HisTrap FF crude Kit

Instructions

HisTrap™ FF crude Kit is a kit for convenient, fast, and efficient purification of histidine-tagged recombinant proteins. HisTrap FF crude Kit contains everything necessary to perform complete purifications without using complicated equipment. It also eliminates timeconsuming buffer preparation and column packing.

The HisTrap FF crude columns included in the kit are prepacked with Ni Sepharose™ 6 Fast Flow, a pre-charged IMAC medium with low nickel (Ni^{2+}) ion leakage and compatibility with a wide range of buffers, reducing agents, denaturants, and detergents.

The specific design of the column allows direct loading of homogenized unclarified cell lysate without the need for filtration and/or centrifugation. This decreases the total purification time that may be important when working with sensitive target proteins.

CAUTION
Contains nickel. May produce an allergic reaction.

Code No.	
28-4014-77	HisTrap FF crude Kit

Code No.		
	containing:	
	HisTrap FF crude columns	3 × 1 ml
	Phosphate buffer, 8× stock solution, pH 7.4	2 × 50 ml
	2 M Imidazole, pH 7.4	50 ml
	Syringe, 5 ml	1

Connector kit

Connectors supplied	Usage	No. supplied
1/16" male/ luer female	Connection of syringe to top of HiTrap column	1
Tubing connector flangeless/M6 female	Connection of tubing (e.g. Peristaltic Pump P1) to bottom of HiTrap column[1]	1
Tubing connector flangeless/M6 male	Connection of tubing (e.g. Peristaltic Pump P1) to top of HiTrap column[2]	1
Union 1/16" female/M6 male	Connection to original FPLC System through bottom of HiTrap column	1
Union M6 female/1/16" male	Connection to original FPLC System through top of HiTrap column	1
Stop plug female, 1/16"	Sealing bottom of HiTrap column	2, 5 or 7

1 Union 1/16" female/M6 male is also needed.

2 Union M6 female/1/16" male is also needed.

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1 Introduction

HisTrap FF crude Kit is designed for rapid, mild affinity purification of histidine-tagged proteins. Using HisTrap FF crude Kit, histidine-tagged proteins can convenient be prepared to high purity in one step.

Histidine-tagged proteins can be purified directly from unclarified cell lysates and are recovered from the column under mild elution conditions that preserve their antigenicity and functionality.

The kit contains three ready to use 1-ml HisTrap FF crude columns (precharged with Ni^{2+}), buffer concentrates, a 5-ml syringe and connectors. The kit provides a sufficient volume of buffer concentrates to perform 10–12 purifications when operated with a syringe. The special design of the column, together with an optimized matrix, provides fast, easy, and reproducible separations in a convenient format. The possibility for direct loading of unclarified cell lysates decreases the total purification time and may increase the possibility to purify sensitive target proteins without losing their activity.

2 General Considerations

HisTrap FF crude columns are supplied precharged with Ni^{2+} ions, and will selectively retain proteins if complex-forming amino acid residues, in particular histidine, are exposed on the surface of the protein. Additional histidines, such as in the case of $(histidine)_6$-tag, increases the affinity for Ni^{2+} and generally makes the histidinetagged protein the strongest binder among other proteins, in for example an *E. coli* lysate.

Histidine-tagged proteins can be eluted from HisTrap FF crude with buffers containing imidazole. Imidazole at low concentrations is commonly used in the binding and wash buffers to minimize binding of unwanted host cell proteins. For the same reason it is important to also include imidazole in the sample (generally, at the same concentration as in the wash buffer). At somewhat higher concentrations, imidazole may also decrease the binding of histidine-tagged proteins. The imidazole concentration must therefore be optimized to ensure the best balance of high purity (low binding of host cell proteins) and high yield (binding of histidine-tagged target protein). This optimal concentration is different for different histidine-tagged proteins, and is usually slightly higher for Ni Sepharose 6 Fast low than for similar IMAC media on the market (see Data File, 11-0008-86, Ni Sepharose 6 Fast Flow. Use a highly pure imidazole, such as the imidazole provided in the kit; such imidazole gives essentially no absorbance at 280 nm.

