Product Information

Product Name:	Ni IDA Beads 6FF
Product Code:	SA052025/SA052100
Size(s):	25ml & 100ml

Product Description:

Ni IDA Beads 6FF is an immobilized metal affinity resin designed for the purification of 6xHis-tagged proteins expressed in expression vectors, such as *E.coli*, yeast, insect and mammalian cells. Ni IDA Beads 6FF is composed of the coupling of 90µm beads of highly cross-linked 6% agarose to immobilised iminodiacetic acid (IDA) chelating ligand. The chelating group is pre-charged with nickel ions (Ni2+) for binding to 6xHis-tagged proteins.

In comparison with Ni NTA Beads 6FF, Ni IDA Beads 6FF has a higher binding capacity and better cost-effectiveness. The high flow properties of Ni IDA Beads 6FF make it excellent for scaled up purification of histidine-tagged protein.

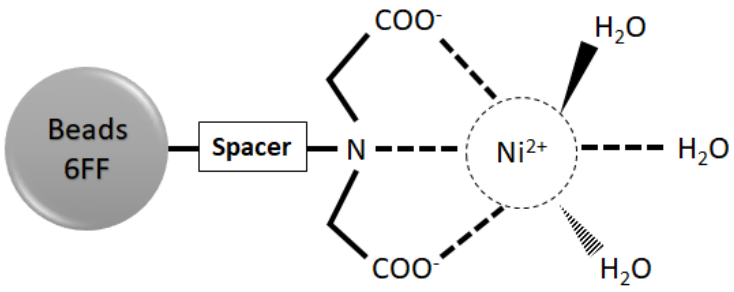

Figure 1: Chemical structure of Ni IDA Beads 6FF

Product Specification:

Matrix:	Highly cross-linked 6% agarose
Binding Capacity:	>40 mg 6xHis-tagged protein/mL medium
Particle Size:	45 - 165µm
Maximum Linear Flow Rate:	600cm/h (20ml/min) using a 16/20 column with 5cm bed height
Maximum Pressure:	3bar (0.3 MPa)
Recommended Flow Rate:	<150cm/h
pH Stability:	Short term: pH 2 – 14
	Long term: pH 3 – 12
Storage Conditions	2 – 8ºC, 1XPBS with 20% ethanol

Test Items	Specifications

Appearance	Blue, green
Volume	50% suspension
Binding Capacity	>50mg 6x His-tagged protein/ml medium
Microbial contamination	<100 Colony Forming Units/ml suspension

Operation Protocol

1. Buffer Preparation

The basic principle for the recommended buffers is to have a low concentration of imidazole in Lysis and Wash Buffer and a high concentration of imidazole in Elution Buffer. Water and chemicals of high purity should be used. It is recommended to filter all buffers through a 0.22μm or 0.45μm filter before usage. **Table 1**presents the recommended buffer for 6xHis-tagged protein purification under native conditions.

Table 1: Recommended buffer for the 6xHis-tagged protein purification under native conditions

Lysis Buffer (1L)	50mM NaH_2PO_4 (7.80g $NaH_2PO_4 \cdot 2H_2O$) 300mM NaCl (17.54g NaCl) 10mM imidazole (0.68g imidazole) Adjusts to pH 8.0 with NaOH solution.
Wash Buffer (1L)	50mM NaH_2PO_4 (7.80g $NaH_2PO_4 \cdot 2H_2O$) 300mM NaCl (17.54g NaCl) 20mM imidazole (1.36g imidazole) Adjusts to pH 8.0 with NaOH solution.
Elution Buffer (1L)	50mM NaH_2PO_4 (7.80g $NaH_2PO_4 \cdot 2H_2O$) 300mM NaCl (17.54g NaCl) 250mM imidazole (17.g imidazole) Adjusts to pH 8.0 with NaOH solution.

2. Sample Preparation

2.1. Protein Expression in *E.coli*

2.1.1. Colonies were cultured in LB medium. According to the instructions, add IPTG for a period of time.

2.1.2. Harvest the cells from an appropriate volume of bacterial cultures by centrifuging at 7,000rpm at 4°C for 10 - 15 minutes. Discard the supernatant and determine the weight of the pellet. Resuspend the pellet in 1:10 ratio (w/v) with Lysis Buffer, before adding lysozyme (0.2 - 0.4mg/ml cell paste. If the host cell has pLysS or pLysE, there can still be an absence of lysozyme) and PMSF (1mM/ml cell paste).

2.1.3. If there is a high concentration of nucleic acids in the cell suspension, it is recommended to add 10μg/ml RNase A and 5μg/ml DNase I to the sample. Sonicate the cell suspension/lysate on ice.

2.1.4. Centrifuge the homogenized lysate at 15,000rpm for 20mins at 4°C. Set the supernatant aside.

2.2. Protein Expression in Yeast, Insect or Mammalian Cells

2.2.1. Harvest the cells from an appropriate volume of culture by centrifuging at 5,000rpm at 4ºC for 10 - 15 minutes. Set the supernatant aside.

2.2.1.1. If there is an absence of EDTA, histidine and reductant in the supernatant, it can be directly purified

2.2.1.2. If there is a presence of EDTA, histidine and reductant in the supernatant, it has to be dialysed to 1XPBS at 4ºC.

2.2.2. If there is a large volume of supernatant, precipitation may be required. This can be performed by adding ammonium sulfate and subsequent dialysis to 1XPBS under 4ºC.

3. Packing Ni IDA Beads 6FF

3.1. Assemble the column (and packing reservoir if required)

3.2. Flush the column with water to remove the air from the end-piece and the adaptor. Ensure that there is no air trapped under the column bed support. Close the column outlet, while covering the bed support with water.

