GE Healthcare

FAQ

Ni Sepharose High Performance
HisTrap HP
HisTrap HP Kit

Q When should I use Ni Sepharose™ 6 Fast Flow instead of Ni Sepharose™ High Performance?

A Both Ni Sepharose 6 Fast Flow and Ni Sepharose High Performance are optimized for purification of histidine-tagged proteins and the media are pre-charged with Ni^{2+}.

Ni Sepharose 6 Fast Flow is designed for fast, reliable scale-up of histidine-tagged protein purification. The medium is also well suited for batch/gravity flow purification and multi-well plate screening.

Ni Sepharose High Performance is optimized for high performance purification of histidine-tagged proteins with a chromatography system, such as ÄKTAdesign™ from GE Healthcare.

Q What is the protein binding capacity of Ni Sepharose High Performance?

A *Dynamic binding capacity:* At least 40 mg $(histidine)_6$-tagged protein/ml medium

Dynamic binding capacity conditions:

Sample:	1 mg/ml $(histidine)_6$-tagged pure proteins (M_r 28 000 or M_r 43 000) in binding buffer ($QB_{10\%}$ determination) or $(histidine)_6$-tagged protein bound from *E. coli* extract
Column volume:	0.25 ml or 1 ml
Flow rate:	0.25 ml/min or 1 ml/min
Binding buffer:	20 mM sodium phosphate, 0.5 M NaCl, 5 mM imidazole, pH 7.4
Elution buffer:	20 mM sodium phosphate, 0.5 M NaCl, 500 mM imidazole, pH 7.4

Note: Dynamic binding capacity is protein dependent.

Q What are the general running conditions recommended?

A **Recommended starting conditions:**

Binding buffer: 20 mM sodium phosphate, 0.5 M NaCl, 20–40 mM imidazole, pH 7.4

Elution buffer: 20 mM sodium phosphate, 0.5 M NaCl, 500 mM imidazole, pH 7.4

Note: The concentration of imidazole that will give optimal purification results (in terms of purity and yield) is protein-dependent, and is usually slightly higher for Ni Sepharose High Performance than for similar IMAC media on the market. This can be useful during the washing step as a higher concentration of imidazole in the wash buffer may wash out contaminants that have bound to the medium. This can result in elution of a more pure target protein.

Higher concentration of imidazole will not cause any problems in terms of increased baseline absorbance if a high purity imidazole is used, such imidazole gives essentially no absorbance at 280 nm.

Q Will the high capacity of Ni Sepharose High Performance result in higher non-specific binding?

A The non-specific binding of proteins is a result of several factors. Other proteins will only bind more to Ni Sepharose High Performance if these non-specific proteins are interacting directly with the Ni^{2+} or the metal chelating ligand. In addition, non-specific proteins can potentially be washed off at higher concentrations of imidazole, while keeping the target protein bound to the medium.

Q With the higher binding capacity, do you have to use harsh conditions to remove the target protein?

A Harsh conditions are not necessary to remove most target proteins. The proteins tested are all eluted below 500 mM imidazole; frequently between 100-300 mM imidazole. The protein is efficiently eluted from the column, resulting in high yields.

Q How many times can you run the Ni Sepharose High Performance while maintaining the same capacity?

A This is sample dependent. Reusing Ni Sepharose High Performance depends on the nature of the sample. To prevent cross-contamination, only reuse the column when purifying identical histidine-tagged proteins.

Q How do you measure the amount of Ni^{2+} in the eluent?

A This quantitative measurement assay is as follows:

The medium is run with 10 column volumes of a buffer with pH 4.0 and then the Ni^{2+} still bound on the column is measured spectroscopically and compared with the amount Ni^{2+} loaded.

Q Can you strip and regenerate the Ni Sepharose High Performance?

A Ni Sepharose High Performance can be stripped with 50 mM EDTA in 20 mM sodium phosphate, M NaCl, pH 7.4 and recharged with Ni^{2+} or other divalent cations, such as Cu^{2+}, Co^{2+}, Zn^{2+}, etc.

Q How can I strip and recharge Ni Sepharose High Performance?

A Recommended stripping buffer: 20 mM sodium phosphate, 0.5 M NaCl, 50 mM EDTA, pH 7.4.

1. Strip the column by washing with 5 to 10 column volumes of stripping buffer.
2. Washing with 5 to 10 column volumes of binding buffer.
3. Wash with 5 to 10 column volumes of distilled water.
4. Re-charge the column.

To recharge the water-washed column:

1. Load 0.5 column volumes of 0.1 M metal salt solution in distilled water on the column. Metal chloride and sulfate salts (e.g., 0.1 M $NiSO_4$) are commonly used.
2. Wash with 5 column volumes of distilled water.
3. Wash with 5 column volumes of binding buffer.

Note: It is important to wash with binding buffer as the last step to get the correct pH.

Q How is the Ni^{2+} leakage at low pH?

A At pH 4, the loss of Ni^{2+} is $< 5\%$ on Ni Sepharose High Performance (reproducible) compared to average 9% for Ni-NTA Superflow from Qiagen (wide batch to batch variations).