The optimal imidazole concentration can be determined by stepwise elution of the protein with buffers containing increasing concentrations of imidazole. Stepwise elution, as described in the *Optimization Protocol, on page 12* is technically simple and fast, and is suitable for syringe operation. The results from this stepwise elution will provide information on which two imidazole concentrations (wash and elution) are the most suitable for purifications of identical protein samples. The concentration of imidazole needed in the sample and the wash buffer to prevent binding of unwanted host cell proteins is generally more critical to determine than

the concentration needed for elution where 500 mM in most cases can be used for the latter purpose. When high yield is more important than optimum purity, the *Basic Purification Protocol, on page 9* may be used directly, without prior optimization.

If the histidine-tagged proteins are expressed as inclusion bodies, see *Histidine-tagged protein is eluted during sample loading/wash:, on page 21*.

3 Sample Preparation

For optimal conditions for growth, induction, and cell lysis of your recombinant histidine-tagged clones, please refer to established protocols. The following is a general protocol for sample preparation:

Step	Action
1	Harvest cells from the culture by centrifugation (e.g. by centrifugation at 7 000–8 000 × g for 10 minutes or 1000–1500 × g for 30 minutes at 4°C).
2	Discard the supernatant. Place the bacterial pellet on ice.

Recommended four-step protocol for cell lysis

The protocol below has been used successfully in our own laboratories, but other established procedures may also work.

Step	Action
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 (see *Optimization Protocol, on page 12*).
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 min at room temperature or 4°C depending on the sensitivity of the target protein.
3	Mechanical lysis[1]: **a.** Sonication on ice, approx. 10 min or **b.** Homogenization with a French press or other homogenizer or **c.** Freeze/thaw, repeated at least five times
4	**Adjust the pH of the lysate:** Do not use strong bases or acids for pH adjustment (precipitation risk). Apply the unclarified lysate on the column directly after preparation.

[1] Mechanical lysis time may have to be extended compared with standard protocols to secure an optimized lysate for sample loading (to prevent clogging of the column and back pressure problems). 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 homogenized unclarified cell lysate is frozen before use, precipitation and aggregation may increase. New sonication of the lysate can then prevent increased back pressure problems when loading on the column.*

Note: *To minimize binding of host cell proteins, the sample should have the same concentration of imidazole as the binding and wash buffers.*
The concentration of imidazole is protein dependent (see Chapter 2 General Considerations, on page 4).

4 Protein Purification Protocols

Column preparation

Step	Action
1	Fill the syringe with deionized water. Remove the stopper and connect the column to the syringe with the provided luer connector "drop-to-drop" to avoid introducing air into the column.
2	Remove the snap-off end. Wash the column with 5 ml distilled water. If air becomes trapped in the column, wash the column with deionized water until the air disappears.

Note: *Leakage of nickel ions from HisTrap FF crude columns is low under all normal conditions. For very critical applications, the minimal leakage during purification can be even further diminished by performing a blank run before loading sample, see below.*

Note: *Ni Sepharose 6 Fast Flow is compatible with reducing agents (see Chapter 9 Appendix A, on page 23). However, removal of any weakly bound Ni^{2+} ions by performing a blank run without reducing agents (as described below), before applying buffer/sample including reducing agents, is recommended. Do not store HisTrap FF crude columns with buffers including reducing agents.*

Blank run

Use binding and elution buffers without reducing agent.

Step	Action
1	Wash the column with 5 ml of deionized water (to remove the 20% ethanol).
2	Wash with 5 ml of elution buffer.
3	Equilibrate with 5–10 ml of binding buffer.

Basic Purification Protocol

When high yield is more important than optimum purity, use the following protocol. When optimum purity is required proceed to the *Optimization Protocol, on page 12*.

Step	Action
1	Prepare 24 ml binding buffer. Mix 3 ml 8× Phosphate buffer stock solution with 0.24 ml 2 M imidazole and add water up to 24 ml. Check pH and adjust to pH 7.4–7.6 if necessary. This buffer will contain 20 mM phosphate (1×), 500 mM NaCl, and 20 mM imidazole.

Step	Action
2	Prepare 8 ml elution buffer. Mix 1 ml 8× Phosphate buffer stock solution with 2 ml 2 M imidazole and add distilled water up to 8 ml. Check pH and adjust to pH 7.4–7.6 if necessary. This buffer will contain 20 mM phosphate, 500 mM NaCl, and 500 mM imidazole.

