GE Healthcare

FAQ

HisTrap FF crude

Q What is HisTrap™ FF crude?

A HisTrap FF crude is a HiTrap column prepacked with Ni Sepharose™ 6 Fast Flow for purification of histidine-tagged proteins. The special design of the column allow direct loading of sonicated cell lysates without a clarification step.

The column tube is made of biocompatible polypropylene. The columns are delivered with a stopper on the inlet and a snap-off end on the outlet. Valco (1/16") fittings are standard making it easy to connect the columns directly to ÄKTAdesign™ systems without any use of connectors.

Note: HisTrap FF crude columns, similar to all other HiTrap coumns, cannot be opened or repacked.

The columns are simple to operate with a syringe and the supplied luer adaptor, a pump, or a chromatography system such as ÄKTAdesign.

ÄKTAdesign systems include preset method templates for HisTrap FF crude, which further simplifies operation. UNICORN™ column list will be updated with these columns in the next version.

Q What is the application for HisTrap FF crude?

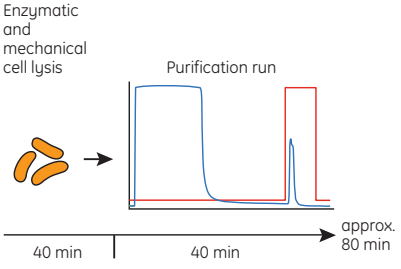
A Purification of histidine-tagged proteins directly from lysozym/Dnase-treated and sonicated cell lysates.

Direct loading of the sonicated cell lysate on the prepacked column without any clarification steps such as centrifugation and/or filtration.

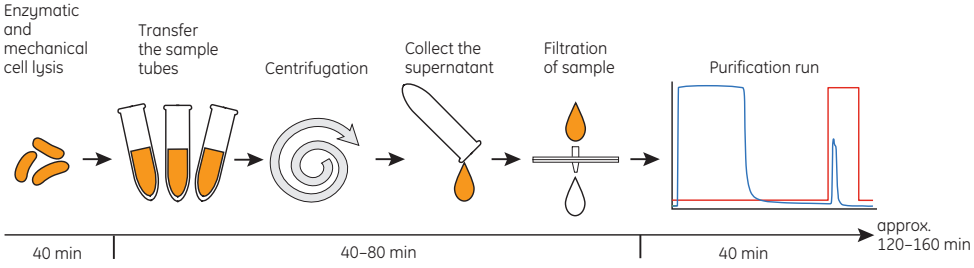
Q What is the benefit with HisTrap FF crude?

A
- Time saving, that will minimize the risk of degradation and oxidation of sensitive samples.
- Convenience

HisTrap FF crude



Conventional IMAC



A What column sizes are available for HisTrap FF crude?

Q 1 ml and 5 ml.

Q When should I use the new HisTrap FF crude versus HisTrap FF?

A

- The new HisTrap FF crude is optimized for purification of histidine-tagged proteins directly from sonicated (and lysozyme and Dnase treated) cell lysates.
- HisTrap FF crude should be used to save time and reduce the manual operations before the purification run starts. No centrifugation and filtration steps are necessary before sample loading on the HisTrap FF crude column.
- When the histidine-tagged target protein is sensitive for degradation by proteases in the cell lysate, it is optimal to use HisTrap FF crude. The cell lysate can be loaded directly onto the column after treatment with lysozyme, Dnase and sonication or homogenisation. No centrifugation or filtration steps are necessary. Therefore, oxidation or degradation of the target protein by proteases present in the cell lysate is minimized, based on the efficiency of the procedure with the new HisTrap FF crude.
- HisTrap FF should be loaded with sonicated and clarified (centrifuged and/or filtered) cell lysate to prevent too high backpressure when sample loading.

Q What are the critical parameters for loading sonicated lysate directly onto the HisTrap FF crude column?

A As a general rule it is recommended to extend the mechanical treatment to ensure a more complete lysis (keep the sample on ice to prevent overheating).

- Addition of lysozyme and Dnase to the sample to minimize viscosity due to DNA.
- That the sonication is very well done.

We have seen that the more sonicated the sample is the larger sample volume can be loaded without backpressure problems.

Note: It is important to keep the sample on ice during the sonication to prevent overheating and denaturation of the target protein.

- Load the sample directly after sonication.

If the sample has been freezed or been standing to long in the lab before loading on the column an extra sonication step is recommended.

See the recommended protocol for cell lysis below.

