



# HisCap Smart 6FF

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## 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.

**HisCap Smart 6FF** is one of a range of prepacked, ready-to-use columns for affinity chromatography. It is prepacked with 1ml and 5ml of **Ni Smart Beads 6FF**. Five different packing sizes are available. **HisCap Smart 6FF** has the standard interface and can be operated with a peristaltic pump or liquid chromatography systems, such as ÄKTA. It is fast, simple and easy operation.

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

Item	Description
Matrix Spherical	Highly cross-linked 6% agarose
Static Binding Capacity	> 10mg 6×His-tagged protein/ml medium
Particle size	45-165µm
Column Size	1ml 0.7X2.5cm 5ml 1.6X2.5cm
Maximum Pressure	0.3MPa, 3bar
Storage Solution	1×PBS containing 20% ethanol
Storage Temperature	4-30℃

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

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 filtering the buffers by passing them through a 0.22µm or 0.45 µm filter before use.

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

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

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

**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 7,000rpm(7,500×g) for 10-15min at 4 °C, 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 pretreatment when the samples solution contain the tolerance reagent and low target protein concentration.

## 2.3 Sample Purification

**HisCap Smart 6FF** is a prepacked, ready to use column for purification of 6X His-tagged proteins. The prepacked column provides fast, simple and easy separations in a convenient format.

- 1) Fill the syringe or pump tubing with binding buffer. 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 10 column volumes of binding buffer at 1 ml/min or 5 ml/min for 1 ml and 5 ml column respectively.
- 3) Apply the sample, using a syringe fitted to the connector or by pumping it onto the column.
- 4) Wash with 5 to 10 column volumes of binding buffer or until no material appears in the effluent.
- 5) Elute with 5 column volumes of elution buffer. Other volumes may be required if the interaction is difficult to break.

## 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 15-20min. 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 1.5M 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.
	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°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