Note:

The high salt concentration in the buffer stock solution may cause salt crystals to form at low temperature. These crystals will dissolve at room temperature. We therefore recommend that the buffer stock solutions be allowed to reach room temperature before use. The formation of salt crystals that dissolve at room temperature does not affect the performance of the product.

3	Using the syringe, equilibrate the column with 10 ml binding buffer.
4	Apply the unclarified lysate with the syringe. Collect the flowthrough fraction. A pump (e.g. Peristaltic Pump P-1) is convenient for large volumes (more than 15 ml) using a flow rate of max. 3 ml/min. Typical loading volumes of unclarified lysate (highly dependent on specific sample, sample pretreatment, and temperature at sample loading): Up to 100 ml.

Step	Action

Note:

Sample loading at 4°C may increase the viscosity of the sample. An adverse effect of increased sample viscosity is that maximum back pressure for the column is reached at a lower sample volume loading on the column. Large volumes may increase back pressure, making the use of a syringe more difficult.

5 Wash with 10 ml binding buffer. Collect the wash fraction.

6 Elute with 5 ml elution buffer. Avoid dilution of the eluate by collecting it in 1 ml fractions.

7 Check the purification by running an aliquot of the collected samples on SDS-PAGE. The purified protein is most likely found in the second and third milliliter of the elution step.

Note:

For A_{280} nm measurement, use the elution buffer as a blank. If imidazole needs to be removed use HiTrap Desalting, HiPrep™ 26/10 Desalting or PD-10 Desalting Columns.

8 After the protein has been eluted, regenerate the column by washing it with binding buffer (according to step 3).

The column is now ready for a new purification. The reuse of HisTrap FF crude depends on the nature of the sample and should only be performed with identical histidine-tagged proteins to prevent cross-contamination.

Optimization Protocol

When optimum purity is needed, the following general protocol for stepwise gradient elution should be used. The next time the same protein is to be purified, the number of steps can be reduced to those described under the Basic Purification Protocol with the optimal imidazole concentrations selected here.

Step	Action
1	Prepare buffers according to Table 1.

Table 1. Mixing table for one purification.

Imidazole concentration in buffer	Phosphate buffer 8× stock solution pH 7.4	2 M Imidazole pH 7.4	Deionized water
20 mM	3.0 ml	0.24 ml	to 24 ml
40 mM	1.0 ml	0.16 ml	to 8 ml
60 mM	1.0 ml	0.24 ml	to 8 ml
100 mM	1.0 ml	0.40 ml	to 8 ml
300 mM	1.0 ml	1.20 ml	to 8 ml
500 mM	1.0 ml	2.00 ml	to 8 ml

Use 1 × Phosphate buffer including 20 mM imidazole as binding buffer and five steps ranging up to 500 mM imidazole as elution buffers. Check pH after mixing and adjust to pH 7.4–7.6 if necessary. For buffers with closer steps of imidazole concentrations, and for other volumes, see *Chapter 10 Appendix B, on page 28*.

Step	Action

Note:

The high salt concentration in the buffer stock solution may cause salt crystals to form at low temperature. These crystals will dissolve at room temperature. We therefore recommend that the buffer stock solutions be allowed to reach room temperature before use. The formation of salt crystals that dissolve at room temperature does not affect the performance of the product.

2 Wash the column according to Column preparation.

3 Equilibrate the column with 10 ml binding buffer (1 × Phosphate buffer, 20 mM imidazole, pH 7.4), using the syringe.

4 Apply the sample. Collect the flowthrough fraction.

5 Wash with 10 ml binding buffer. Collect the wash fraction.

6 Start elution with 5 ml 1 × Phosphate buffer containing 40 mM imidazole. Avoid dilution by collecting the eluate in 1 ml fractions.

7 Proceed with the next imidazole concentration, for example elute with 5 ml 1 × Phosphate buffer containing 60 mM imidazole. Collect the eluate in 1 ml fractions as above.

8 Proceed with the buffers of increasing imidazole concentration, as described in steps 6 and 7. The purified protein is most likely found in the second and third milliliter of one of the elution steps.

Step	Action
9	Check the different fractions, for example by SDS-PAGE and/or Western blotting.

