ChangZhou Smart-Lifesciences Biotechnology Co., Ltd.
No.8,Lanxiang Road,West Taihu science and Technology Industrial Park,
Wujin District,Changzhou City,Jiangsu Province,China.
Market：0086-519-83820182 Support：0086-519-83736881 www.smart-lifesciences.com

Ni Smart Beads 6FF

Index

1. Product Description

Ni Smart Beads 6FF is a new immobilized metal ion affinity chromatography(IMAC) medium precharged with nickel ions. **Ni Smart Beads 6FF** is designed mainly for capture and purification of histidine-tagged proteins secreted into eukaryotic cell culture supernatants. The strong nickel ion binding also provides very high resistance to EDTA and reducing agents like DTT. **Ni Smart Beads 6FF** enable direct loading of large sample volumes without having to remove agents that cause stripping of nickel ions from conventional IMAC medium. **Ni Smart Beads 6FF** is stable in all buffers commonly used in IMAC.

Table 1. Characteristics of **Ni Smart Beads 6FF**

Item	Description
Matrix Spherical	Highly cross-linked 6% agarose
Static Binding Capacity	＞10 mg 6×His-tagged protein/ml medium
Particle size	45-165 μm
Maximum Pressure	0.3 MPa, 3 bar
Storage Solution	1×PBS containing 20% ethanol
Storage Temperature	4-30℃

Table 2. Chemical compatibilities for **Ni Smart Beads 6FF**

Reagent	Stability
0.01M HCl ,0.01M NaOH	One week
10mM EDTA, 1M NaOH, 5mM DTT, 5mM TCEP, 20mM β-mercaptoethanol, 6M Guanidine HCl	24 hours
500mM imidazole， 100mM EDTA	2 hours
30% isopropanol	20 minutes

2. Purification Procedure

2.1 Buffer Preparation

Water and chemicals used for buffer preparation should be of high purity. It is recommended to filter the buffers by passing them through a 0.22 μm or 0.45 μm filter before use.

Binding Buffer:20 mM sodium phosphate,0.5 M NaCl, pH7.4

Wash Buffer: 20 mM sodium phosphate, 0.5 M NaCl, 0-5 mM imidazole, pH7.4

Elution Buffer: 20 mM sodium phosphate, 0.5 M NaCl, 250 mM imidazole, pH7.4

Note: 1) It is not recommended to include imidazole in sample and Binding buffers. To minimize host cell proteins in the elution, it is recommended to include imidazole at low concentrations in the wash buffer . However ,for some target proteins, even a small increase of the imidazole concentration in the wash buffer may lead to partial elution.

2) Addition of salt, for example 0.5 to 1.0 M NaCl in buffers eliminates ion-exchange effects.

3) Alternatively, the proteins may be eluted by other methods or combinations of methods, for example by lowering the pH within the range 2.5 to 5.0.

2.2 Sample Preparation

1) Before sample loading, remove the cell by centrifugation at 7,000rpm for 10-15 min at 4℃, otherwise clogging of the column may occur.
2) For optimal binding, it is not recommended to include imidazole in sample and Binding buffer.
3) It is not be pretreatment when the samples solution contain the tolerance reagent and low target protein concentration.

2.3 Packing Columns

Ni Smart Beads 6FF is easy to pack and use, and its high flow properties make it excellent for industrial scaling-up. The method of packing the column is described below.
1) Remove air from the column dead spaces by flushing the end-piece and adapter with packing buffer. Make sure no air has been trapped under the column net.
2) Close the column outlet leaving the net covered with packing buffer.
3) Resuspend the beads stored in its container by shaking (avoid stirring the sedimented medium). Pouring the slurry down a glass rod held against the column wall will minimize the introduction of air bubbles. If using a packing reservoir, immediately fill the remainder of the column and reservoir with packing buffer. 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.
4) Open the bottom outlet of the column and set the pump to run at the desired flow velocity. Ideally, **Ni Smart Beads 6FF** is packed at a constant pressure of approximately 3 bar (0.3 MPa). If the packing equipment does not include a pressure gauge, use a packing flow velocity of approximately 400 cm/h (10 cm bed height, 25℃, low viscosity buffer). If the recommended pressure or flow velocity can not be obtained, use the maximum flow velocity the pump can deliver. This should also give a reasonable well-packed bed. Do not exceed 75% of the packing flow velocity in subsequent chromatographic procedures.
5) Maintain packing flow velocity for at least 3 bed volumes. When the bed has stabilized, mark the bed height on the column and close the bottom outlet and stop the pump. If using a packing reservoir, disconnect the reservoir and fit the adapter to the column. If using the column, carefully place the top filter on top of the bed before fitting the adapter.
6) With the adapter inlet disconnected, push the adapter down into the column until it reaches the mark, allowing the packing solution to flush the adapter inlet. Lock the adapter in position.
7) Connect the pump, open the bottom outlet and continue packing. The bed will be further compressed at this point and a space will be formed between the bed surface and the adapter.
8) Close the bottom outlet. Disconnect the column inlet and lower the adapter approximately 2 mm into the bed. Connect the pump. The column is now ready to use.

