BeaverBeads™ His-tag Protein Purification

Product Introduction

Beaver 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, Beaver 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 hour, 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.

Product Information

Product name	BeaverBeads™ IDA-Nickel	BeaverBeads™ IDA-Cobalt
Bead size	30μm~150μm	
Chelating metal ion	Ni^{2+}	Co^{2+}
Metal ion density	30~50 μmol/mL beads	
Protein binding ability[1]	30~40 mg/mL （100% beads）	20~30 mg/mL （100% beads）
Operating temperature	2~30℃	
Suspension concentration[2]	10%（v/v）magnetic beads suspension	
Preservation	20%（v/v）ethanol	
Quality guarantee period	Stored at 2 ~ 8 ℃, Quality guarantee period of 2 years	

Note:

1：The binding amount of magnetic beads protein is related to the target protein characteristics, where only reference values are given.

2：1 mL magnetic beads contain 100 μL of magnetic beads.

3: Reference the appendix information for the tolerability of bead sovlent.

Application

It is suitable for the purification about soluble His-tag protein that secretion or intracellular expression by bacterium, yeast, insect and mammalian cells; also can be used for purification of denatured protein (denature inclusion body then carry out purification operation).

Operational Process

1. Preparation of buffer solution

The binding performance of the target protein and the magnetic beads will directly affect the purification efficiency of the target protein. The preparation of various buffers will also affect the recovery and purity of the target protein to a certain extent. Therefore, before the protein is purified, the user should design the experiment and screen out the buffer for the target protein, including Binding Buffer, Washing Buffer, Elution Buffer.

The most common elution method of His-tag protein purification beads is increasing the concentration of imidazole, before usage: when the best elution of imidazole concentration is uncertain, recommend to separately add 10 mM、20 mM、50 mM、100 mM、200 mM、300 mM、400 mM、500 mM imidazole to buffer, separately elute from low concentration to high concentration, after the magnetic seperation, then collect protein supernatant and use the SDS-PAGE electrophoresis to identify elution result.

For users reference: the below buffer systems are applicable to majority of His-tag protein purification.

- Binding Buffer： 20 mM Phosphate Buffer，500 mM NaCl，5~50 mM Imidazole，pH7.4
- Washing Buffer： 20 mM Phosphate Buffer，500 mM NaCl，50~100 mM Imidazole，pH7.4
- Elution Buffer： 20 mM Phosphate Buffer，500 mM NaCl，500 mM Imidazole，pH7.4

2. Sample treatment

This User's Manual provides below three sample treatments:

(1) Escherichia coli, yeast and other intracellular expression of protein: dilute the expression cells with appropriate amount of Binding Buffer, then add protease inhibitors (eg. the final concentration of 1 mM PMSF); ice bath and ultrasound to lysis cell to make crude protein samples. If the sample is too viscous, adding the appropriate amount of nuclease to the crude sample as needed and place it for 30 min on ice to degrade the nucleic acid. In addition, users can centrifuge the crude protein sample according to the actual demand.

(2) Extracellular expression protein: Take extracellular expression supernatant and dilute with equal amount of Binding Buffer to make crude protein samples.

(3) Animal cells intracellular expression protein: Take appropriate amount of animal cells, wash with plenty of PBS once and discard the supernatant; resuspend with 1% (v / v) Triton X-100 or 1% (v / v) NP-40 Binding Buffer ; add protease inhibition (eg. the final concentration of 1 mM PMSF); then ice bath for 10 mins to make crude protein samples.

3. BeaverBeads pretreatment

In general, the application amount of the bead is calculated by the target protein and the magnetic bead binding yield. For example, using E.coli to express a target protein. 2g wet weight of the cell is obtained from 500ml fermentation broth. Through the pre-experiment estimation, target protein production is 10~20mg. Users will need 5ml 10% magnetic suspension for the purification of target protein. Below are detail operation processes:

(1) Put BEAVER magnetic beads on the vortex mixer to mix, and then use a pipette to take 5 mL of magnetic beads suspension to the 15 mL centrifuge tube then magnetic separation, discard the supernatant.

