HiPurA® Ni-NTA Protein Binding Magnetic Beads

Product Name	Product Code	Kit Packing
HiPurA® Ni-NTA Protein Binding Magnetic Beads	ML244-2ML	2 ml
	ML244-5ML	5 ml

Introduction:

Protein purification is an essential pre-requisite for proteomics. Protein purification procedures can vary from simple one-step precipitation procedures to large scale production processes. The most accepted of these methods is affinity purification where the protein of interest is purified through its specific binding properties to an immobilized ligand. Affinity tags are very efficient tools to purify heterologous proteins from different sources because the single step purification process involves mild elution conditions and do not interfere with the structure and function of the recombinant protein. For the purification of recombinant proteins additional amino acids or a whole protein is often added and is known as fusion tags. One of the most common fusion tag is 6xHis or polyHis tag (six histidine residues) which binds to nickel. Polyhistidine-tag is an amino acid motif in proteins that consists of at least six histidine (His) residues, often at the N- or C-terminus of the protein. Polyhistidinetags are often used for affinity purification of Polyhistidine-tagged recombinant proteins expressed in *Escherichia coli*. Affinity purification using a polyhistidine-tag usually results in relatively pure protein when the recombinant protein is expressed in prokaryotic organisms. Expressed His-tagged proteins can be purified and detected easily because the histidine residues bind metal ions like nickel immobilized on a column matrix under specific buffer conditions and bound proteins are eluted out by changing the pH or by adding a competitive molecule like imidazole.
Ni-NTA based magnetic beads or agarose resins uses nitrilotriacetic acid (NTA), a tetradentate chelating ligand. NTA binds Ni2+ ions by four coordination sites.

Description:

HiPurA® Ni-NTA Protein Binding Magnetic Beads are designed for efficient and rapid purification of 6XHis-Tagged proteins. These beads have nitrile-triacetic acid (NTA) groups with charged nickel covalently bond to the surface of magnetic beads. These beads having a high affinity for 6xHis-tagged proteins, under magnetic conditions captures the 6xHis-tagged proteins facilitating the remove of other proteins/ impurities from the crude lysate. The captured his-tagged proteins can be eluted off the magnetic beads by changing the pH or by adding a competitive molecule like imidazole for further use in downstream applications.
As compared to the traditional agarose resins, these beads are faster, easier, and more efficient for purifying the proteins because of the four metal-binding chelation sites.

ISO 9001-2015 CERTIFIED
WHO GMP CERTIFIED
ISO 13485-2016 CERTIFIED

Registered Office
HiMedia Laboratories Pvt Ltd.
Plot No. C-40, Road No. 21Y, MIDC, Wagle Industrial Area,
Thane, (West) 400604, Maharashtra, INDIA.
Customer Care No.: 00-91-22-6116 9797
Tel : 00-91-22-6147 1919, 6903 4800
Fax : 6147 1920
Web : www.himedialabs.com
Email : info@himedialabs.com
mb@himedialabs.com

These beads are compatible with native or denaturing conditions and have been optimized for use in manual process with a magnetic stand, or automated process with an instrument.
HiPurA® Ni-NTA Protein Binding Magnetic Beads are suspended in 20% ethanol and has a binding capacity of ~5mg His-tagged protein per ml.

Application:

HiPurA® Ni-NTA Protein Binding Magnetic Beads can be used for effective magnetic based purification of His-Tagged protein from the crude lysate.

Properties:

- Mean Bead Diameter: Spherical, 1.1~1.4 µm
- Ligand: Nitilotriacetic acid (NTA)
- Binding Capacity: ~ 5.0 to 5.2 mg His-tagged protein per mL
- Bead Concentration: ~50 mg/mL in 20% ethanol
- Density: 1.0 g/cm^3 at 20°C (68°F).

Material required but not provided:

1. For manual purification: Magnetic Stand (Recommended Product Code: MBLA001).
2. For automated purification: Insta NX® Mag24 (Product code: LA1120).
3. 1.5 ml Micro Centrifuge Tube (Recommended Product Code: PW146).
4. Gel Rocker/ End-over end shaker.
5. Micro-Pipettes and tips.
6. 5 ml or 10 ml cartridge and combs for Insta NX® Mag24.
7. Vertical Electrophoresis Apparatus (For PAGE analysis, Recommended Product Code: LA1070).

Recommended buffers (For native conditions):

1. Equilibration (Recommended: 100 mM sodium phosphate, 300 mM sodium chloride, 0.05% Tween®-20, 30 mM imidazole) *
2. Binding/ Wash Buffer (Recommended: 100 mM sodium phosphate, 300 mM sodium chloride, 0.05% Tween®-20, 50 mM imidazole) *
3. Elution Buffer (Recommend: 100 mM sodium phosphate, 300 mM sodium chloride, 250 mM imidazole) *

***Note:** The buffers mentioned are for recommendations. For better yield and lower nonspecific binding, imidazole concentration and/or salt concentration can be changed required for specific proteins.

Procedure

A. Manual Purification of the His-Tagged proteins:

Sample Preparation: Prepare the protein sample by mixing the Crude lysate with equal volume of Equilibration buffer (For Example, Mix 100 µl of Crude lysate with 100 µl of Equilibration buffer).

