HiPurA® Mag24 His-tagged Protein Purification Kit (Magnetic Bead Based)

Product Name	Product Code	Kit Packing
HiPurA® Mag24 His-tagged Protein Purification Kit (Magnetic Bead Based)	MBPP001M-5PR	5PR

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 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® Mag24 His-tagged Protein Purification Kit (Magnetic Bead Based) is designed for efficient and rapid purification of 6XHis-Tagged proteins using magnetic beads using automated system. 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.

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Application:

HiPurA® Mag24 His-tagged Protein Purification Kit (Magnetic Bead Based) is to be used for effective magnetic based purification of His-Tagged protein from the recombinant bacterial sample.

Kit Content:

Sr. No.	Product Code	Materials Provided	Quantity	Storage
			5 PR	
1	ML244	HiPurA® Ni-NTA Protein Binding Magnetic Beads	0.5 ml	2-8 °C
2	DS1892	Equilibration/ Wash Buffer	12 ml	2-8 °C
3	DS1893	Elution Buffer	6 ml	2-8 °C
4	DS0114	3M Imidazole	2 ml	2-8 °C
5	DS1864	5ml Cartridge for Insta NX® Mag24	5 No	RT
6	DS2813	10ml Cartridge for Insta NX® Mag24	1 No	RT
7	DS1896	Magnetic Rod Tip for Insta NX® Mag24	2 No	RT
8	DBCA016	Collection Tubes (Uncapped)	5 No	RT

NOTE: This kit allows for 5 experimental runs using 400 µl initial sample volume and 1 run using 5 ml & 10 ml sample volume respectively.

Sample Volume	Cartridge Code to be used	Number of samples that can be processed per 5PR kit
400 µl	DS1864	5
1 ml	DS1864	3
5 ml	DS1864	1
10 ml	DS2813	1

General Preparation and Instructions:

1. Imidazole needs to be added to the Equilibration/ Wash Buffer and Elution buffer to prepare Equilibration Buffer, Wash Buffer and Elution Buffer respectively. (Buffers may need some optimization depending upon the specific protein).

The following table can be referred for preparing buffers with different imidazole concentrations (The mentioned recipe is for 10 ml total volume of the buffers):

Imidazole Final Conc. (mM)	Equilibration/ Wash Buffer/ Elution Buffer (ml)	3M Imidazole (µl)
10	9.97	33.3
20	9.93	66.6
25	9.92	83.3
30	9.90	100
40	9.87	133.3
50	9.83	166.6
60	9.80	200
75	9.75	250
150	9.50	500

200	9.33	667
250	9.17	834
350	8.83	1166
500	8.33	1668

For most proteins the following imidazole concentration is recommended:
Equilibration Buffer - 30mM imidazole
Wash Buffer - 50mM imidazole
Elution Buffer - 250mM 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.

2. Dilute the crude samples (1:1) with equilibration buffer to maintain the proper pH and ionic strength for optimal binding.
3. Vortex or mix the beads evenly prior to use.
4. The amount and quality of protein produced depend on factors like protein expression level, conformation, and solubility characteristics. To get the best results, small scale purification can be performed.
5. Do not freeze the magnetic beads and keep away from magnet.

Material required but not provided:

1. Insta NX® Mag24 (Product code: MBLA020)
2. 1.5 ml or 2.0 ml Micro Centrifuge Tube (Recommended Product Code: PW146 or MBLA017).
3. Micro-Pipettes and tips.
4. Vertical Electrophoresis Apparatus (For PAGE analysis, Recommended Product Code: LA1070).
5. Components for casting of PAGE gels.

Procedure:

1. Set up the assay as the below table depending upon the sample volume.

- **Set up for 400 µl sample volume**:

Step No.	Well No.	Well Name	Content	Volume
1	2	Equilibration	Equilibration buffer	40 µl of beads + 360 µl Equilibration buffer of equilibration buffer
2	1	Sample Binding	Sample prepared in equilibration buffer	400 µl of sample
3	3	Washing 1	Wash buffer	400 µl Wash buffer
4	4	Washing 2	Wash buffer	400 µl Wash buffer
5	5	Elution 1	Elution buffer	100 µl of Elution buffer
6	6	Elution 2	Elution buffer	100 µl of Elution buffer

