HiPur Ni Smart

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.

HiPur Ni Smartis a prepacked ready to use column. It is packed with 20 ml **Ni Smart Beads 6FF**.The arrow on the column label indicates the recommended flow direction.It can be adapted to all kinds of chromatography system, such as ÄKTA. It is easy to operate.

Table 1. Characteristics of **HiPur Ni Smart**

Item	Description
Size	20 ml
Column size	1.6 ×10 cm
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	2-8℃

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

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 Sample Purification

HiPur Ni Smart is a prepacked, ready to use column for purification. The prepacked column provides fast, simple and easy separations in a convenient format.

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 stopper at the column outlet and connect the column to the chromatographic system.

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) Apply the pre-treated sample, using a Loop fitted to the connector or by pumping it onto the column.

5) Wash with Wash Buffer until the absorbance reaches the baseline or no material appears in the effluent (Generally at least 10-15 column volumes).

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.

2.4 Analysis

Identify the fractions containing the target 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
HiSelect Ni Smart	SA036C47	4.7 ml
HiPur Ni Smart	SA036C20	20 ml