His-tag Protein Purification Kit-IDA-Co

Cat No: AKIT-His-IDA-Co

Description

This product is based on metal ion chelated magnetic particles as the main component, and also contains the main buffer and reagent components required for the use of magnetic particles. According to the operation process of this manual, part of the concentrated buffer can be directly used for the magnetic bead method of histidine label protein purification and magnetic bead regeneration, etc., to solve the complicated, time-consuming and laborious problem of buffer preparation for users, and easily realize rapid small amount of protein purification and high throughput purification screening. Users can also refer to this manual, according to the different needs of their own experiments, prepare various buffer and reagent components.

Abvigen metal ion chelated magnetic particles are superparamagnetic, which is a new functional material designed for efficient and rapid purification of histidine label protein. It is used for the purification of histidine label protein without high-speed centrifugation or filtration of crude protein samples. No need to control the flow rate, let alone expensive chromatographic equipment, fast solid-liquid, liquid-liquid separation through magnetic separation, simple operation process. It is suitable for purification of soluble histidine label protein secreted or expressed in bacteria, yeast, mammalian cells, etc., and can also be used for purification of denaturetic protein.

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Characteristics

1 Kit composition

Name	Composition	Total amount	Dosage/time	Number of times
IDA-Co	10% (V/V) particle suspension (20% ethanol solution)	5 mL	0.5 mL	10
10× Phosphate Buffer (For Binding Buffer/Washing Buffer preparation)	200 mM Sodium Phosphate, pH 7.4	50 mL	5 mL	10
10× Imidazole Buffer (For Washing/Elution Buffer preparation)	200 mM Sodium Phosphate, 5 M Imidazole, pH 7.4	50 mL	5 mL	10
NaCl	1.46 g/tube	20 tubes	2 tubes	10
Stripping Buffer	50 mM Tris-HCl, 500 mM NaCl, 100 mM EDTA, pH 7.4	30 mL	10 mL	3
Particles Washing Buffer	0.5 M NaOH, 2 M NaCl	15 mL	5 mL	3
Co^{2+} Recharge Buffer	100 mM $NiSO_4$	15 mL	5 mL	3
Storage Buffer	20% ethanol	50 mL	5 mL	10

2 Solvent tolerance of magnetic particles

Solvent type	Solvent name	Tolerable conc.	Remark
Reductant	DTE	5 mM	Before using a reducing agent, wash the magnetic particles with a non-reducing solution. The use of solutions containing reducing agents for prolonged treatment of magnetic particles should be avoided.
	DTT	5 mM	
	β-mercaptoethanol	20 mM	
	TCEP	5 mM	
	Reduced Glutathione	10 mM	
Denaturant	Urea	8 M	_
	Guanidine Hydrochlride	6 M	_
Surfactant	Triton X-100	2%	_
	Tween 20	2%	_
	NP-40	2%	_
	Cholate	2%	_

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Buffer solution	CHAPS	1%	_
	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	_
	Imidazole	500 mM	_
	Ethanol	20%	_
Other solution	NaCl	1.5 M	_
	Na_2SO_4	100 mM	_
	Glycerin	50%	_
	EDTA	1 mM	Add to protein samples only and cannot be used in buffers.
	Citrate	60 mM	

Operation Process

In general, the amount of magnetic particles is calculated by the user according to the information of the target protein production and magnetic bead load. For example, the target protein production is estimated to be 10 ~ 20 mg through pre-experiment, and the binding amount of Particles IDA-Ni with the target protein is 3~ 4 mg /mL, and the user needs to take 5 mL magnetic bead suspension for the purification of the target protein. The amount of buffer is adjusted according to the amount of magnetic particles used.