- **Set up for 1 ML sample volume:**

Step No.	Well No.	Well Name	Content	Volume
1	2	Equilibration	Beads in equilibration buffer	900 µl Equilibration buffer + 100 µl of magnetic beads
2	1	Sample Binding	Sample prepared in equilibration buffer	1000 µl
3	3	Washing 1	Wash buffer	1000 µl Wash buffer
4	4	Washing 2	Wash buffer	1000 µl Wash buffer
5	5	Elution 1	Elution buffer	250 µl of Elution buffer
6	6	Elution 2	Elution buffer	250 µl of Elution buffer

- **Set up for 5 ML sample volume:**

Step No.	Well No.	Well Name	Content	Volume
1	2	Equilibration 1	Equilibration buffer	400 µl of beads + 600 µl Equilibration buffer of equilibration buffer
2	1	Sample Binding	Sample prepared in equilibration buffer	5000 µl sample
3	3	Washing 1	Wash buffer	1000 µl Wash buffer
4	4	Washing 2	Wash buffer	1000 µl Wash buffer
5	5	Elution 1	Elution buffer	1000µl of Elution buffer
6	6	Elution 2	Elution buffer	1000 µl of Elution buffer
7	7	Elution 3	Elution buffer	1000 µl of Elution buffer
8	8	Elution 4	Elution buffer	1000 µl of Elution buffer

- **Set up for 10 ML sample volume:**

Step No.	Well No.	Well Name	Content	Volume
1	2	Equilibration 1	Equilibration buffer	400 µl of beads + 600 µl Equilibration buffer of equilibration buffer
2	1	Sample Binding	Sample prepared in equilibration buffer	10,000 µl sample
3	3	Washing 1	Wash buffer	1000 µl Wash buffer
4	4	Washing 2	Wash buffer	1000 µl Wash buffer
5	5	Elution 1	Elution buffer	1000 µl of Elution buffer
6	6	Elution 2	Elution buffer	1000 µl of Elution buffer

2. Setup the protocol on Insta NX® Mag24 machine and load the cartridge in the machine.
3. Press run to start the purification assay.
4. After the completion of run, collect the flow through, wash and eluates in a collection tube for further analysis and down streaming.

   Note: If any leftover magnetic beads are observed in any of the collected fractions, use a magnetic stand to separate out the beads and re-collect the fraction.

When scaling up the assay, adjust the volumes of Magnetic Beads, Equilibration, Wash, and Elution Buffer as per volume of the sample.

Note: The protein of interest is usually visible on a gel or detectable by UV/assay for a limited number of the collected eluate fractions. The intensity of your protein typically decreases with each subsequent eluate; the highest concentration is often found in the first, second or third elution fraction, depending on the initially protein concertation in the crude lysate.

***Software protocol name in Insta NX® Mag24 for His-tagged protein purification:**

1. For 400 µl sample: MAG24NiNTA400uL
2. For 1 ML sample: MAG24NiNTA1ML
3. For 5 ML sample: MAG24NiNTA5ML
4. For 10 ML sample: MAG24NiNTA10ML

Trouble Shooting

Troubles	Solutions
Low protein yield	1. Optimize the concentration of imidazole in the elution buffer. 2. Check for the pH and composition of the all buffers.
No protein is present in the eluate	1. Check the protein's expression level before purification by a fraction of the crude lysate on an SDS-PAGE gel and perform a western analysis with a primary antibody targeting your affinity tag. 2. Check the DNA construct sequence and that the protein coding region is in frame.
Protein is degraded during purification	1. Use protease inhibitors in all the buffers used for purification as well as lysis buffer.
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.
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.

***Note: The presence of beads exclusively at the rim and tips of the tube, with no contamination of the eluted protein well, will not compromise the purity or yield of the protein. Carefully transfer the eluted fraction to a new tube**

Storage conditions:

HiPurA® Mag24 His-tagged Protein Purification Kit (Magnetic Bead Based) has to be stored at 2-8°C. Under recommended condition, the kit is stable for 6 months.
Do not freeze the beads provided in the kit and keep away from magnet.

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 kit is expected when the kit is stored at recommended temperature and within the expiry period.

Safety Information

HiPurA® Mag24 His-tagged Protein Purification Kit (Magnetic Bead Based) 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.

Symbol

	Manufacturer		Do not use if package is damaged
	Batch code		Temperature limit
	Date of manufacture (YYYY-MM)		Consult instructions for use
	Use-by date (YYYY-MM)		Catalogue number

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