Ni Smart Beads

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1. Product Description

Ni Smart Beads is a new immobilized metal ion affinity chromatography (IMAC) medium precharged with nickel ions. **Ni Smart Beads** 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** 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** is stable in all buffers commonly used in IMAC.

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

Item	Description
Matrix	4% agarose
Static Binding Capacity	＞10mg 6×His-tagged protein/ml medium
Particle size	45-165 μm
Maximum Pressure	0.1 MPa, 1 bar
Storage Solution	1×PBS containing 20% ethanol
Storage Temperature	4-30℃

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

Solution	Time
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, pH 7.4

Wash Buffer: 20 mM sodium phosphate, 0.5 M NaCl, 0-5 mM imidazole, pH 7.4

Elution Buffer: 20 mM sodium phosphate, 0.5 M NaCl, 250 mM imidazole, pH 7.4

Note: 1) It is not recommended to include imidazole in sample and equilibration buffers. To minimize host cell proteins in the eluate, 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 pH within the range 2.5 to 5.0.

2.2 Sample Preparation

1) Before sample loading, remove the cell by centrifugation at 7,000 rpm (7,500×g) 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 equilibration buffer.
3) It is not be pretreatment when the samples solution contain the tolerance reagent and low target protein concentration.

2.3 Column Purification

1) Mix the slurry by gently inverting the bottle several times to completely suspend the **Ni Smart Beads.** Close the column outlet leaving the net covered with packing buffer. Transfer the slurry to the column.
2) Allow the resin to settle down and the buffer to drain from the column. Add 5 column volumes Binding buffer to the column to equilibrate the beads.
3) Load the sample to the column. Collect the flow-through for measuring the binding efficiency to the beads, i.e. by SDS-PAGE.
4) Wash the column with 10 column volumes wash buffer or until the absorbance of the effluent at 280 nm is stable.
5) Elute the target protein with elution buffer and collect the eluate.
6) Equilibrate the column with 5 column volumes of binding buffer, distilled water and 1×PBS containing 20% ethanol. Finally store the beads with 1×PBS containing 20% ethanol at 4℃.

2.4 Analysis

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

3. Cleaning-in-Place

A 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, as they were not completely removed during the sample clarification steps.

Remove the strong hydrophobic binding protein, lipoprotein and lipid

Wash the column using 5-10 column volumes 30% isopropanol contacting for 15-20 min. Or you can choose the 2CV 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 distilled water

Remove the proteins combined with ion interacting

Wash the column with 1.5 M 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 1 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.
	Elution conditions are too mild.	Increase imidazole concentration in elution buffer. Or decrease buffer pH.
	Protein degradation or purification cause the his-tag to be removed.	Operate at 4℃. 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 high	Perform the purification at 4℃.
	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