Note:

For A_{280} measurements, use the elution buffers as blanks. If imidazole is to be removed, use HiTrap Desalting, HiPrep 26/10 Desalting or PD-10 Desalting Columns.

10 After the protein has been eluted, re-equilibrate the column with binding buffer (according to step 3).

The column is now ready for a new purification. The reuse of HisTrap FF crude depends on the nature of the sample and should only be performed with identical histidine-tagged proteins to prevent cross-contamination.

The results of the above experiment provides information about the optimal binding and elution buffers. The optimum elution buffer is the one that eluted the histidine-tagged protein. The optimum binding (wash) buffer is the one from the step before, with a lower concentration of imidazole. Using the highest possible concentration of imidazole in the binding buffer will give the highest purity of the purified protein. Use these buffers for the next purification of an identical protein. The concentration of imidazole needed to prevent nonspecific binding of host cell proteins (without any elution of histidine-tagged protein) is generally more important to determine than the concentration needed for elution. 500 mM can in most cases be used for elution.

Scaling up

HisTrap FF crude is also available in a 5-ml format, see *Chapter 8 Ordering information, on page 21*.

Pump operation

The purification process can also be performed using different chromatography systems, such as ÄKTA™ design, FPLC System or a low-pressure laboratory pump (e.g. Peristaltic Pump P-1). This would be an alternative in some situations, for example when large sample volumes have to be passed through the column. The necessary connectors are listed below. Connect the column to the pump or chromatography system with the connectors supplied. Use a flow rate of 1–4 ml/min.

System/ equipment	Connector
Syringe	1/16" male/luer female
FPLC System	1/16" female/M6 male and M6 female/1/16" male
ÄKTA design systems	No connector needed

5 Column Cleaning and Storage

Stripping and Cleaning

To remove the Ni^{2+} ions prior to cleaning, strip the column with 10 ml 20 mM sodium phosphate, 500 mM NaCl, 50 mM EDTA, pH 7.4. To remove precipitated proteins, debris, etc., remove first nickel ions by stripping the column, fill the column with 1 M NaOH, and incubate for up to 2 hours. Wash out the dissolved proteins with 10 ml water, and a buffer with pH ~ 7 until the pH of the flowthrough reaches pH ~ 7.

Recharging with nickel ions is done by washing with 10 ml distilled water, followed by 0.5 ml 0.1 M $NiSO_4$ (dissolved in distilled water), 5 ml distilled water, and 5 ml binding buffer (to adjust pH).

Storage

For storage, fill the HisTrap FF crude column with 20% ethanol. Seal the column with the provided stop plugs to avoid dehydration The recommended storage temperature for the kit is 4°C to 30°C.

6 Troubleshooting

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

Note: *When using urea or Gua-HCl (as described below), protein unfolding generally takes place. Refolding on-column (or after elution) is protein-dependent.*

Tip: *To minimize dilution of the sample, solid urea or Gua-HCl can be added.*

Tip: *Samples containing urea can be analyzed directly by SDS-PAGE whereas samples containing Gua-HCl must be buffer-exchanged to a buffer with urea before SDS-PAGE.*

Increased back pressure:

- Increase the efficiency of the mechanical cell disruption (for example increase 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 copurification 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+} (see *Chapter 3 Sample Preparation,*). Alternatively, draw the lysate through a syringe needle several times.
- Freeze/thaw of the unclarified lysate may increase precipitation and aggregation. Sonication of the thawed lysate can prevent increased back pressure problems when loading on the column.
- If the purification has been performed at 4°C, move to room temperature if possible (sample viscosity is reduced at room temperature).
- Decrease flow rate during sample loading.

Column has clogged:

- If cleaning-in-place is unsuccessful, replace the column. Optimize sample pretreatment before the next sample loading.

Protein is difficult to dissolve or precipitates during purification:

- **The following additives may be used:** 2% Triton™ X-100, 2% Tween™ 20, 2% NP-40, 2% cholate, 1% CHAPS, 1.5 M NaCl, 50% glycerol, 20 mM β-mercaptoethanol, 1–3 mM DTT or DTE (up to 5 mM is possible but depends on the sample and the sample volume), 5 mM TCEP, 10 mM reduced glutathione, 8 M urea, or 6 M Gua-HCl. Mix gently for 30 min to aid solubilization of the tagged protein (inclusion bodies may require longer mixing). Note that Triton X-100 and NP-40 (but not Tween) have a high absorbance at 280 nm. Furthermore, detergents cannot be easily removed by buffer exchange.
- **The protein might be insoluble (inclusion bodies):** The protein can usually be solubilized (and unfolded) from inclusion bodies using common denaturants such as 4–6 M Gua-HCl, 4–8 M urea, or strong detergents.

