Nickel Magnetic Beads for His Tag Protein Purification

Notice!

Magnetic beads should be stored at 4°C, do not freeze.
Do not dry the magnetic beads.

1. Package Information

Component	Cat#: B23600	Cat#: B23601	Cat#: B23602	Cat#: B23603	Cat#: B23604
Nickel Magnetic Beads For His Tag Protein Purification	500 ul	5 mL	10 mL (5 mL X2)	25 m (5mL X5)	50 mL (5 mL X10)

1. Diameter of the bead is about 50 μm~100 μm.
2. Metal ions ligand is Ni2+ and density of metal ions is 30~50 μ mol/mL beads.
3. Binding capacity to proteins is about 3~4 mg/mL (10% beads suspension). The protein binding capacity is provided as a reference. True binding capacity is correlated with the nature of the target proteins.
4. Working temperature is between 4°C~30°C.

2. Storage Information

Nickel Magnetic Beads For His Tag Protein Purification is provided as 10% (v/v) beads suspension (1 mL suspension contains 100 μL beads) in the storage buffer of 20% Ethanol. This product can be stored at 4°C~30°C, and it is suggested to be stored at 4°C for long-term stability.

3. Notices for This Product

1. Read the User Manual carefully before the first use.
2. Avoid freezing, drying and high-speed centrifugation during use and storage of beads.
3. Shake the beads thoroughly to keep them in a homogeneous suspension before use.
4. Choose well-qualified pipette tips and centrifuge tubes in case of bead loss from adherence or leakage when mixing.
5. When mixing the beads and the solutions, aspirate the beads with the pipette repeatedly or vortex briefly, if the solution is too thick to resuspend the beads manually.
6. To analyze the purification process and optimize the protein purification procedure, keep the removed supernatant (elution) separated by the magnetic beads and assay for detection if necessary.
7. The beads can be reused, but it is necessary to regenerate the beads when the purification capability decreases.
8. Purify the same kinds of proteins when reusing the beads; choose new beads when purifying different proteins.
9. This product should be used with the magnetic separator.
10. This product can be stably stored at 4°C for two years.
11. The product can only be used for research purposes.

4. Reference Protocol for His-tag Protein Purification

The binding capability of the target proteins and the metal beads has a direct effect on the purification efficiency. The preparation of the buffers has a similar effect on the recovery ratio and the purity of the target proteins. Users should design a preliminary experiment before a large-scale purification to select the appropriate buffer, which should include binding buffer (Buffer A), washing buffer (Buffer B) and elution buffer (Buffer C). A reference is provided here depicting a His-tag protein purification procedure with a relatively strong binding capability (Figure 1).

4.1. Preparation of Buffer

Buffer A (Binding Buffer): 20 mM Sodium Phosphate, 500 mM NaCl, 5~50 mM Imidazole, pH 7.4
Buffer B (Washing Buffer): 20 mM Sodium Phosphate, 500 mM NaCl, 50~100 mM Imidazole, pH 7.4
Buffer C (Elution Buffer): 20 mM Sodium Phosphate, 500 mM NaCl, 500 mM Imidazole, pH 7.4

The Buffer system above is apropriate for most His- tag protein purifications. Nonspecific binding can be reduced and binding capacity and purity of the target proteins can be increased by adding Imidazole into Buffer A. For the first trial, add 5~10 mM Imidazole and adjust the concentration of Imidazole in buffer according to purification results. Additionally, users may add other ingredients to the buffer such as Glycerin and surfactants if necessary.

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4.2. Preparation of Samples

This User Manual provides preparation of samples as follows:

1. Intracellular- expressed proteins in bacteria, yeasts and other cells: dilute the cells with Buffer A supplemented with a protease inhibitor (like BiotoolTM Protease Inhibitor Cocktail), perform cell lysis in an ultrasonic ice bath to generate the crude protein samples. Once the samples are thick, add nuclease and place on ice for 30 min to degrade the nucleic acids. If the yield of the target proteins is expected to be low, centrifuge the crude proteins to concentrate them.
2. Extracellular- expressed proteins: harvest the supernatant containing the extracellular-expressed proteins and add equal amount of Buffer A to generate the crude protein samples.
3. Intracellular- expressed proteins in animal cells: wash animal cells once with PBS, centrifuge, and discard the supernatant; resuspend the cells with Buffer A containing 1% (v/v) Triton X-100 or 1% (v/v) NP-40 (attached table 1), add a protease inhibitor, lastly, place the tube on ice for 10 min to generate the crude protein samples.