3.3. Resuspend the medium. Pour the slurry into the column in a single continuous motion. To minimise the introduction of air bubbles, pour the slurry down a glass rod while holding it against the column wall.
Note: If a packing reservoir is in use, fill the remaining column and reservoir with water immediately. Mount the adaptor/lid of the packing reservoir and connect the column to a pump. Avoid trapping air bubbles under the adaptor or in the inlet tubing.

3.4. Open the bottom outlet and set the pump to run at the desired flow rate.

3.5. Maintain the packing flow rate for at least 3 bed volumes after obtaining a constant bed height. Mark the bed height on the column.

3.6. Stop the pump and close the column outlet.
Note: If a packing reservoir is in use, disconnect the reservoir and fit the adaptor to the column.

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

3.8. Before starting the equilibration process, connect the column to a pump or a chromatography system. Adjust the adaptor if required.

4. Sample Purification

4.1. Add 5 column volumes of Lysis Buffer into the column to equilibrate the beads. Drain the Lysis Buffer

4.2. Apply the sample into the column. Collect the flow-through to measure the binding efficiency to the beads through SDS-PAGE analysis

4.3. Wash the column with either 10 column volumes of Wash Buffer or until the absorbance reading of the effluent is stabilised at 280nm.

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

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

5. Results Analysis

Fractions containing the His-tagged proteins can be identified using UV absorbance, SDS-PAGE or western blot

6. Cleaning-in-place

The column used in the protein purification process usually contains insoluble substances and cell debris that are non-specifically absorbed to the matrix. Cleaning-in-Place eliminates the remaining unremoved materials after regeneration and prevents the progressive accumulation of contaminants. For the reuse of the column, these contaminant have to be cleaned from the column.

i. Removal of strong hydrophobic binding protein, lipoprotein and lipid:

a. Wash the column with 5 - 10 column volumes of 30% isopropanol. The contact time is 15 - 20 minutes. Alternatively, wash the column with 2 column volumes of acidic or alkaline solution containing detergents (i.e. 0.1M acetic acid solution contains 0.1 - 0.5% non-ion detergent). The contact time is 1 - 2 hours.
b. Wash the column with 10 column volumes of distilled water.

ii. Removal of ionic bound proteins:

a. Wash the column with 1.5M NaCl solution. The contact time is 10 - 15 minutes.
b. Wash the column with 10 column volumes of distilled water.

7. Regeneration

Ni IDA Beads 6FF can be reused several times without regeneration. When the back pressure is too high or when the binding capacity is significantly low, the metal ions have to be stripped away and the Ni IDA Beads have to be recharged.

Wash the column with the following process:

i. Wash the column with 2 column volumes of 0.2M acetic acid and 6M guanidine hydrochloride. Rinse with 5 column volumes of distilled water;
ii. Wash the column with 3 column volumes of 2% SDS. Rinse with 5 column volumes of distilled water
iii. Wash the column with 5 column volumes of 70% ethanol. Rinse with 5 column volumes of distilled water.
iv. Wash the column with 5 column volumes of 100mM EDTA (pH 8.0). Rinse with 5 column volumes of distilled water
v. Wash the column with 5 column volumes of 100mM $NiSO_4$. Rinse with 5 column volumes of distilled water.

The medium can be used immediately after regeneration

Storage

Ni IDA Beads 6FF have to be suspended in an equal volume of 1x PBS containing 20% ethanol at 2-8°C.

Troubleshooting Guide

Problems	Possible Causes	Solutions
The back pressure exceeds 1bar	The column is clogged	• Cleaning-in-place (Refer **above Section 6**) • Increase the centrifugation speed or filter the sample
	The sample is too viscous	Increase sonication or ad DNase I (5μg/ml with 1mM Mg^{2+}). Incubate on ice for 15mins
	The buffer is too viscous	Add more homogenization buffer to dilute the sample.
There are no proteins eluted	There is a very low expression of target	• Estimate the amount of proteins in the extract, flow through, eluted fraction and pellet upon centrifugation to check on the protein expression levels. • Apply large sample volumes
	The target protein is present in the flow through	• Reduce the imidazole concentration in the Lysis buffer sample and Wash Buffer • Increase buffer pH
	The elution conditions are too mild	• Increase the imidazole concentration in the Elution buffer • Decrease buffer pH • By using a 10 – 100mM EDTA solution, nickel ions can be stripped from the resin. The target protein may be eluted together with Ni^{2+}
	Protein degradation or purification can cause the removal of histidine tag	• Perform at 4°C. Add protease inhibitors • Develop a new construct with the attachment of His-tag to other terminus
The His-tagged protein is not pure	There is insufficient washing	Increase the volume of Wash buffer
	There is an association between the His-tagged protein and protein contaminant	• Adjusting the pH and imidazole concentration to optimize the wash condition • An additional chromatography (i.e. ion exchange, hydrophobic interaction or size exclusion) step can be added
The colour of medium becomes shallow	The nickel ions was stripped	Chelate the nickel ions through “Regeneration” (refer **above Section 7**)

The medium is brown	The buffer contains DTT	Use Ni NTA Beads or Ni NTA Beads 6FF when the reductant concentration is below 2mM.
Protein precipitates during purification	The temperature is too low	Protein purification should be performed at room temperature
	Aggregation of protein	Addition of solubilizing agents (i.e. 0.1% Triton™ X-100, Tween-20 and ≤20% glycerol) to the samples and buffers to maintain protein solubility.