Prepare buffers containing 20 mM sodium phosphate, 8 M urea, or 6 M Gua-HCl, and suitable imidazole concentrations, pH 7.4–7.6. Buffers with urea should also include 500 mM NaCl. Use these buffers for sample preparation, as well as binding buffer and as elution buffer. For sample preparation and binding buffer, use 10–20 mM imidazole or the concentration selected during optimization trials (including urea or Gua-HCl). To minimize dilution of the sample, solid urea or Gua-HCl can be added.

No histidine-tagged protein in the purified fractions:

- **Elution conditions are too mild (histidine-tagged protein still bound):** Elute with an increasing imidazole gradient or decreasing pH to determine the optimal elution conditions.
- **Protein has precipitated in the column:** Decrease amount of sample, or decrease protein concentration by eluting with linear imidazole gradient instead of imidazole steps. Try detergents or change NaCl concentration, or elute under denaturing (unfolding) conditions (use 4–8 M urea or 4–6 M Gua-HCl).
- **Nonspecific hydrophobic or other interaction:** Add a nonionic detergent to the elution buffer (e.g. 0.2% detergent) or increase the NaCl concentration.
- **Protein found in the flowthrough:** Concentration of imidazole in the sample and/or binding buffer is too high; decrease imidazole concentration.
- **Protein found in the flowthrough:** Histidine tag may be insufficiently exposed; perform purification of unfolded protein in urea or Gua-HCl as for inclusion bodies. To minimize dilution of the sample, solid urea or Gua-HCl can be added.
- **Protein found in the flowthrough:** Buffer/sample composition is not optimal; check pH and composition of sample and binding buffer. Ensure that the concentration of chelating or strong reducing agents in the sample as well as the concentration of imidazole is not too high.

The eluted protein is not pure (multiple bands on SDS polyacrylamide gel):

- **Partial degradation of tagged protein by proteases:** Add protease inhibitors (use EDTA with caution, see *Table 2, on page 24*).
- **Contaminants have high affinity for nickel ions:** Elute with a stepwise or linear imidazole gradient to determine optimal imidazole concentrations to use for binding and for wash; add imidazole to the sample in the same concentration as the binding buffer. Wash thoroughly before elution with binding buffer containing the highest possible imidazole concentration (chosen imidazole concentration must not cause elution of the histidine-tagged protein). A shallow imidazole gradient (20 column volumes or more), may separate proteins with similar binding strengths. If optimized conditions do not remove contaminants, further purification by ion exchange chromatography (HiTrap Q HP or HiTrap SP HP) and/or gel filtration (Superdex™ Peptide, Superdex 75, or Superdex 200) may be necessary.
- **Contaminants are associated with tagged proteins:** Add detergent and/or reducing agents before sonicating cells. Increase detergent levels (e.g. up to 2% detergent or 2% Tween 20), or add glycerol (up to 50%) to the wash buffer to disrupt nonspecific interactions.

Histidine-tagged protein is eluted during sample loading/wash:

- **Buffer/sample composition is not optimal:** Check pH and composition of sample and binding buffer. Ensure that the concentration of chelating or strong reducing agents in the sample, as well as the concentration of imidazole is not too high.
- **Histidine tag is partially obstructed:** Purify under denaturing conditions (use 4–8 M urea or 4–6 M Gua-HCl).
- **Column capacity is exceeded:** If HisTrap FF crude 1-ml columns have been used, change to a larger column, HisTrap FF crude 5 ml.

7 Intended use

The HisTrap FF crude Kit is intended for research use only, and shall not be used in any clinical or in vitro procedures for diagnostic purposes.

