His-tag Protein Purification Magnetic Particles, 30-150 μm-IDA-Ni
PRODUCT DATA SHEET

His-tag Protein Purification Magnetic Particles, 30-150 μm-IDA-Ni

Cat No: AM-His-3000-IDA-Ni

Description

Abvigen His-tag protein purification beads posses superparamagnetism, it is a new functional material designed for efficient and rapid purification of His-tag protein. It can extract the target protein with high purity from the biological samples in one step using magnetic separation method and it greatly simplifies the purification process and improves the efficiency of purification. This method is suitable for scientific research and industrial areas to facilitate the purification of his-tag protein.

Compared with the traditional tomographic method which use metal chelate agarose prepacked column, His-tag Protein Purification beads do not need to carry out high-speed centrifugation and filter filtration on the crude protein samples for a long time, or control the flow rate, and do not need the expensive chromatography equipment. The specific binding of the sample to the magnetic beads, following washing and target protein elution become very simple, fast and easy to operate. For skilled operators, highly purified protein can be obtained within 1 h, and parallel processing of high throughput and large amount samples can be easily achieved to save researchers time and cost.

The His-tag product line includes two metal ion chelate beads consist of Nickel and Cobalt, they have different properties at combining ability of target proteins and nonspecific adsorption. In addition, users can choose different metal ion chelate beads from different purpose.

Characteristics

Magnetic bead size range	30 ~ 150 μm
Chelating metal ions	Ni^{2+}
Metal ion density	30 ~ 50 μmol/mL magnetic beads
Protein binding capacity	30 ~ 40 mg/mL (100% magnetic beads)
Operating temperature	2 ~ 30°C
Suspension concentration	10% (v/v) magnetic beads suspension
Preservative solution	Pure Water (recommend to swtich to 20% V/V ethanol solution)

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Quality guarantee period	Stable storage at 2 ~ 8°C, shelf life of 2 years (can be stored or transported for a short time at room temperature)

Note 1: The binding amount of magnetic globin is related to the characteristics of the target protein and is only used as a reference value

Note 2: 1 mL magnetic bead suspension contains 100 μL magnetic beads

Note 3: Please refer to the appendix for information on solvent tolerance of magnetic beads

Scope of Application

It is suitable for the purification of soluble histidine label proteins secreted or expressed in bacteria, yeast, insects and mammalian cells, and can also be used for the purification of denaturated proteins (inclusion bodies need to be purified after denaturation).

Operation Process

1. Buffer preparation

The binding properties of the target protein and the magnetic beads will directly affect the purification efficiency of the target protein, and the preparation of various buffers will also affect the recovery and purity of the target protein to a certain extent. Therefore, before large-scale protein purification, users should design their own experiments to screen out Buffer liquid systems suitable for the purification of target proteins, including Binding Buffer, Washing Buffer and Elution Buffer.

Increasing the concentration of imidazole is the most common elution method for histidine label purification magnetic beads. When the optimal concentration of imidazole is uncertain, it is recommended to add 10 mM, 20 mM, 50 mM, 100 mM, 200 mM, 300 mM, 400 mM, 500 mM to the buffer solution for the first time. Elution was performed from low concentration to high concentration. After magnetic absorption, protein supernatant was collected and the elution results were identified by SDS-PAGE electrophoresis.

The buffer liquid provided below is suitable for the purification of most histidine label proteins for user reference.

Binding Buffer: 20 mM Phosphate Buffer, 500 mM NaCl, 5 ~ 50 mM Imidazole, pH 7.4

Washing Buffer: 20 mM Phosphate Buffer, 500 mM NaCl, 50 ~ 100 mM Imidazole, pH 7.4

Elution Buffer: 20 mM Phosphate Buffer, 500 mM NaCl, 500 mM Imidazole, pH 7.4

2. Sample treatment

This User's Manual provides the following three sample handling methods:

(1) Protein expressed in E. coli, yeast and other cells: the expressed cells were diluted with an appropriate amount of Binding Buffer, added with protease inhibitors (such as PMSF with a final concentration of 1 mM), then the cells were suspended, and the cells were lysed by ice bath ultrasound to become crude protein samples. If the sample is too thick, an appropriate amount of nuclease can be added to the crude sample as needed, and the ice bath is 30 min to degrade the nucleic acid. In addition, the user can also centrifuge the protein sample according to the actual needs.

(2) Extracellular expression protein: extracellular expression supernatant was taken and diluted with an equal amount of Binding Buffer to form a crude protein sample.

(3) Intracellular expression of protein in animal cells: Appropriate amount of animal cells were taken, washed once with appropriate amount of PBS, and the supernatant was discarded; The crude protein samples were then suspended with a Binding Buffer containing 1% (V/V) Triton X-100 or 1% (V/V) NP-40, added with protease inhibitors (such as PMSF with a final concentration of 1 mM), and bathed in ice for 10 min.

