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Ni NTA Beads 6FF

Product Number: SA005010

Shipping and Storage

Store product at 2°C-8°C. Do not freeze.The product is shipped at ambient temperature.

Store the Ni NTA Beads 6FF for longer periods of time in an equal volume of 1X PBS containing 20% ethanol at 2-8°C.

Description

Ni NTA Beads 6FF can be used to purify 6xHis-tagged proteins expressed in series of expression vectors, such as E.coli, yeast, insect cells, and mammalian cells. Ni NTA Beads 6FFconsists of 90μm beads of highly cross-linked 6% agarose, to which Nitilotriacetic acid (NTA) has been coupled. The chelating group has then been charged with nickel ions (Ni^{2+}). This form is very stable octahedral structure of nickel ions in the center, which can protect the nickel ions from attack of the competitive small molecule. The structure of Ni-NTA is compatible with a certain concentration of reducing agents, denaturing agents, detergents and other additives. Ni NTA Beads 6FF is highly stable and expand the range of suitable operating conditions.

In addition, the medium is easy to pack and use, and its high flow properties make it excellent for scaling-up. The key characteristics of the medium are listed in Table 1. A variety of compounds that are compatible with Ni NTA Beads 6FFare listed in Table 2.

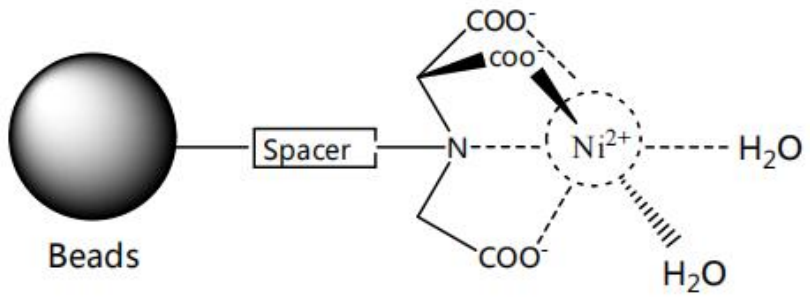

Figure1.Schematic illustration of the chemical structure of Ni-NTA on the support matrix.

Table 1. Ni NTA Beads 6FFcharacteristics.

Matrix	Highly cross-linked 6% agarose
Static binding capacity	>40mg 6x His-tagged protein/mL medium
Particle size	45μm–165μm
Maximum pressure	0.3 MPa, 3 bar
Storage solution	1x PBS containing 20% ethanol
Storage temperature	2°C-8°C

Table 2.Chemical compatibilities for Ni NTA Beads 6FF.

Reducing agents	5 mM DTE 0.5-1mM DTT 20 mM β-mercaptoethanol 5 mM TCEP 10 mM reduced glutathione (GSH)
Denaturing agents[1)]	8 M urea 6 M Gua-HCl
Detergents	2% Triton™ X-100 (nonionic) 2% Tween™ 20 (nonionic) 2% NP-40 (nonionic)

	2% cholate (anionic) 1% CHAPS (zwitterionic)
Other additives	500 mM imidazole 20% ethanol 50% glycerol 100 mM Na_2SO4 1.5 M NaCl 1 mM EDTA[2)] 60 mM citrate
Commonly used buffer	50 mM sodium phosphate, pH 7.4 100 mMTris-HCl, pH 7.4 100 mMTris-acetate, pH 7.4 100 mM HEPES, pH 7.4 100 mM MOPS, pH 7.4 100 mM sodium acetate, pH 4

Note:1)Tested for one week at 40°C.

2)The strong chelator EDTA up to 1 mM has been used successfully in some cases. Generally, chelating agents should be used with caution (and only in the sample, not in the buffers). Any metal-ion stripping may be counteracted by addition of a small excess of $MgCl_2$ before centrifugation/filtration of the sample. Note that stripping effects may vary with applied sample volume.

Protocol

1. Buffer Preparation

Generally, the concentration of imidazole in Lysis Buffer and Wash Buffer should be low, while it is high in Elution Buffer. Water and chemicals used for buffer preparation should be of high purity. It is recommended to filter all buffers before use by passing through a 0.22μm or 0.45μm filter. Ni NTA Beads 6FF can be used for His-tagged protein purification under both native conditions and denaturing conditions, which may require different buffers. See Tables 3 and 4.

Table 3. Recommended buffers for His-tagged protein purification under native conditions.