(2) Put 5 ml Binding Buffer into the above centrifuge tube, flip the centrifuge tube upside down several times to make the beads resuspended. Then magnetic separation and discard the supernatant. Repeat washing twice.

（*Note: In order to reduce the loss of magnetic beads, cover the centrifuge tube lid tightly when the solution is clear. Keep the centrifuge tube in the magnetic separator and flip the separator and centrifuge tube up and down several times to make the lid's residual beads washed. Put it aside to let the solution become clear again; the same as below）

4.The binding of target protein and BeaverBeads

(1) Use 10 mL Binding Buffer to suspend 2 g wet weight cells and then crush and crack to form a crude protein sample; add the crude protein sample to a centrifuge tube which is containing pretreated beads, put the centrifuge tube on the vortex mixer for 15 seconds;

(2) Place the centrifuge tube on a rotary mixer. The mixture was rotated for 20 to 30 min at room temperature (if needed, it could be rotated for one hour at a low temperature of 2 to 8 ° C, preventing degradation of the target protein);

(3) Place the centrifuge tube on a magnetic separator for magnetic separation, and remove the supernatant to a new centrifuge tube for subsequent detection. Take out the centrifuge tube from the magnetic separator for

following washing steps.

5. BeaverBeads washing

(1) Add 10 mL washing buffer to the centrifuge tube with beads, gently flip it several times to make the beads resuspended, then magnetic separation, remove the washing fluid to the new centrifuge tube for sampling test, repeat this step once.

(2) Add 10 mL washing buffer to the centrifuge tube with beads to make the beads resuspended, and then transfer them to a new centrifuge tube to avoid contamination of the target protein with nonspecific adsorbate on the wall of the original centrifuge tube; then make magnetic separation and remove the supernatant to the cleaning solution collection tube.

6.The elution of target protein

(1) (The user can change the elution volume to adjust the target protein concentration) Add 2 ~10mL Elution Buffer B to the centrifuge tube, gently flip it several times to make the beads suspend, then magnetic separation, then collect the eluent into a new centrifuge tube to make the purified target protein sample;

(2) If needed, repeat the above steps once and collect the sample into a new centrifuge tube to test if the target protein elutes completely.

7. After processing of BeaverBeads

(1) Add 5 mL Elution buffer to the centrifuge tube with beads, flip it up and down several times to make the beads suspend, then magnetic separation and remove the supernatant.

(2) Repeat the above step twice.

(3) Add 5 mL ddH_2O, flip it up and down several times to make the beads suspend, then magnetic separation and remove the supernatant.

(4) Repeat the above step twice.

(5) Add Storage Buffer into the beads to make the total volume of 5mL, store at 2~30 ° C （long term preservation, store at 2~8 ° C), it can be used again for purification of the same protein.

8. Magnetic beads regeneration

If magnetic beads are used for more than three times, their ability to bind target protein may be significantly reduced, it is recommended to regenerate the beads.

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 （The chemicals are poisonous and can cause allergic reactions, must pay attention during using）

Storage Buffer：20%（v/v）ethanol

Taking 5 mL 10% (v/v) magnetic suspension as an example, the operation of magnetic bead regeneration is described in detail;

(1) Magnetic separation of magnetic bead suspension, remove the supernatant, then take the centrifuge tube away from the magnetic separator, and add 5 mL **ddH_2O** to the centrifuge tube, flip the centrifuge tube up and down several times, make the magnetic beads resuspended, magnetic separation, remove the supernatant.

(2) Add 5 mL **Stripping Buffer**, flip the centrifuge tube up and down several times to make the magnetic beads resuspended, rotate at room temperature and mix 5 min, then magnetic separation and remove the supernatant. Repeat this step 1 times.

(3) Add 5 mL **ddH_2O**, flip the centrifuge tube up and down several times to make the magnetic beads resuspended, then magnetic separation and remove the supernatant, repeat this step 2 times.