Q What is the recommended protocol for cell lysis?

A The protocol below have been used in our own laboratories, but other procedures may also work.

4-step protocol:

1. Dilution of cell paste: Add 5–10 ml of binding buffer for each gram of cell paste.

To prevent the binding of host cell proteins with exposed histidines, it is essential to include imidazole at a low concentration in the sample and binding buffer.

2. Enzymatic lysis: 0.2 mg/ml lysozyme, 20 µg/ml DNAse, 1 mM MgCl2, 1 mM Pefabloc™ SC or PMSF (final concentrations). Stir for 30 minutes at room temperature or +4 °C depending on the sensitivity of the target protein.
3. Mechanical lysis*:

   Sonication on ice, 5–10 min

   or

   Homogenisation with French press or other homogeniser

   or

   Freeze/thaw, repeated at least 5 times
4. Finally adjust the pH of the lysate. Do not use strong bases or acids for pH-adjustment (precipitation risk).

Start applying the crude lysate on the column directly after preparation.

* Mechanical lysis time may have to be extended compared to normally used protocols to secure an optimized lysate for sample loading (to prevent clogging of the column and back pressure problems). Remember that different proteins have different sensitivity to cell lysis and caution has to be taken to avoid frothing and overheating of the sample.

Note: If the sonicated or homogenised crude cell lysate is freezed before use precipitation and aggregation may increase. New sonication of the lysate can then prevent increased back pressure problems when loading on the column.

Q What volumes of sonicated cell lysate can be loaded on HisTrap FF crude columns?

A *HisTrap FF crude 1 ml:* < 100 ml

HisTrap FF crude 5 ml: < 500 ml

Note: These are general recommendations. The maximum volume is dependent on the specific sample.

It may be necessary to extend the sonication time to prevent backpressure problems when loading sample to the column. Loading of sample at +4 °C may increase the viscosity of the sample and therefore it may be necessary to decrease the sample volumes when running at +4 °C or if possible increase the sonication time.

Q How do I set the pressure limit setting on ÄKTAdesign systems to prevent reaching the maximum pressure too early?

A **Note:** When setting the total pressure limit the back pressure of the system (including the flow restrictor) should also be added to the maximum back pressure of the column. For example, when using an ÄKTAdesign system with a flow restrictor, the pressure limit should be set to 0.5 MPa (5 bar), where the column and the flow restrictor give a pressure of 0.3 MPa and 0.2 MPa, respectively. The contribute from the ÄKTAdesign system itself is usually very low (not measurable), unless extremely narrow tubings are used.

Q What is the matrix for Ni Sepharose 6 Fast Flow?

A The matrix is a highly cross-linked spherical 6% agarose with a mean particle size of 90 µm.

Q What is the metal ion capacity for Ni Sepharose 6 Fast Flow?

A Metal ion capacity: ~15 µmol Ni^{2+}/ml medium

Q What is the protein binding capacity for Ni Sepharose 6 Fast Flow?

A Dynamic binding capacity: Approx. 40 mg $(Histidine)_6$-tagged protein/ml medium

Dynamic binding capacity conditions:

Sample:	1 mg/ml $(Histidine)_6$-tagged pure protein (M_r 43 000) in binding buffer ($QB_{10\%}$ determination) or $(Histidine)_6$-tagged protein (M_r 28 000) bound in *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 6 Fast Flow than for similar IMAC media on the market (the same as for Ni Sepharose High Performance). 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 6 Fast Flow result in higher binding of unwanted proteins?**

A The binding of unwanted proteins is a result of several factors. Other proteins will only bind more to the new medium if these unwanted proteins are interacting directly with the Ni^{2+} or the metal chelating ligand. In addition, unwanted proteins can potentially be washed off at intermediate 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 **Can you strip and regenerate Ni Sepharose 6 Fast Flow medium?**

A Ni Sepharose 6 Fast Flow can be stripped with 50 mM EDTA in 20 mM sodium phosphate, 0.5 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 many times can you run without stripping while maintaining the same capacity?**

A The column does not have to be stripped between each purification, if the same protein is going to be purified. In our labs three purifications of the same crude cell lysate was performed on the same column without any loss in binding capacity purification.

Note: This is sample dependent. Reusing HisTrap FF crude depends on the nature of the sample. To prevent cross-contamination, only reuse the column when purifying identical histidine-tagged proteins.