8 Ordering information

Product	No. Supplied	Code No.
HisTrap FF crude Kit	1 kit	28-4014-77

Related products	No. Supplied	Code No.
HisTrap FF crude Kit	5 × 1 ml	11-0004-58
	100 × 1 ml[1]	11-0004-59
	5 × 5 ml	17-5286-01
	100 × 5 ml[1]	17-5286-02

Related products	No. Supplied	Code No.
HiTrap Desalting	5 × 5 ml	17-1408-01
HiPrep 26/10 Desalting	1 × 53 ml	17-5087-01
	4 × 53 ml	17-5087-02
PD-10 Desalting Column	30	17-0851-01

[1] Special pack size delivered on specific customer order.

Accessories	No. Supplied	Code No.
1/16" male/luer female[1]	2	18-1112-51
Tubing connector flangeless/M6 female[1]	2	18-1003-68
Tubing connector flangeless/M6 male[1]	2	18-1017-98
Union 1/16" female/M6 male[1]	6	18-1112-57
Union M6 female /1/16" male[1]	5	18-3858-01
Union luerlock female/M6 female	2	18-1027-12
HiTrap/HiPrep, 1/16" male connector for ÄKTA design	8	28-4010-81
Stop plug female, 1/16"[2]	5	11-0004-64
Fingertight stop plug, 1/16"[3]	5	11-0003-55

[1] One connector included in each HiTrap package.

[2] Two, five, or seven stop plugs female included in HiTrap packages depending on the product.

[3] One fingertight stop plug is connected to the top of each HiTrap column at delivery.

Related literature	Code No.
Recombinant Protein Purification Handbook, Methods and Principles	18-1142-75
Affinity Chromatography Handbook, Principles & Methods	18-1022-29
Convenient Protein Purification, HiTrap Column Guide	18-1129-81
Affinity Chromatography Columns and Media, Selection guide	18-1121-86

9 Appendix A

Description & characteristics

Medium

HisTrap FF crude is packed with Ni Sepharose 6 Fast Flow. Ni Sepharose 6 Fast Flow consists of 90 µm particles of highly crosslinked agarose to which Ni^{2+} ions are bound by a stable chelating group. This coupling technique gives negligible metal-ion leakage, high binding capacity, and high performance. The medium is compatible with all commonly used aqueous buffers and denaturants, such as 6 M Gua-HCl, and 8 M urea. EDTA, EGTA, and other strong chelators should be used with caution during purification (see *Table 2, on page 24*). The medium is optimized for purification of histidine-tagged proteins.

Column

The column is made of polypropylene, which does not interact with biomolecules. Each column is delivered with a stop plug on the inlet and a snap-off end on the outlet. The columns have porous top and bottom frits that allow high flow rates. Separations can be easily performed using the syringe supplied or alternatively, a low pressure laboratory pump, for example Peristaltic Pump P-1 or an ÄKTA design chromatography system.

The column cannot be opened or refilled.

Note: *To prevent leakage it is essential to ensure that the connector is tight.*

Table 2. Characteristics of HisTrap FF crude 1 ml.

Matrix	Highly cross-linked spherical agarose, 6%
Column volume	1 ml
Column dimensions	0.7 × 2.5 cm
Dynamic binding capacity[1]	At least 40 mg (histidine)6-tagged protein per column
Average particle size	90 µm
Pre-charged metal ion	Ni^{2+}
Maximum flow rate	4 ml/min
Recommended flow rate	1–4 ml/min
Maximum back pressure[2]	0.3 MPa, 3 bar
Compatibility during use	Stable in all commonly used aqueous buffers, reducing agents and denaturants. See *Table 3, on page 27*.

Chemical stability[3]	0.01 M HCl, 0.1 M NaOH. Tested for 1 week at 40°C. 1 M NaOH, 70% acetic acid. Tested for 12 hours. 2% SDS. Tested for 1 hour. 30% 2-propanol. Tested for 30 minutes.
pH stability[3]	
Short term (at least 2 hours)	2 to 14
Long term (< 1 week)	3 to 12
Storage	4°C to 30°C in 20% ethanol (antimicrobial agent)
Avoid	Chelating agents e.g. EDTA, EGTA, citrate

[1] Dynamic binding capacity conditions:

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

Note:
Dynamic binding capacity is protein-dependent

[2] H_2O at room temperature.

[3] Ni^{2+}-stripped medium.