1. Buffer preparation

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

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

Binding Buffer: 20 mM Sodium Phosphate, 500 mM NaCl, 5~50 mM Imidazole, pH7.4

WashingBuffer: 20 mM Sodium Phosphate, 500 mM NaCl, 50~100 mM Imidazole, pH7.4

Elution Buffer: 20 mM Sodium Phosphate, 500 mM NaCl, 500 mM Imidazole, pH7.4

This product has been pre-prepared for users with 10×Phosphate Buffer and 10×Imidazole Buffer and related salts. 1×Phosphate Buffer and 1×Imidazole Buffer can be prepared by directly adding ddH_2O. On this basis, Binding Buffer, Washing Buffer and Elution Buffer suitable for target protein purification were prepared.

Methods for preparing 1×Phosphate Buffer and 1×Imidazole Buffer

Name	Composition	Volume	Preparation method
1×Phosphate Buffer	20 mM Sodium Phosphate, 500 mM NaCl, pH 7.4	50 mL	5 mL 10×Phosphate Buffer + 1 tube of NaCl + 40 mL ddH_2O, dissolved to 50 mL.
1×Imidazole Buffer	20 mM Sodium Phosphate, 500 mM NaCl, 500 mM Imidazole, pH 7.4	50 mL	5 mL 10×Imidazole Buffer + 1 tube of NaCl+40 mL ddH_2O, dissolving to 50 mL.

Users can prepare Binding Buffer and Washing Buffer containing Imidazole of different concentrations according to actual dosage in the following table. 1×Imidazole Buffer can be used directly as the Elution Buffer.

Formulation of Imidazole solutions containing different concentrations

Final conc. of Imidazole (mM)	1×Phosphate Buffer(mL)	1×Imidazole Buffer(mL)	Buffer volume (mL)
5	49.5	0.5	50
10	49	1	50
20	48	2	50
40	46	4	50
80	42	8	50
100	40	10	50
200	30	20	50

2. Sample handling

This manual provides the following three sample handling methods:

(1) Protein expressed in E. coli, yeast and other cells: add 5~10 mL Binding Buffer per gram of cells, add protease inhibitors (such as PMSF with a final concentration of 1 mM), resuspension cells, and ultrasonic lysis of cells in ice bath to form crude protein samples. If the sample is too thick, an

appropriate amount of nuclease can be added to the crude sample as needed and placed on ice for 30 min to degrade 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) Protein expressed in animal cells: Take an appropriate amount of animal cells, wash them once with an appropriate amount of PBS (self-provided), discard the supernatant, suspend them with an appropriate amount of Binding Buffer containing 1% (V/V) Triton X-100 or 1% (V/V) NP-40, and add protease inhibitors (such as PMSF with a final concentration of 1 mM). When placed on ice for 10 min, it is a crude protein sample.

3. Magnetic particle pretreatment

The purification of the histidine label protein expressed by Escherichia coli was illustrated as an example. The bacteria with 2 g wet weight were harvested with 500 mL fermentation solution, and the target protein yield was estimated to be 10-20 mg by pre-experiment. The user needed to take 5 mL magnetic bead suspension for the purification of the target protein.

(1) The metal ion chelated magnetic particles were placed on the vortex mixer and thoroughly mixed, and 5 mL magnetic bead suspension was taken by pipette into 15 mL centrifuge tube for magnetic separation, the supernatant was discarded, and the centrifuge tube was removed from the magnetic separator.

[Note: Place the centrifugal tube on the magnetic separator, so that the magnetic particles are adsorbed on the tube wall until the solution is clarified; In the process of magnetic separation, in order to reduce the loss of magnetic particles 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 can be washed with the residual magnetic particles on the centrifugal tube cover, and let it stand for a moment, so that the solution can be clarified again; The description of this operation is omitted below.]

(2) Add 5 mL Binding Buffer into the centrifuge tube equipped with magnetic particles, and turn the centrifuge tube up and down several times to re-suspend the magnetic particles, perform magnetic separation, and remove the supernatant. Repeat washing 2 times.

4. The target protein binds to the magnetic particle

(1) The 2 g wet weight bacteria 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 particles, and the centrifuge tube was placed in a vortex mixer for 15 s.