Q **How many times can we use the medium without stripping and re-charging?**

A No general answer is possible as this is totally dependent on the sample, the cleaning procedures, clogging of filters etc. However, with un-clarified cell lysates stripping and cleaning of the column will be required more often than with clarified samples.

Q **With stripping and re-charging, will the capacity be back to the original level?**

A Depending on the nature of the sample, the capacity of the column can be regenerated in some cases with stripping and recharging alone. However, cleaning of the column with NaOH (as described in the instructions) may be necessary to regenerate the column binding capacity if very crude samples have been run.

Note: The column should be stripped before cleaning.

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 with a spectroscopic method and compared with the amount Ni^{2+} loaded.

Q What is the pH stability of Ni Sepharose 6 Fast Flow, prepacked in HisTrap FF crude?

A pH stability: short term (< 2 hours): 2–14
long term (< 1 week): 3–12

Q What different additives can I use together with Ni Sepharose 6 Fast Flow, prepacked in HisTrap FF crude?

A Tests performed in our laboratories show that Ni Sepharose 6 Fast Flow is compatible with the following compounds, at least at the concentrations given.

Additives:	Buffer substances:	Reducing agents*:	Denaturing agents:
500 mM imidazole	50 mM sodium phosphate, pH 7.4	5 mM DTE	8 M urea**
20% ethanol	100 mM Tris-HCl, pH 7.4	5 mM DTT	6 M guanidine hydrochloride**
50% glycerol	100 mM Tris-acetate, pH 7.4	20 mM ß-mercaptoethanol	**Detergents:**
100 mM Na_2SO_4	100 mM HEPES, pH 7.4	5 mM TCEP	2% Triton™ X-100 (nonionic)
1.5 M NaCl	100 mM MOPS, pH 7.4	10 mM reduced glutathion	2% Tween™ 20 (nonionic)
1 mM EDTA***	100 mM sodium acetate, pH 4**		2% NP-40 (nonionic)
60 mM citrate***			2% cholate (anionic)
			1% CHAPS (zwitterionic)

* For best results, a blank run including reducing agents in the buffers is recommended prior to the purification of the target protein. (See below).

** Tested for 1 week at +40 °C.

*** The strong chelator EDTA has been used successfully in some cases at 1 mM. Generally, chelating agents should be used with caution (and only in the sample, not the buffer). Any metal ion stripping may be counteracted by addition of a small excess of $MgCl_2$ to the sample. Note that stripping effects may vary with applied sample volume.

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

A At pH 4, the loss of Ni^{2+} is < 5% on Ni Sepharose 6 Fast Flow (very 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 leakage to a real minimum (below <5 %)?

A Leakage of Ni^{2+} from Ni Sepharose 6 Fast Flow is low under all conditions and resistance towards reducing agents is thus high.

Note: Before using buffers/sample including reducing agents it is recommended to perform a blank run without reducing agents before loading sample (to remove any weakly bound Ni^{2+} ions and minimize change of color of the medium):

Blank run: Use binding and elution buffer without reducing agents

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

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 binding of unwanted proteins) 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 binding of unwanted proteins. Higher concentrations of NaCl (500 mM) generally reduce the level of non-specific binding due to charge interactions.

Q How does 1 mM EDTA affect the binding capacity and nickel leakage?

A 1 mM EDTA has been used successfully in some cases (only in samples, not in buffers), but it is not recommended.

Q How does 5 mM DTT affect the binding capacity and nickel leakage?

A DTT, tested up to 5 mM, does not affect the binding capacity or nickel ion leakage of the Ni Sepharose 6 Fast Flow. There is no effect on the performance of the medium.

Note: Performing a blank run before loading sample/buffers including reducing agents will minimize the change of color of the medium.

Q Is it possible to use high concentrations of Tris-HCl buffer?

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

Q How does this compatibility to Tris buffers compare to other suppliers?

A 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 strip and recharge the medium?

A Recommended stripping buffer:

20 mM sodium phosphate, 0.5 M NaCl, 50 mM EDTA, pH 7.4

Strip the column by washing with 5–10 column volumes of stripping buffer. Follow this by washing with 5–10 column volumes of binding buffer and 5–10 column volumes of distilled water before re-charging the column.

To recharge the water-washed column, load 0.5 column volumes of 0.1 M metal salt solution in distilled water on the column. Metal chloride and sulphate salts, e.g. 0.1 M $NiSO_4$, are commonly used. Wash with 5 column volumes distilled water, and 5 column volumes binding buffer.