Buffers

The phosphate buffer and the imidazole solution included in HisTrap FF crude Kit have been prepared using the highest quality salts and water and filtered through a 0.45 µm filter.

Note: *The high salt concentration in the buffer stock solution may cause salt crystals to form at low temperature. These crystals will dissolve at room temperature. We therefore recommend that the buffer stock solutions be allowed to reach room temperature before use. The formation of salt crystals that dissolve at room temperature does not affect the performance of the product.*

Denaturing conditions

The buffers described above refer to native conditions. For denaturing conditions use 20 mM sodium phosphate, 8 M urea or 6 M Gua-HCl and imidazole concentrations in the range 10 mM–500 mM, pH 7.4. Buffers with urea should also contain 500 mM NaCl.

Buffers recipes

If larger volumes of solutions are needed than supplied in the kit they can be prepared as follows:

- **8× Phosphate buffer, pH 7.4**

  To 1.42 g Na2HPO4 × $2H_2O$ (177.99 g/mol), 1.11 g NaH_2PO_4 × H_2O (137.99 g/mol), and 23.38 g NaCl (58.44 g/mol), add distilled water to 90 ml and dissolve completely. Adjust the pH to 7.4. Add distilled water to 100 ml and filter through a 0.45 µm filter.

  This gives a final concentration of 160 mM phosphate and 4 M NaCl.

- **2 M Imidazole, pH 7.4**

  To 13.62 g imidazole (68.08 g/mol) add distilled water to 90 ml and dissolve completely. Adjust the pH to 7.4 using conc. HCl. Add distilled water to 100 ml and filter through a 0.45 µm filter. Use a highly pure imidazole, that has essentially no absorbance at 280 nm.

Table 3. Ni Sepharose 6 Fast Flow is compatible with the following compounds, at least at the concentrations given.

Reducing agents[1]	5 mM DTE
	5 mM DTT
	20 mM β-mercaptoethanol
	5 mM TCEP
	10 mM reduced glutathione
Denaturing agents	8 M urea[2]
	6 M guanidine hydrochloride[2]
Detergents	2% Triton X-100 (nonionic)
	2% Tween 20 (nonionic)
	2% NP-40 (nonionic)
	2% cholate (anionic)
	1% CHAPS (zwitterionic)
Additives	500 mM imidazole
	20% ethanol
	50% glycerol
	100 mM Na_2SO_4
	1.5 M NaCl
	1 mM EDTA[3]
	60 mM citrate[3]

Buffer substances	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[2]

[1] See notes and *Blank run, on page 9*.

[2] Tested for 1 week at 40°C.

[3] 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 buffers). Any metal ion stripping may be counteracted by addition of a small excess of $MgCl_2$. Note that stripping effects may vary with applied sample volume.

10 Appendix B

Buffers, Mixing Table for 50 ml Buffer

Imidazole concentration in buffer (mM)	Phosphate buffer 8× stock solution pH 7.4 (ml)	2 M Imidazole pH 7.4 (ml)	Deionized water (ml)
0	6.25	0	to 50 ml
10	6.25	0.25	to 50 ml
20	6.25	0.50	to 50 ml
30	6.25	0.75	to 50 ml
40	6.25	1.00	to 50 ml
50	6.25	1.25	to 50 ml
60	6.25	1.50	to 50 ml
70	6.25	1.75	to 50 ml
80	6.25	2.00	to 50 ml
90	6.25	2.25	to 50 ml

Imidazole concentration in buffer (mM)	Phosphate buffer 8× stock solution pH 7.4 (ml)	2 M Imidazole pH 7.4 (ml)	Deionized water (ml)
100	6.25	2.50	to 50 ml
150	6.25	3.75	to 50 ml
200	6.25	5.00	to 50 ml
250	6.25	6.25	to 50 ml
300	6.25	7.50	to 50 ml
400	6.25	10.00	to 50 ml
500	6.25	12.50	to 50 ml

To obtain the imidazole concentration indicated in the first column, mix Phosphate buffer 8 × stock solution, 2 M imidazole and distilled water according to the table. Check pH and adjust to pH 7.4–7.6 if necessary. These buffers will contain 20 mM phosphate, 500 mM NaCl, and the concentrations of imidazole indicated. For one purification, 24 ml of the binding buffer and 8 ml of each elution buffer are sufficient.

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