(2) Place the centrifuge tube on the rotating mixer and rotate the mixture at room temperature for 20-30 min (if necessary, rotate the mixture at a low temperature of 2-8°C for 1 h to prevent the 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 particle washing

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

(2) 10 mL Washing Buffer was added into the centrifuge tube with magnetic particles, the magnetic particles were re-suspended, the magnetic bead suspension was transferred to the new centrifuge tube (to avoid non-specific adsorption of protein on the wall of the original centrifuge tube to contaminate the target protein), magnetic separation was carried out, and the supernatant was removed to 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 mLElution Buffer, gently turning the centrifuge tube several times to make the magnetic particles 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.

(3) Target proteins were detected by SDS-PAGE or Western blotting. If the protein concentration needs to be measured, Elution Buffer zeroing can be used to determine the protein concentration, or dialysis, ultrafiltration and other methods can be used to remove Imidazole before concentration determination.

7. Post-processing of magnetic particles

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

(2) Repeat the above steps two times.

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

(4) Repeat the above step (3) twice.

(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 particles

The ability of magnetic particles to bind to the target protein may be significantly reduced if the particles are used for 3 or more consecutive times, and it is recommended that magnetic particles be regenerated.

Taking 5 mL 10% (V/V) magnetic bead suspension as an example, the regeneration operation of magnetic bead 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 to make 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 particles, 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 particles are re-suspended, magnetic separation, remove the supernatant, repeat this step twice.

(4) Alkali treatment (optional step) : Add 5 mL Particles Washing Buffer, turn the centrifuge tube up and down several times to re-suspend the magnetic particles, rotate and mix at room temperature for 5 min, magnetic separation, and remove the supernatant. Add 5 mL ddH_2O, turn the centrifugal tube up and down several times, so that the magnetic particles are re-suspended, magnetic separation, and the supernatant is removed. Repeat the DDHO washing step 3 to 5 times until the washing solution is neutral.

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(5) Add 5 mL Recharge Buffer, turn the centrifugal tube up and down several times to re-suspend the magnetic particles, rotate and mix at room temperature for 20 min, magnetic separation, and remove the supernatant. (Recharge Buffer: 100 mM $NiSO_4$, which has certain toxicity and may cause allergic reactions, please pay attention when using!)

(6) Add 5 mL ddH_2O, turn the centrifugal tube up and down several times, so that the magnetic particles are re-suspended, magnetic separation, and the supernatant is removed. Repeat this step four times.

(7) Add Storage Buffer to the magnetic bead so that the total volume is 5 mL and stored 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 particles, users can optimize the purification process from the following aspects to improve the recovery and purity of the target protein.

(1) the concentration of Imidazole in the sample solution and Binding Buffer, as well as the composition and concentration of other added reagents;

(2) sample treatment method, sample solution volume, concentration, etc.;

(3) The amount of magnetic particles used in purification;

(4) Temperature and time of incubation of sample solution with magnetic particles;

(5) The time and number of washing magnetic particles;

(6) Imidazole concentration, elution time, volume, and frequency of elution of target protein, etc.

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 particles;

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

(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.