Lysis Buffer, 1 L	50 mM NaH_2PO4(7.80 g $NaH_2PO4·2H_2O$) 300 mMNaCl(17.54 g NaCl) 10 mM imidazole (0.68 g imidazole) Adjust the buffer pH to 8.0 with NaOH solution
Wash Buffer, 1 L	50 mM NaH_2PO4(7.80 g $NaH_2PO4·2H_2O$) 300 mMNaCl(17.54 g NaCl) 20 mM imidazole(1.36 g imidazole) Adjust the buffer pH to 8.0 with NaOH solution
Elution Buffer, 1 L	50 mM NaH2PO4(7.80 g $NaH_2PO4·2H_2O$) 300 mMNaCl(17.54 g NaCl) 250 mM imidazole (17.0 g imidazole) Adjust the buffer pH to 8.0 with NaOH solution

Table 4. Recommended buffers for His-tagged protein purification under denaturing conditions.

Lysis Buffer, 1 L	8 M Urea (480.50 g urea) 100 mM NaH2PO4(15.60 g $NaH_2PO4·2H_2O$) 100 mMTris·HCl(15.76 g Tris·HCl) Adjust the buffer pH to 8.0 with HCl solution

Wash Buffer, 1 L	8 M Urea (480.50 g urea) 100 mM NaH2PO4(15.60 g NaH2PO4·2H2O) 100 mMTris·HCl(15.76 g Tris·HCl) Adjust the buffer pH to 6.3with HCl solution
Elution Buffer, 1 L	8 M Urea(480.50 g urea) 100 mM NaH2PO4(15.60 g NaH2PO4·2H2O) 100 mMTris·HCl(15.76 g Tris·HCl) Adjust the buffer pH to 4.5 with HCl solution

2. Sample Preparation

2.1. Recombinant native protein expressed in E.coli or yeast

2.1.1. Single colonies should be cultured in LB medium. According to the instruction, add the inducers for a period of time.

2.1.2. Harvest cells from an appropriate volume of bacterial culture by centrifugation at 7,000rpm for 10-15min at 4°C. Discard supernatant and determine weight of pellet. Resuspend pellet in 1:10 ratio (w/v) in lysis buffer and add lysozyme (0.2-0.4mg/ml cell paste, if the host cell containing pLysS or pLysE, it can be without lysozyme) and PMSF (1mM/ml cell paste).

2.1.3. If high concentration of cell suspension, it is consider to add 10μg/ml RNase A and 5μg/ml DNase I. Sonicate the cell suspension/lysate on ice.

2.1.4. Centrifuge the homogenized lysate at 10,000rpm for 20min at 4°C to clarify sample. Save supernatant.

2.2. Native protein expressed in yeast, insect or mammalian cells

2.2.1. Harvest cells from an appropriate volume of culture by centrifugation at 5,000rpm for 10-15min at 4°C. Save supernatant.

2.2.2. If the supernatant contains no EDTA, histidine and reductant, proceed to purification directly. Otherwise the supernatant needsto be dialyzedagainst 1x PBS under 4°C.

2.2.3. If supernatant is of a large volume, it may require precipitation by adding ammonium sulfate and subsequent dialysis against 1xPBS under 4°C.

2.3. Inclusion bodies from E.coli

2.3.1. Harvest cells from an appropriate volume of bacterial culture by centrifugation at 7,000rpm for 10-15min at 4°C. Discard supernatant and determine weight of pellet.

2.3.2. Resuspend pellet in 1:10 ratio (w/v) with Lysis Buffer. Sonicate the cell suspension/lysate on ice.

2.3.3. Centrifuge the homogenized sample at 10,000rpm for 20min at 4°C to pellet the inclusion.

2.3.4. Resuspend pellet in 1:10 ratio (w/v) with denaturing Lysis Buffer(containing 8M urea). Sonicate, as needed, to dissociate the pellet.

2.3.5. Analyze the concentration of the target protein and continue with purification protocols under denaturing conditions.

2.4. Packing Ni NTA Beads 6FF

2.4.1. Assemble the column (and packing reservoir if necessary).

2.4.2. Remove air from the end-piece and adapter by flushing with water. Make sure no air has been trapped under the column bed support. Close the column outlet leaving the bed support covered with water.

2.4.3. Resuspend the medium and pour the slurry into the columnin a single continuous motion. Pouring the slurry down aglass rod held against the column wall will minimize the introduction of air bubbles.

2.4.4. If using a packing reservoir, immediately fill the remainder of the column and reservoir with water. Mount the adapter or lid of the packing reservoir and connect the column to a pump. Avoid trapping air bubbles under the adapter or in the inlet tubing.

2.4.5. Open the bottom outlet of the column and set the pump torun at the desired flow rate.

2.4.6. Maintain packing flow rate for at least 3 bed volumes after a constant bed height is reached. Mark the bed height

on the column.

2.4.7. Stop the pump and close the column outlet.

2.4.8. If using a packing reservoir, disconnect the reservoir and fit the adapter to the column.

2.4.9. With the adapter inlet disconnected, push the adapter down into the column until it reaches the mark. Allow the packing solution to flush the adapter inlet. Lock the adapter in position.