3. Magnetic bead pretreatment

In general, the amount of magnetic beads used is calculated by the user based on the target protein yield and magnetic bead load information. For example, Escherichia coli is used to express a target protein, and 2 g wet weight bacteria are harvested from 500 mL fermentation solution. The target protein yield is estimated to be 10 ~ 20 mg through pre-experiment, and the user needs to take 5 mL magnetic bead suspension for the purification of the target protein. The following is an example to explain in detail:

(1) The magnetic bead product was thoroughly mixed on the vortex mixer, and 5 mL magnetic bead suspension was taken by pipette into 15 mL centrifugal tube for magnetic separation*. The superliquid was discarded and the centrifugal tube was removed from the magnetic separator.

(2) Add 5 mL Binding Buffer into the centrifuge tube equipped with magnetic beads, and turn the centrifuge tube up and down several times to re-suspend the magnetic beads; Magnetic separation is performed and the supernatant is removed. Repeat washing 2 times.

(Note*: In the process of magnetic separation, in order to reduce the loss of magnetic beads during use, after the solution is clarified, close the centrifugal tube cover, keep the centrifugal tube still on the magnetic separator, and turn the magnetic separator and the centrifugal tube up and down several times, so that the clarified solution is boiled and the residual magnetic beads on the centrifugal tube cover are left to stand for a moment, so that the solution is re-clarified; Same as below.

4. The target protein binds to the magnetic bead

(1) The bacteria with 2 g wet weight were suspended with 10 mL Binding Buffer, and after crushing and cracking, the target crude protein samples were added into a centrifuge tube equipped with pre-treated magnetic beads, and the centrifuge tube was placed in a vortex mixer for 15 s for oscillation.

(2) Place the centrifuge tube on the rotary mixer and rotate it for 20 ~ 30 min at room temperature (if necessary, rotate it for 1 h at a low temperature of 2-8°C to prevent degradation of the target protein).

(3) Place the centrifugal tube on the magnetic separator for magnetic separation, remove the supernatant to the new centrifugal tube for subsequent detection, and remove the centrifugal tube from the magnetic separator for subsequent washing steps.

5. Magnetic bead washing

(1) Add 10 mL Washing Buffer into the centrifuge tube equipped with magnetic beads, gently turn the centrifuge tube several times to make the magnetic beads re-suspended, magnetic separation, and remove the washing solution into the new centrifuge tube for sampling and testing. Repeat this step once.

(2) Add 10 mL Washing Buffer into the centrifuge tube fitted with magnetic beads to re-suspend the magnetic beads, transfer the suspension of magnetic beads to the new centrifuge tube (to avoid non-specific adsorption of proteins on the wall of the original centrifuge tube to contaminate the target proteins), magnetically separate, and remove the supernatant from the cleaning solution collection tube.

6. Target protein elution

(1) The user can adjust the concentration of the target protein by changing the Elution volume as required, adding 2~10 mL Elution Buffer, gently turning the centrifuge tube several times to make the magnetic beads suspended and magnetic separation, and collecting the eluent into the new centrifuge tube, that is, the purified target protein sample.

(2) If necessary, the above steps can be repeated once to collect the sample into a new centrifuge tube to test whether the target protein is completely eluted.

7. Post-processing of magnetic beads

(1) Add 5 mL Elution Buffer into the centrifugal tube equipped with magnetic beads, turn the centrifugal tube up and down several times, so that the magnetic beads are suspended, magnetic separation, and the supernatant is removed.

(2) Repeat the above steps two times.

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(3) Add 5 mL ddH_2O to the centrifuge tube, turn the centrifuge tube up and down several times, so that the magnetic beads are suspended, magnetic separation, and the supernatant is removed.

(4) Repeat the above steps two times.

(5) Add Storage Buffer to the magnetic bead so that the total volume is 5 mL, and store it at 2-30°C (long-term storage at 2-8°C), which can be used for the next purification of the same protein.

8. Regeneration of magnetic beads

If the magnetic bead is used more than three times continuously, its ability to bind the target protein may be significantly reduced, and it is recommended to carry out magnetic bead regeneration treatment.

Stripping Buffer: 20 mM Sodium Phosphate, 500 mM NaCl, 100 mM EDTA, pH 7.4

Beads Washing Buffer (optional): 0.5 M NaOH, 2 M NaCl

Recharge Buffer: 100 mM $NiSO_4/CoCl_2$ (This chemical has certain toxicity and may cause allergic reactions, please pay attention when using it)

Storage Buffer: 20% (V/V) ethanol

Taking 5 mL 10% (V/V) magnetic bead suspension as an example, the magnetic bead regeneration operation is explained in detail:

(1) Magnetic separation of the magnetic bead suspension, removal of the supernatant, removal of the centrifuge tube from the magnetic separator, adding 5 mL **ddH_2O** to the centrifuge tube, turning the centrifuge tube up and down several times, so that the magnetic bead re-suspension, magnetic separation, removal of the supernatant.