Operation Flow Chart of Histidine Label Protein Purification

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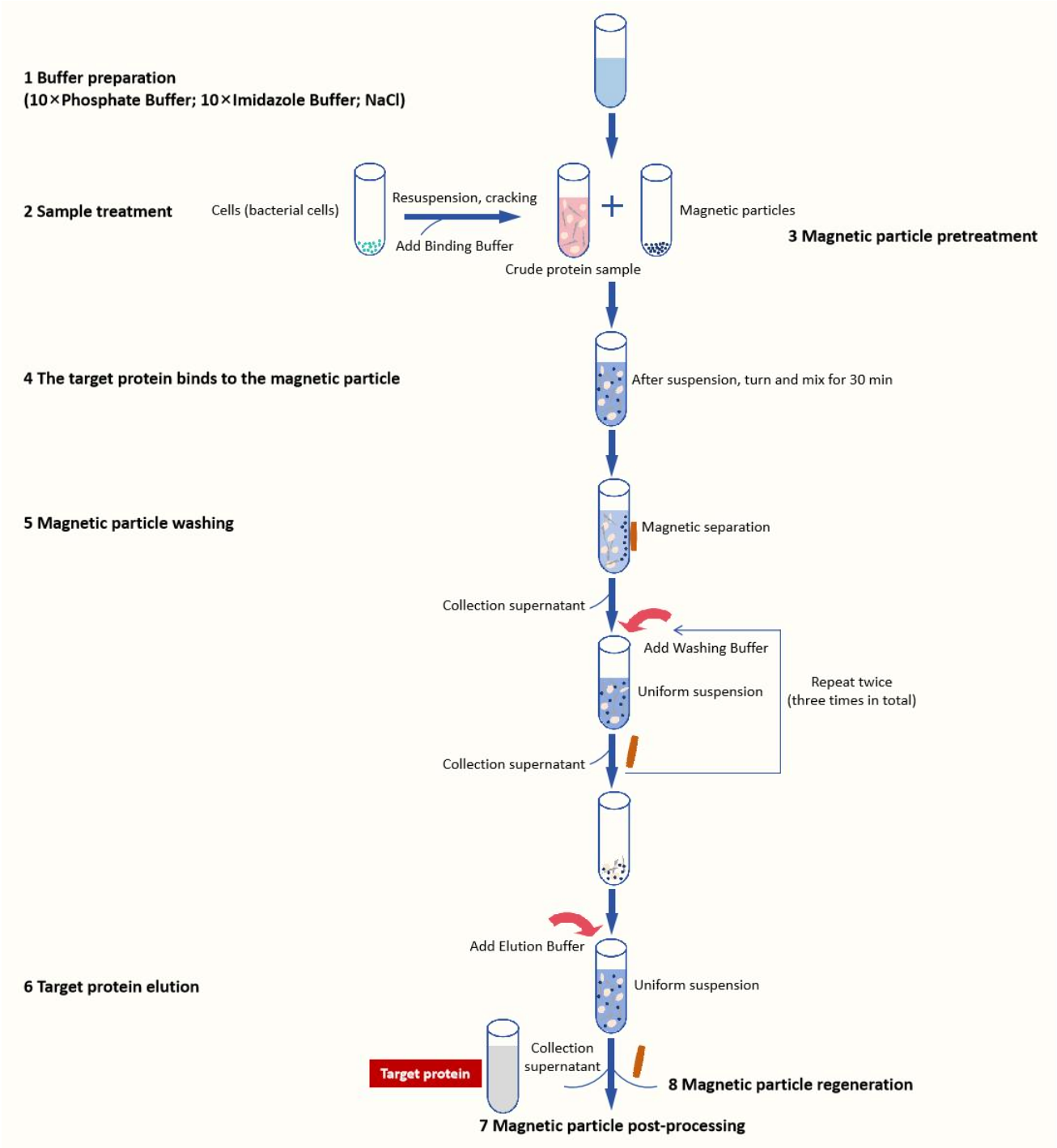

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Applications

It can be directly used for the magnetic bead method of histidine label protein purification and magnetic bead regeneration and other operations, to solve the complicated, time-consuming and laborious buffer preparation problems for users, easy to achieve fast small amount of protein purification and high throughput purification screening.

Features

The target protein can be purified directly from the crude sample, greatly reducing the purification time

The concentration and volume of the target protein can be easily controlled

High purity target protein can be obtained by one step purification

Parallel operation has high stability and facilitates high throughput and large-scale protein purification

Higher target protein yield can be obtained

It can be reused and the regeneration process is simple

Storage

This product should be stored at 2 - 8°C. **DO NOT FREEZE**.

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 particles;

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

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

5. During the mixing process of magnetic particles 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 particles;

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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 particles are reused, it is recommended to purify the same protein, and when purifying different kinds of proteins, it is recommended to use new magnetic particles;

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

10. This product can be stably stored at 2°C ~ 8°C with a shelf life of two years;

11. This product is for research use only.

Ordering Information

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