1. **Mixing and equilibration of the magnetic beads:**
    - Transfer 40 µl of HiPurA™ Ni-NTA Protein Binding Magnetic Beads in 1.5 ml of microcentrifuge tube. Add 160 µl of Equilibration buffer to beads and resuspend the beads by pipetting or vortexing for up to 10 seconds.
    - Place the tube on magnetic stand for 30-60 seconds to immobilize the beads at the tube wall. Carefully discard the supernatant and remove the tube from the magnetic stand.
    - Add 400 µl of Equilibration buffer to the beads and mix by pipetting or vortexing for 15 second and then place the tube on magnetic stand. Discard the supernatant and remove the tube from the magnetic stand. The beads are ready for purification of His-tagged proteins.
2. **Sample Binding:**
    - Add 400 µl of prepared protein sample to tube with magnetic beads, mix by pipetting or vortex for 10 second. Incubate the tube with beads on gel rocker or end over end shaker for 30 minutes at room temperature for binding.
    - Place the tube on the magnetic stand for 30-60 seconds to immobilize the beads at the tube wall. Carefully collect or discard the supernatant and then remove the tube from magnetic stand.

Note: It is recommended to collect and store the clear supernatant as Flow through for further analysis.

3. **Washing Step:**
    - Add 400 µl of wash buffer and resuspend the beads by pipetting or vortexing for 10 seconds.
    - Place the tube on the magnetic stand for 30 – 60 seconds to immobilize the beads on the tube wall and collect or discard the supernatant.
    - Repeat the wash step once for a total of two washes.

Note: The wash supernatant can be collected and stored for further analysis.

4. **Elution of purified protein:**
    - Add 50 µl* of Elution Buffer to the tube with beads and resuspend by pipetting and vortexing for 10 second. Ensure that all the beads are submerged in the elution buffer.
    - Incubate the beads with elution buffer for 15 minutes on gel rocker or end over end shaker for 15 minutes at room temperature.

- Place the tube on the magnetic stand for 30 – 60 seconds to immobilize the beads on the tube wall and carefully collect the supernatant containing the His-Tagged protein. **
- If required repeat the elution step once for a total of two eluates. ***

Note:

*Elution buffer volume can be varied depending upon the protein concentration of the crude lysate.

**If the beads are stuck on the wall prior to collecting the eluate, short spin the tube with beads for collecting beads at end of the tube, then place the tube on magnetic stand.

***The first eluate from the elution step contains majority of the purified His-tagged protein.

The eluates along with the flow through and washes can be directly analyzed by SDS-PAGE or the protein content of the eluates can be by BCA Assay, Bradford assay.

B. Procedure for Automated Purification of the His-Tagged proteins:

Note: The following protocol is optimized for use with the Insta NX® Mag24. The protocol can be changed according to your needs.

1. Set up the assay as the below table:

Step No.	Well No.	Well Name	Content	Volume	Time and Speed
1	2	Equilibration 1	Equilibration buffer	40 µl of beads + 160 µl Equilibration buffer of equilibration buffer	30 seconds/Medium
2	3	Equilibration 2	Beads in equilibration buffer	400 µl Equilibration buffer	30 seconds/Medium
3	1	Sample Binding	Sample prepared in equilibration buffer	400 µl	30 minutes/Slow
4	4	Washing 1	Wash buffer	400 µl Wash buffer	15 seconds/Slow
5	5	Washing 2	Wash buffer	400 µl Wash buffer	15 seconds/Slow
6	6	Elution 1	Elution buffer	100 µl of Elution buffer	15 minutes/Medium
7	7	Elution 2	Elution buffer	100 µl of Elution buffer	10 minutes/Medium

2. Fill the cartridge wells as per the above table.
3. Setup the protocol on Insta NX® Mag24 machine and load the cartridge in the machine.
4. Press run to start the purification assay.
5. After the completion of run, collect the flow through, wash and eluates in a press tube for further down streaming.

When scaling up the assay, adjust the volumes of Equilibration, Wash, and adjust Elution Buffer as per volume of magnetic beads slurry.

Trouble Shooting:

Troubles	Solutions
Low protein yield	1. Optimize the incubation time and temperature depending on the sample. 2. Optimize the concentration of imidazole in the elution buffer. 3. Check for the pH and composition of the all buffers. 4. Thoroughly suspend the beads during binding and elution steps.
Protein is degraded during purification	1. Use protease inhibitor cocktail (Recommended product Code: ML051) in all the buffers used for purification.
The beads are adhering on the tip or tube	1. Increase Tween 20 concentration in equilibration/ wash buffer. 2. Decrease salt concentration in equilibration/ wash buffer.
Immobilization of the beads is hard on the magnetic stand	1. Make Sure the beads are in direct contact with the magnet.
Protein cannot be quantified using Bradford or BCA assay	1. Imidazole in elution buffer may interfere with assay. Either dilute the samples or dialyze to the optimal imidazole concentration of protein quantification reagent used. 2. Check if the beads are in the eluates which may interfere.

Storage conditions:

HiPurA® Ni-NTA Protein Binding Magnetic Beads has to be stored at 2-8°C. Under recommended condition, the beads are stable for 6 months.

Warning and Precautions

Not for Medicinal Use. Read the SDS carefully before beginning the protocol. Wear protective gloves/protective clothing/eye protection/face protection. Follow good clinical laboratory practices while handling clinical samples. Standard precautions should be followed as per established guidelines. Safety guidelines may be referred in safety data sheets of the product.

Performance and Evaluation

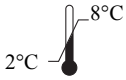
Performance of the solution is expected when the solution is stored at recommended temperature and within the expiry period.

Safety Information

HiPurA® Ni-NTA Protein Binding Magnetic Beads is for laboratory use only, not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves and safety goggles when handling. Not compatible with disinfecting agents containing bleach. Please refer the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.

Technical Assistance

At HiMedia we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance, mail at mb@himedialabs.com.



Storage temperature

Do not use if package is damaged

HiMedia Laboratories Private Limited,
Reg. Off: Plot No. C-40, Road No. 21Y,
MIDC, Wagle Industrial Area, Thane,
(West) 400604, Maharashtra, INDIA.
Web: www.himedialabs.com

05/2027

PIML244_0/0524 ML244-00