(2) Add 5 mL **Stripping Buffer**, turn the centrifuge tube up and down several times, re-suspend the magnetic beads, rotate at room temperature for 5 min, magnetic separation, and remove the supernatant. Repeat this step once.

(3) Add 5 mL **ddH_2O**, turn the centrifugal tube up and down several times, so that the magnetic beads are re-suspended, magnetic separation, remove the supernatant, repeat this step twice.

(4) Alkali treatment: Add 5 mL **Beads Washing Buffer**, turn the centrifuge tube up and down several times to re-suspend the magnetic beads, rotate and mix at room temperature for 5 min, magnetic separation, and remove the supernatant. Add 5 mL **ddH_2O** and turn the centrifugal tube up and down several times to re-suspend the magnetic beads, magnetic separation, and remove the supernatant. Repeat the **ddH_2O** washing step 3~5 times until the washing solution is neutral.

(5) Add 5 mL **Recharge Buffer**, turn the centrifuge tube up and down several times to re-suspend the magnetic beads, rotate and mix at room temperature for 20 min, magnetic separation, and remove the supernatant.

(6) Add 5 mL **ddH_2O** and turn the centrifugal tube up and down several times to re-suspend the magnetic beads, magnetic separation, and remove the supernatant. Repeat this step more than 4 times to ensure complete removal of nickel ions.

(7) Add **Storage Buffer** to the magnetic bead so that the total volume is 5 mL, stored at 2~30°C (long-term storage, placed at 2~8°C).

Optimization of Protein Purification Process

The above operation process is suitable for the purification of most of the histidine label proteins. According to the different binding properties of the target protein and metal ion chelated magnetic beads, users can optimize the purification process from the following aspects to improve the recovery and purity of the target protein.

Reference methods to improve the recovery rate of target protein:

(1) Reduce the concentration of Imidazole in the sample solution and Binding Buffer;

(2) Add surfactants and other substances to the sample solution and other buffers;

(3) Add appropriate protease inhibitors to prevent target protein degradation;

(4) Increase the amount of magnetic beads;

(5) Prolong the incubation time of protein and magnetic beads;

(6) Prolong the elution time of the target protein or increase the elution frequency.

Reference methods to improve the purity of the target protein:

(1) Increase the concentrations of Imidazole and NaCl in sample solution and Binding Buffer;

(2) Add surfactants and other substances to the sample and buffer;

(3) Add appropriate protease inhibitors to prevent target protein degradation;

(4) Extend the washing time and increase the number of washes;

(5) Use gradient Imidazole concentration to elute target proteins.

Notes

1. Please read this user manual carefully before using this product for the first time;

2. Freezing, drying and high-speed centrifugation should be avoided during the use and preservation of magnetic beads;

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3. Before using this product, be sure to fully oscillate to keep the magnetic beads in uniform suspension;

4. Please choose a good quality pipette head and centrifugal tube to avoid leakage of magnetic beads during the wall attachment or mixing process;

5. During the mixing process of magnetic beads and solution, if the solution is thick and cannot be re-suspended by turning over the centrifugal tube, the pipette can be used to repeatedly blowing or short-term vortex mixing to fully re-suspended magnetic beads;

6. Users can retain the supernatant removed by magnetic separation according to actual needs for sampling and testing, in order to analyze the purification process and optimize the protein purification process;

7. This product can be reused, when the purification performance is reduced, it is recommended to be recycled;

8. When used magnetic beads are reused, it is recommended to purify the same protein, and when purifying different kinds of proteins, it is recommended to use new magnetic beads;

9. This product should be used with a magnetic separator;

10. This product is for research use only.

Appendix Information

Solvent tolerance of magnetic beads

Solvent type	Solvent name	Tolerable concentration	Note
Reducing agent	DTE	5 mM	Before using reducing agent, wash the magnetic beads with a non-reducing agent solution. The use of reducing agent solutions to treat magnetic beads
	DTT	5 mM	
	β-mercaptoethanol	20 mM	
	TCEP	5 mM	
	Reduced Glutathione	10 mM	

			for long periods of time should be avoided
Denaturant	Urea	8 M	
	Guanidine Hydrochlride	6 M	
Surface active agent	Triton X-100	2%	
	Tween 20	2%	
	NP-40	2%	
	Cholate	2%	
	CHAPS	1%	
Buffer solution	Sodium Phosphate, pH 7.4	50 mM	
	HEPES	100 mM	
	Tris-HCl, pH 7.4	100 mM	
	Tris-Acetate, pH 7.4	100 mM	
	MOPS, pH 7.4	100 mM	
	Sodium Acetate, pH 4.0	100 mM	
Other solutions	Imidazole	1.0 M	
	Ethanol	20%	
	NaCl	1.5 M	
	Na_2SO_4	100 mM	
	Glycerin	50%	
	EDTA	1 mM	It is limited to protein samples and cannot be used in buffers
	Citrate	60 mM	

Ordering Information

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