2.4.10. Connect the column to a pump or a chromatography system and start equilibration. Re-adjust the adapter if necessary.

2.5. Sample Purification

2.5.1. Add 5 column volumes Lysis Buffer to the column to equilibrate the beads.

2.5.2. Apply the sample to the column. Collect the flow-through to measure the binding efficiency to the beads, i.e. by SDS-PAGE.

2.5.3. Wash the column with 10 column volumes Wash Buffer or until the absorbance of the effluent at 280nm is stable.

2.5.4. Elute the target protein with Elution Buffer and collect the eluate.

2.5.5. Equilibrate the column with 5 column volumes of Lysis Buffer, distilled water and 1XPBS containing 20% ethanol. Finally store the beads with 1XPBS containing 20% ethanol at 4°C.

2.6. Analysis

2.6.1. Examine and identify the fractions containing the His-tagged protein. Use UV absorbance, SDS-PAGE, or Western blotting.

Cleaning-in-Place

A column used to purify protein from cell extract usually has buildup of insoluble substances and cell debris, which are non-specifically absorbed onto the matrix support and cannot be completely removed during washing steps. If the column is to be reused, these contaminants should be cleaned from the column. Cleaning-in-Place helps eliminating materials that cannot be removed by regeneration and preventing progressive buildup of contaminants.

To remove strongly bound hydrophobic proteins, lipoproteins and lipids:

1. Wash the column using 5-10 CV of 30% isopropanol contacting for 15-20 min. Or apply 2 CV of acidic or alkaline solution containing detergent(i.e. 0.1 M acetic acid solution contains 0.1-0.5% non-ionic detergent), for 1-2 hours.
2. Finally wash the column with 10CV distilled water.

To remove the proteins engaged with ionic interaction:

1. Wash the column with 1.5 M NaCl for 10-15 min.
2. Finally wash the column with 10 column volumes distilled water.

Regeneration

In general, Ni NTA Beads 6FFmay be used a number of times before it becomes necessary to recharge them with metal ions. When the back pressure is too high or the capacity significantly lower, it need to strip the metal ions and recharge the Ni NTA Beads 6FF as the following procedure.

Wash the column with one of the following solutions:

1. 0.2M acetic acid with 6 M Guanidine Hydrochloride, 2 column volumes;
2. Rinse with 5 column volumes of distilled water;
3. 2% SDS, 3 column volumes;
4. Rinse with 5 column volumes of distilled water;
5. 70% ethanol, 5 column volumes;
6. Rinse with 5 column volumes of distilled water;
7. 100 mM EDTA (pH 8.0), 5 column volumes;
8. Rinse with 5 column volumes of distilled water;

9. 100mM NiSO4, 5 column volumes;
10. Rinse with 5 column volumes of distilled water;

After regeneration, the medium can be used immediately.

Trouble Shooting Guide

Problem	Probable cause	Solution
Back pressure exceeds 1 bar	Column is clogged	Cleaning in place(Section 3). Increase the centrifugation speed or filtering the sample.
	Sample is too viscous	Increase sonication or add DNase I (5μg/ml with 1mM Mg^{2+}).Incubate on ice for 15min.
	Buffer is too viscous	Dilute sample by adding more homogenization buffer.
No protein is eluted	Expression of target protein in extract is very low	Check expression level of protein by estimating the amount in the extract, flow through, elute fraction and pellet upon centrifugation. Or apply larger sample volume.
	Target protein is found in the flow through	Reduce imidazole concentration in lysis buffer sample and wash buffer. Or increase buffer pH.
	Elution conditions are too mild.	Increase imidazole concentration in Elution buffer. Or decrease buffer pH. Strip nickel ion by using 10-100mM EDTA solution, at the same time you can obtain target protein.
	Target protein is degraded	Perform purification at 4°C in the presence of protease inhibitors.
	The His-tag is missing	Make a new construct with his-tag attached to other terminus.
His-tagged protein is not pureHis-tagged protein is not pure	Insufficient wash	Increase the volume of Wash Buffer.
	Association between the His-tagged protein and protein contaminants.	Optimize washing condition by tweaking the pH, salt concentration, and imidazole concentration. Add an additional chromatography step, which can be of either ion exchange, hydrophobic interaction, or size exclusion.
Medium color is shallow.	The nickel ion was stripped.	Add an additional chromatography step, which can be of either ion exchange, hydrophobic interaction, or size exclusion.
The medium are brown.	The buffer contains too much DTT.	Reduce the concentration of DTT below 2mM.
Protein precipitates during purification	Temperature is too low.	Perform the purification at room temperature.
	Aggregate formation	Add solubilizing agents to the samples and buffers, for example 0.1% Triton X-100, Tween-20 and ≤20% glycerol to maintain protein solubility.