Note: Loss of metal ions is more pronounced at lower pH.

Q My target protein is extremely sensitive to Ni^{2+}. How do I reduce the Ni^{2+} leakage to a real minimum?

A Leakage of Ni^{2+} from Ni Sepharose High Performance is low under all conditions and resistance towards reducing agents is thus high.

Note: If using buffers/sample including reducing agents it is recommended to perform a blank run without reducing agents in the buffers before loading sample (to remove any weakly bound Ni^{2+} ions):

Blank run: Use binding and elution buffer *without* reducing agents

1. Wash the column with 5 column volumes of distilled water.
1. Wash with 5 column volumes of binding buffer.
2. Wash with 5 column volumes of elution buffer.
3. Equilibrate with 10 column volumes of binding buffer.

Q What different additives can I use together with Ni Sepharose High Performance and HisTrap™ HP?

A Tests performed in our laboratories show that Ni Sepharose High Performance is compatible with the following compounds at the concentrations given.

Reducing agents	5 mM DTE 5 mM DTT 20 mM ß-mercaptoethanol 5 mM TCEP 10 mM reduced glutathione	
Denaturing agents	8 M urea* 6 M guanidine hydrochloride*	
Detergents	2% Triton™ X-100 2% Tween™ 20 2% NP-40 2% cholate 1% CHAPS	Non-ionic detergent Non-ionic detergent Non-ionic detergent Anionic Detergent Zwitterionic Detergent
Additives	500 mM imidazole 20% ethanol 50% glycerol 100 mM Na_2SO_4 1.5 M NaCl 1 mM EDTA** 60 mM citrate**	
Buffer solutions	50 mM sodium phosphate, pH 7.4 100 mM Tris-HCl, pH 7.4 100 mM Tris-acetate, pH 7.4 100 mM HEPES, pH 7.4 100 mM MOPS, pH 7.4 100 mM sodium acetate, pH 4*	

* Tested for 1 week at +40 °C

** Generally, chelating agent should be used with caution (and only in the sample, not in buffers). Any metal ion stripping may be counteracted by addition of a small excess of $MgCl_2$ before centrifugation or filtration of the sample. Note that stripping effects may vary with applied sample volume.

Q **How do I remove imidazole from my target protein after elution?**

A If imidazole needs to be removed from the protein, use HiTrap™ Desalting 5 ml (can be coupled in series to increase the sample volume), a PD-10 column, or HiPrep™ 26/10 Desalting, depending on the sample volume.

Q **Do you see an improved purity (less non-specific binding) with high concentrations of NaCl?**

A In most cases, concentration of ions, imidazole, and other additives in the binding and wash buffers will influence the level of non-specific binding. Higher concentrations of NaCl (500 mM) generally reduce the level of non-specific binding.

Q **Is it possible to use high concentrations of Tris-HCl buffer and how does this compatibility to Tris buffers compare to other suppliers?**

A These are the Tris buffers systems that have been tested and work well.

50 mM Tris-HCl, pH 7.4

100 mM Tris-HCl, pH 7.4

100 mM Tris-acetate, pH 7.4

Tris buffers in high concentrations can adversely affect the binding of histidine-tagged target proteins to nickel medium from other suppliers; therefore, lower concentrations of Tris buffers (less than 50 mM) or phosphate buffers are generally used.

Q **How can I clean Ni Sepharose High Performance?**

A **Note:** Strip off the metal ions before cleaning (to prevent precipitation of metal salt)

To remove precipitated proteins, hydrophobically bound proteins and lipoproteins:

1. Washing the column with 1 M NaOH, contact time usually 1 to 2 hours (12 hours or more for endotoxin removal).
2. Wash the column with approximately 10 column volumes of binding buffer.
3. Wash with 5 to 10 column volumes of distilled water.

To remove strongly hydrophobically bound proteins, lipoproteins and lipids:

1. Wash the column with 5 to 10 column volumes 30% isopropanol for about 15 to 20 minutes.
2. Wash the column with approximately 10 column volumes of distilled water.
3. Re-charge the medium or wash with 5 column volumes 20% ethanol for storage.

Alternatively:

1. Wash the column with 2 column volumes of detergent in a basic or acidic solution. Use, for example, 0.1% to 0.5% nonionic detergent in 0.1 M acetic acid, contact time 1 to 2 hours.
2. After treatment, always remove residual detergent by washing with 5 column volumes of 70% ethanol.
3. Wash the column with approximately 10 column volumes of distilled water.
4. Re-charge the medium or wash with 5 column volumes 20% ethanol for storage.

To remove ionically bound proteins:

1. Wash the column with 1.5 M NaCl solution, contact time 10 to 15 minutes.
2. Wash the column with approximately 10 column volumes of distilled water.
3. Re-charge the medium or wash with 5 column volumes 20% ethanol for storage.

Q Is it possible to use Ni Sepharose High Performance by gravity or in 96- or 384-well multiwell plates for screening?

A Yes it is possible, but for gravity usage we recommend using Ni Sepharose 6 Fast Flow.

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