HiPurA® Mag32 His-tagged Protein Purification Kit (Magnetic Bead Based)

Product Name	Product Code	Kit Packing
HiPurA® Mag32 His-tagged Protein Purification Kit (Magnetic Bead Based)	MBPP003M-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® Mag32 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 removal 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.
HiPurA® Ni-NTA Protein Binding Magnetic Beads provided in the kit are suspended in 20% ethanol and has a binding capacity of ~5mg His-tagged protein per ml.

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

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Application:

HiPurA® Mag32 His-tagged Protein Purification Kit (Magnetic Bead Based) can be used for effective magnetic based purification of His-Tagged protein from the crude lysate.

Properties of HiPurA® Ni-NTA Protein Binding Magnetic Beads:

- 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.

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	10 ml	2-8 °C
3	DS1893	Elution Buffer	2 ml	2-8 °C
4	DS0114	3M Imidazole	1 ml	2-8 °C
5	DS1913	Cartridge for Insta NX® Mag32	5 No	RT
6	DS1914	Magnetic Rod Tip for Insta NX® Mag32	2 No	RT

NOTE: This kit allows for 5 experimental runs using 400 µl initial sample volume and 2 runs using 1 ml sample volume.

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 used to make buffers with different imidazole concentrations:

Table 2:

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. 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.
4. Do not freeze the magnetic beads and keep away from magnet.

Material required but not provided:

1. Insta NX® Mag32 (Product code: LA1096) and Insta NX® Mag32^{Plus} (Product code: MBLA019)
2. 1.5 ml or 2.0 ml Micro Centrifuge Tube (Recommended Product Code: PW146 or MBLA017).
3. Gel Rocker/ End-over end shaker.
4. Micro-Pipettes and tips.
5. Vertical Electrophoresis Apparatus (For PAGE analysis, Recommended Product Code: LA1070).
6. Components for casting of PAGE gels.

Procedure:
The following protocol is optimized for use with the Insta NX® Mag32 for a minimum volume of 400 µl sample and maximum volume of 1000 µl sample.

1. Set up the assay as the below table (**For 400 µl sample**):

Step No.	Well No.	Well Name	Content	Volume	Time and Speed
1	1	Equilibration	Beads in equilibration buffer	360 µl Equilibration buffer + 40 µl of magnetic beads	30 seconds/Medium
2	2	Sample Binding	Sample prepared in equilibration buffer	400 µl	30 minutes/Slow
3	3	Washing 1	Wash buffer	400 µl Wash buffer	15 seconds/Slow
4	4	Washing 2	Wash buffer	400 µl Wash buffer	15 seconds/Slow
5	5	Elution 1	Elution buffer	100 µl of Elution buffer	15 minutes/Medium
6	6	Elution 2	Elution buffer	100 µl of Elution buffer	10 minutes/Medium

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

Step No.	Well No.	Well Name	Content	Volume	Time and Speed
1	1	Equilibration	Beads in equilibration buffer	900 µl Equilibration buffer + 100 µl of magnetic beads	30 seconds/Medium
2	2	Sample Binding	Sample prepared in equilibration buffer	1000 µl	40 minutes/Slow
3	3	Washing 1	Wash buffer	1000 µl Wash buffer	15 seconds/Slow
4	4	Washing 2	Wash buffer	1000 µl Wash buffer	15 seconds/Slow
5	5	Elution 1	Elution buffer	250 µl of Elution buffer	15 minutes/Medium
6	6	Elution 2	Elution buffer	250 µl of Elution buffer	10 minutes/Medium

2. Fill the cartridge wells as per the above table.
3. Setup the protocol on Insta NX® Mag32 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 collection tube for further analysis and down streaming.

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

***Software protocol name in 1. Insta NX® Mag32/ Insta NX® Mag32^{Plus} for His-tagged protein purification:**

1. For 400 µl sample: Mag32NiNTA400

2. For 1 ML sample: Mag32NiNTA1ML

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.
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® Mag32 His-tagged Protein Purification Kit (Magnetic Bead Based) has to be stored at 2-8^{o}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® Mag32 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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