4.3. Pretreatment of Beads

Usually, the amount of beads used is determined by users' calculations according to the target protein yield and bead capacity. For example, for a protein expressed in E.coli, 2g-wet weight of microbes can be obtained from 500 mL broth. The yield of the target protein will be 10~20 mg by the preliminary experimental estimation, so 5 mL beads suspension is recommended for the target protein purification. Details of this example are presented as follows.

1. Homogenize the Nickel Magnetic Beads by vortexing, and pipette 5mL beads suspension to a centrifuge tube.
2. Place the tube on the magnetic separator, pipette and discard the storage buffer when the liquid becomes clean, remove the tube from the separator.

Note:

Make sure that every time you remove the supernatant by pipetting or pouring only when the tube is still attached to the magnetic separator!

3. Add 5 mL Buffer A to the tube above, manually turn the tube and resuspend the beads; place the tube on the separator for magnetic separation* and discard the supernatant, wash the beads twice.

Note:

in order to decrease loss of beads, during the magnetic separation, close the centrifuge tube cap tightly and keep the tube on the separator until the liquid becomes clear. Manually invert the separator and the tube several times to wash the beads adherent to the cap by the clarified liquid, let the tube stand until the liquid becomes clear again.

4.4. Binding of Target Protein and Beads

1. Suspend 2g-wet weight of microbes in 10 mL Buffer A for lysis, this is the crude protein samples. Add samples to the tube containing the pretreated beads, vortex for 15s.
2. Place the tube on a shaker/agitator, mix at room temperature for 20~30 min (if necessary, mix the tube at 4°C for 1 h to prevent the target protein degradation).
3. Place the tube on the magnetic separator, pipette the supernatant to a centrifuge tube for further assaying. Wash the beads and sample.

4.5. Washing of Beads

1. Add 10 mL Buffer B (Washing Buffer) to the centrifuge containing beads, invert several times to resuspend the beads, perform a magnetic separation, pipette the washing supernatant to another tube for further assaying.
2. Repeat the step (1) once again.
3. Add 10 mL Buffer B to the tube containing the beads to resuspend them, pipette the suspension to another tube to avoid nonspecific-adherent proteins, perform a magnetic separation and pipette the supernatant to the elute tube.

4.6. Elution of Target Proteins

1. Add 2~10 mL Buffer C (Elution Buffer), (adjust the volume based on concentration of the target proteins if necessary), invert gently several times to resuspend the beads, perform a magnetic separation and collect the elution into a new tube. This is the purified target protein samples.
2. If necessary, repeat the step (1) once again and collect the samples into another tube to examine whether the target proteins have been eluted thoroughly.

4.7. After treatment of Beads

1. Add 5 mL Buffer C to the tube containing the beads, invert several times to suspend the beads, perform a magnetic separation and remove the supernatant.
2. Repeat the step (1) twice.
3. Add 5 mL deionized water to the tube, invert several times to suspend the beads, perform a magnetic separation and remove the supernatant.
4. Repeat the step (3) above twice.
5. Add 20% (v/v) ethanol to the beads for a total volume of 5 mL, store the beads at 4~30℃ (4°C for a long storage) for the next purification of the same proteins.

4.8. Regeneration of Beads

After three uses, the binding capability of the beads to the target proteins may decrease, it is recommended to regenerate the beads.

Buffers needed:

Stripping Buffer: 20 mM Sodium Phosphate, 500 mM NaCl, 100 mM EDTA, pH 7.4
Bead Washing Buffer (optional): 0.5 M NaOH, 2 M NaCl
Recharging Buffer: 100 mM NiSO4 (this chemical reagent may lead to an allergic reaction, so pay close attention when using it!)