2.4 Sample Purification

1) Fill the syringe or pump tubing with distilled water. Remove the stopper and connect the column to the syringe (with the provided connector), or pump tubing,“drop to drop”to avoid introducing air into the column. Remove the snap-off end at the column outlet.
2) Wash the column with 3-5 column volumes of distilled water.
3) Equilibrate the column with at least 5 column volumes Lysis Buffer.
4) Load the pre-treated sample by using a Loop fitted to the connector or by pumping it onto the column.
5) Wash the column with10-15 column volumes of wash buffer or until the absorbance reaches the baseline or no material appears.
6) Elute with elution buffer using a stepwise or linear gradient. For one-step elution, 5 column volumes are usually enough. Other volumes may be required if the interaction is difficult to break. Linear gradient elution can be used to separate proteins of different binding strengths with a small gradient, such as 20 column volumes or more.

2.5 Analysis

Identify the fractions containing the His-tagged protein. Use UV absorbance, SDS-PAGE, or western blot.

3. Cleaning-in-Place

The column used to purify protein from cell extract usually contains some soluble substances and cell debris that are nonspecifically absorbed onto the matrix. Cleaning-in-Place eliminates material not removed by regeneration and prevents progressive buildup of contaminants. If the column is to be reused, these contaminants should be cleaned from the column.

- **Remove the precipitated proteins, hydrophobically bound proteins, and lipoproteins**

Wash the column with 1M NaOH, contacting for at least 30minutes. Finally wash with 10 column volumes of equilibration buffer.

- **Remove the strong hydrophobic binding protein, lipoprotein and lipid**

Wash the column with 5-10 column volumes of 30% isopropanol contacting for 15-20min. Or you can choose the 2CV of acidic or alkaline solution containing detergents, for example, 0.1 M acetic acid solution contains 0.1-0.5% non-ionic detergent, contacting for 1-2 hours. Finally wash the column with 10CV of distilled water

- **Remove the proteins combined with ion interacting**

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

4. Troubleshooting

Problem	Probable cause	Solution
Back pressure exceeds 3 bar	Column is clogged	Cleaning in place (Part 3).
		Increase the centrifugation speed or filtering the sample.
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 large sample volume.
	Target protein is found in the flow through	Reduce imidazole concentration in equilibration buffer sample and wash buffer. Increase buffer pH.
	The Protein is not eluted from the medium.	Increase imidazole concentration in elution buffer. Or decrease buffer pH.
	Protein degradation or purification cause the his-tag to be removed.	Operate at 4°C. Add protease inhibitors.
		Make a new construct with his-tag attached to other terminus.
His-tagged protein is not pure	Wash is not enough	Increase the volume of wash buffer.
	Association between the his-tagged protein and protein contaminant.	Optimize the wash condition by adjusting the pH and imidazole concentration.
		Add an additional chromatography step, that is ion exchange, hydrophobic interaction or size exclusion.
Protein precipitates during purification	Temperature is too low	Perform the purification at room temperature.
	Aggregate formation	Add solubilization agents to the samples and buffers, for example 0.1% Triton X-100 , Tween-20 and ≤20% glycerol to maintain protein solubility.

5. Related Products

Product	Cat. No.	Size
Ni Smart Beads	SA035005	5 ml
	SA035025	25 ml
	SA035100	100 ml
	SA035500	500 ml
	SA03501L	1 L
	SA03510L	10 L
Ni Smart Beads 6FF	SA036005	5 ml
	SA036025	25 ml
	SA036100	100 ml
	SA036500	500 ml
	SA03601L	1 L
	SA03610L	10 L
HisCap Smart 6FF	SA036C11	1×1 ml
	SA036C51	5×1 ml
	SA036C15	1×5 ml
	SA036C55	5×5 ml
	SA036CS	3×1 ml+1×5 ml