(4) Alkali treatment: add 5 mL **Beads Washing Buffer**, flip the centrifuge tube up and down several times, make the magnetic beads resuspended, rotate at room temperature 5 min, then magnetic separation, remove the supernatant. Add 5 mL **ddH_2O**, flip the centrifuge tube up and down several times, make the magnetic beads resuspended, magnetic separation, remove the supernatant. Repeat **ddH_2O** washing step 3~5 times, until the washing liquid is neutral.

(5) Add 5 mL **Recharge Buffer**, flip the centrifuge tube up and down several times, make the magnetic beads resuspended, rotate at room temperature and mix 20 min, magnetic separation, remove the supernatant.

(6) Add 5 mL **ddH_2O**, flip the centrifuge tube up and down several times, make the magnetic beads resuspended, magnetic separation, then remove the supernatant. Repeat this step more than 4 times to ensure the nickel ions are removed completely.

(7)Adding **Storage Buffer** to magnetic beads to make the total volume of 5 mL, store at 2~30° C (long term preservation, store at 2~8 ° C).

Optimization of Protein Purification Process

The above procedure is suitable for the purification of most His-tag proteins. Depending on the different binding properties between the target protein and the His-tag protein purified beads, the purification process can be optimized from the following aspects to improve the recovery rate and purity of the target protein.

A reference method to improve target protein recovery rate:

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

(2) Adding surfactants in the sample solution and other buffers;

(3) Adding appropriate protease inhibitors to prevent degradation of target proteins;

(4) Increase the amount of magnetic beads;

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

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

A reference method to improve the purity of target protein:

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

(2) Adding surfactants in the sample and buffer;

(3) Adding appropriate protease inhibitors to prevent degradation of target proteins;

(4) Prolong the washing time and increase the washing times;

(5) The target protein can be eluted by gradient Imidazole concentration.

Note

(1) Be sure to read this user's manual carefully before using this product for the first time;

(2) The freezing, drying and high-speed centrifugating and other operations should be avoided during the usage and storage of the beads ;

(3) Before using this product, be sure to fully oscillate the beads to maintain a uniform suspension;

(4) Please use pipette tip and centrifuge tube with good quality to avoid the stick on the wall or BEAVER beads loss caused by tube leakage during the mixing process;

(5) In the process of mixing the beads and the solution, if the solution is viscous, the magnetic beads cannot be resuspended by fliping the centrifuge tube, using short-term vortex mixing to make the BEAVER beads fully resuspended;

(6) Users can retain the removed supernatant 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 regenerate beads.

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

(9) This product must be used with magnetic separation equipment.

(10) This product is for research use only.

	Product name	Specification	concentration
70501-5	BeaverBeads™ IDA-Nickel	5 mL	10%（v/v）
70501-100	BeaverBeads™ IDA-Nickel	2×50 mL	10%（v/v）
70501-1000	BeaverBeads™ IDA-Nickel	4×250 mL	10%（v/v）
70501-K10	BeaverBeads™ IDA-Nickel Kit-10	Ten reaction	10%（v/v）
70502-5	BeaverBeads™ IDA-Cobalt	5 mL	10%（v/v）
70502-100	BeaverBeads™ IDA-Cobalt	2×50 mL	10%（v/v）
70502-1000	BeaverBeads™ IDA-Cobalt	4×250 mL	10%（v/v）
70502-K10	BeaverBeads™ IDA-Cobalt Kit-10	Ten reaction	10%（v/v）

For support, please visit: www.beaverbio.com/support or email: Service@beaverbio.com

Appendix information

Solvent tolerance of magnetic beads

Solvent type	Solvent	Tolerable concentration	Note
Reducing agent	DTE	5 mM	Before using reductant, please wash the magnetic beads with no reductant solution first. Avoid using reductant solution to treat magnetic beads for a long time.
	DTT	5 mM	
	β-mercaptoethanol	20 mM	
	TCEP	5 mM	
	Reduced Glutathione	10 mM	
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	Limited to add to protein samples, not for buffers.
	Citrate	60 mM	