Example: Take 1 mL 10% (v/v) beads suspension for regeneration are as follows:

1. Perform a magnetic separation of the beads suspension, remove the supernatant, remove the tube from the separator, add 1 mL deionized water to the tube, and invert several times to resuspend the beads, perform the separation again and remove the supernatant.
2. Add 1 mL Stripping Buffer and invert several times to resuspend the beads, spin and mix them at room temperature for 5 min, perform a magnetic separation and remove the supernatant. repeat this step once.
3. Add 1 mL deionized water and invert several times to resuspend the beads. perform a magnetic separation and remove the supernatant, repeat this step twice.
4. Treatment by alkali (optional step) : Add 1 mL Bead Washing Buffer and invert several times to resuspend the beads, spin and mix them at room temperature for 5 min, perform a magnetic separation and remove the supernatant, add 1 mL deionized water and invert for several times to resuspend the beads, perform the separation again and remove the supernatant, repeat the deionized washing step 3~5 times until the eluate becomes neutral.
5. Add 1mL Recharge Buffer and invert several times to resuspend the beads, spin and mix them at room temperature for 20 min, perform a magnetic separation and remove the supernatant.
6. Add 1 mL deionized water and invert several times to resuspend the beads, perform a magnetic separation and remove the supernatant; repeat this step four times.

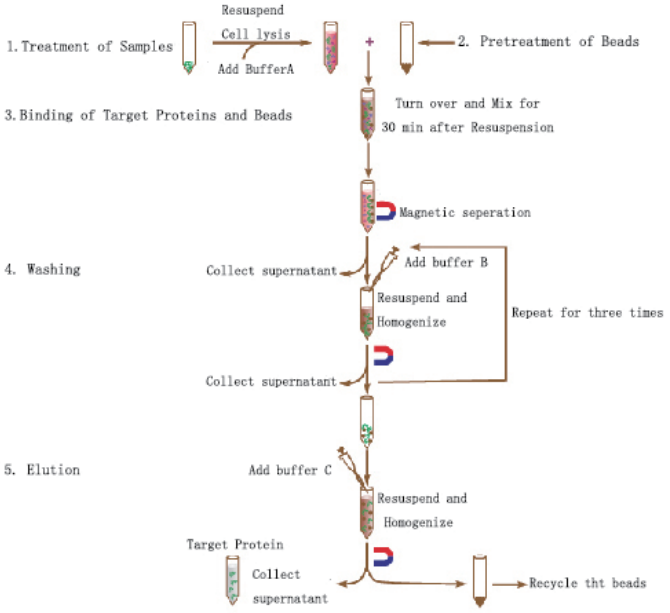

Figure1. Procedures of the Nickel Magnetic Beads For His Tag Protein Purificatio

5. Trouble Shooting

Q1: How to optimize the protein purification procedure?

A1: The operation protocols above are appropriate for purifying most His-tag proteins. To increase the recovery ratio and purity of the target proteins, users may optimize the purification procedures according to the following parameters.

1. The concentration of Imidazole in the samples and Buffer A, and the ingredients and concentration of the other reagents.
2. The treatment modes, volume and concentration of the samples.
3. The amount of beads used in purification.
4. The incubation temperature and time of the samples and the beads.
5. Time and the frequency of washing the beads.
6. The concentration of Imidazole, time, the volume and the frequency when eluting the target proteins.

Q2: How to increase the Recovery Ratio of Target Proteins?

A2: You can try the following measures:

1. Decrease the concentration of Imidazole in the samples and Buffer A.
2. Add surfactants to the samples and the buffer.
3. Add protease inhibitors to prevent the protein degradation.
4. Increase the amount of beads used.
5. Prolong the incubation of the proteins and the beads.
6. Prolong the elution of the proteins or increase times of the elution.

Q3: How to increase the purity of target proteins?

A3: You can try the following measures:

1. Increase the concentration of Imidazole and NaCl in the samples and Buffer A.
2. Add surfactants to the samples and the buffer.
3. Add protease inhibitor to prevent the protein degradation.
4. Prolong the washing of the proteins or increase times of the washing.
5. Elute the target proteins by Imidazole gradient concentration.

Attached table1: Solvent Tolerance

Solvent Category	Solvent Name	Concentration Tolerance	Remarks
Reductant	DTE	5 mM	Before adding solvent, wash the beads with the non-reducing solution first. Avoid treating the beads with the reductant for a long time.
	DTE	5 mM	
	β-mercaptoethanol	20 mM	
	DCEP	5 mM	
	Reduced Glutathione	10 mM	
Denaturant	Urea	8 M	
	Guanidine Hydrochloride	6 M	
Surfactant	Triton X-100	2%	
	Tween 20	2%	
	NP-40	2%	
	Cholate	2%	
	CHAPS	1%	
Buffer	Sodium Phosphate, pH	50 mM	
	7.4		
	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 Buffers	Imidazole	500 mM	
	Ethanol	20%	
	NaCl	1.5 M	
	Na_2SO_4	100 mM	
	Glycerin	50%	