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

Q How can I clean HisTrap FF crude?

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

- Remove precipitated proteins, hydrophobically bound proteins and lipoproteins by washing the column with 1 M NaOH, contact time usually 1–2 hours (12 hours or more for endotoxin removal). Then wash the column with approx. 10 column volumes of binding buffer, followed by 5–10 column volumes of distilled water.
- Remove strongly hydrophobically bound proteins, lipoproteins and lipids by washing the column with 5–10 column volumes 30% isopropanol for about 15–20 minutes. Then wash the column with approx. 10 column volumes of distilled water. Re-charge the medium or wash with 5 column volumes of 20% ethanol for storage. Alternatively, wash the column with 2 column volumes of detergent in a basic or acidic solution. Use, for example, 0.1–0.5% nonionic detergent in 0.1 M acetic acid, contact time 1–2 hours. After treatment, always remove residual detergent by washing with 5 column volumes of 70% ethanol. Then wash the column with approx. 10 column volumes of distilled water. Re-charge the medium or wash with 5 column volumes 20% ethanol for storage.
- Remove ionically bound proteins by washing the column with 1.5 M NaCl solution, contact time 10–15 minutes. Then wash the column with approx. 10 column volumes of distilled water. Re-charge the medium or wash with 5 column volumes 20% ethanol for storage.

Q What is the chemical stability of HisTrap FF crude (Ni Sepharose 6 Fast Flow)?

A **The medium is stable in the following:**

1 week at 40 °C:	0.01 M HCl, 0.1 M NaOH
12 hours:	1 M NaOH, 70% HAc.
1 hour:	2% SDS.
30 min:	30% 2-propanol

Q How do I store HisTrap FF crude?

A Store in 20% ethanol at +4 to +30 °C.

Q What new products are available?

A

HisTrap FF crude 5 x 1 ml	Code No: 11-0004-58
HisTrap FF crude 100 x 1 ml*	Code No: 11-0004-59
HisTrap FF crude 5 x 5 ml	Code No: 17-5286-01
HisTrap FF crude 100 x 5 ml*	Code No: 17-5286-02

- Special pack size delivered on specific customer order. Delivery time from accepted order approx. 20 days.

Connectors included in each HisTrap FF crude package:

1/16" male/luer female 1
Union luerlock female/M6 female 1
Union 1/16" female/M6 male 1
Tubing connector flangeless/M6 male 1
Tubing connector flangeless/M6 female 1
Union M6 female/1/16" male 1
Stop plug female, 1/16" 2, 5, or 7

Q How do I scale-up?

A Scaling up from a HisTrap FF crude 1 ml column to a 5 ml column while keeping the same linear flow rate provides highly consistent results.

Q Do the new HisTrap FF crude columns have the new valco (1/16") fittings?

A Yes. This makes it possible to connect these columns directly into ÄKTAdesign™ without any extra connectors. This will increase the convenience and minimize possible leakage problems.

Q What is the shelf life of the HisTrap FF crude columns?

A A forced shelf life study has been done and shows a shelf life of at least 3 years in +4 to +30 °C.

Q What printed material is available?

A

Data File:	"HisTrap FF crudecolumns", 11-0012-37, AA
Instructions:	HisTrap FF crude 1 ml and 5 ml, 11-0012-38, AA

Troubleshooting

The following tips may be of assistance. If you have any further questions about your HisTrap FF crude column, please visit: www.amershambiosciences.com/hitrap contact our technical support, or your local representative.

Increased back pressure:

- Increase the efficiency of the mechanical cell disruption (for example increase the sonication time). Keep the sample on ice to avoid frothing and overheating as this may denature the target protein. Over-sonication can also lead to co-purification of host proteins with the target protein.
- Increase dilution of the cell paste before sonication or dilute after the sonication to reduce viscosity.
- If the lysate is very viscous due to a high concentration of host nucleic acid, continue sonication until the viscosity is reduced, and/or add an additional dose of DNAse and Mg_{2+}. Alternatively, draw the lysate through a syringe needle several times.
- Freeze/thaw of the crude lysate may increase precipitation and aggregation. New sonication of the lysate can prevent increased back pressure problems when loading on the column.
- If the purification is performed at +4 °C move to room temperature if possible (will reduce viscosity of the sample).
- Decrease flow rate during sample loading.

Column has clogged:

- Replace with a new column. Optimize sample pre-treatment before next sample loading.

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