps if necessary.

**5. 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 2-8ºC.

**6. 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 $NiSO_4$.

(10) 10× bed volumes of ultrapure water.

(11) Store agarose in 20% ethanol at 2-8°C.

## 5 Storage

Store at 2-8°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, house hold, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

## 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. |
| | | Reduce amount of Wash Buffer used. |
| | Protein degradation | Perform all procedure steps on 4°C; add some protease inhibitor. |
| | Insufficient washing | Increase duration of wash. |
| Poor protein purity | 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”. |

## Appendix: Reagents Compatibility

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| Reducing agents | 5 mM DTE |
| | 1 mM DTT |
| | 20 mM β-mercaptoethanol |
| | 5 mM TCEP |
| | 10 mM reduced glutathione |
| Denaturants | 8 M Urea |
| | 6 M Gu-HCl |
| Detergents | 2% Triton X-100 (nonionic) |
| | 2% Tween 20 (nonionic) |
| | 2% NP-40 (nonionic) |
| | 2% Cholate (anionic) |
| | 1% CHAPS (zwitterionic) |
| Others | 500 mM imidazole |
| | 20% ethanol |
| | 50% glycerol |
| | 100 mM $Na_2SO_4$ |
| | 1.5 M NaCl |
| | 1 mM EDTA |
| | 60 mM citrate |
| Buffers | 50 mM sodium phosphate, pH 7.4 |
| | 100 mM Tris-HCl, pH 7.4 |
| | 100 mM Tris-acetate, pH 7.4 |
| | 100 mM HEPES, pH 7.4 |
| | 100 mM MOPS, pH 7.4 |
| | 100 mM sodium acetate, pH 4 |