instructions

HiTrap

HisTrap HP Kit

for purification of His-tagged proteins

Caution!
Contains nickel. May produce an allergic reaction.

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

The HisTrap HP columns included in the kit are prepacked with Ni Sepharose™ High Performance, an IMAC medium with low nickel (Ni^{2+}) ion leakage and compatibility with a wide range of buffers, reducing agents, denaturants, and detergents.

i 71-5027-69 Edition AB

Amersham Biosciences

Code No.	Designation	No. supplied
17-5249-01	HisTrap HP Kit	
	containing:	
	HisTrap HP columns	3 × 1 ml
	Phosphate buffer, 8x stock solution, pH 7.4	2 × 50 ml
	2 M Imidazole, pH 7.4	50 ml
	Syringe, 5 ml	1
	Connectors	
	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"	3
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1. Introduction

HisTrap HP Kit is designed for rapid, mild affinity purification of His-tagged proteins. Using HisTrap HP Kit, His-tagged proteins can be prepared to high purity in one step.
His-tagged proteins can be purified directly from pretreated bacterial lysates and are recovered from the medium under mild elution conditions that preserve their antigenicity and functionality.

The kit contains three ready to use 1-ml HisTrap HP columns (pre-charged 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.

2. General Considerations

HisTrap HP 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 $(His)_6$-tag, increases the affinity for Ni^{2+} and generally makes the His-tagged protein the strongest binder among other proteins, in e.g. an *E. coli* extract.

His-tagged proteins can be eluted from HisTrap HP 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 His-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 His-tagged target protein). This optimal concentration is different for different His-tagged proteins, and is usually slightly higher for Ni Sepharose High Performance than for similar IMAC media on the market (see Data File Ni Sepharose High Performance, 18-1174-40). 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 9 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 (see page 8) may be used directly, without prior optimization.

If the His-tagged proteins are expressed as inclusion bodies, see page 15 and references 1 and 2 for purification and refolding information.

3. Sample Preparation

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

1. Harvest cells from the culture by centrifugation (e.g. by centrifugation at 7 000–8 000 × g for 10 minutes or 1 000–1 500 × g for 30 minutes at +4 °C).
2. Discard the supernatant. Place the bacterial pellet on ice.
3. Using a pipette, completely suspend the cell pellet by adding 3–6 volumes of ice-cold buffer, pH 7–8.5 per volume of cell pellet, e.g. 1 × phosphate buffer, 20 mM imidazole, pH 7.4 (binding buffer), prepared according to Appendix B.

4. Disrupt suspended cells by, for example, sonicating on ice in short bursts. Save an aliquot of the sonicate for SDS-PAGE. Sonicate for the minimum time necessary to disrupt the cells. Avoid over-heating and frothing as this may denature the His-tagged protein and can also lead to co-purification of host proteins with the tagged target protein. Addition of lysozyme and DNase may improve sonication (see Troubleshooting on page 13).
5. Sediment the cell debris by centrifugation (e.g. at 12 000 × g or higher for 10 minutes at +4 °C or room temperature, depending on the stability of the protein). Carefully transfer the supernatant, without disturbing the pellet, to a fresh container. Save aliquots of the supernatant and the cell debris pellet for SDS-PAGE.

   Samples containing 8 M urea can be analyzed by SDS-PAGE directly, but samples containing 6 M Gua-HCl must be buffer exchanged (with HiTrap™ Desalting, PD-10 Desalting Columns, or HiPrep™ 26/10 Desalting columns) against buffer containing 8 M urea before loading on a gel.
6. To avoid clogging of the column it is recommended to centrifuge and/or filter through a 0.22 µm or a 0.45 µm filter to remove cell debris or other particulate material.

Note: Always check pH of the sample before loading on the HisTrap HP column. Adjustment to pH 7–8 can be achieved either by buffer exchange, dilution with binding buffer or by addition of buffer stock solutions. Do not use strong bases or acids for pH adjustment (precipitation risk). Sample should include imidazole (e.g. 20 mM), and 500 mM NaCl.

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 General considerations, page 3).

7. Store the sample at –20 °C. The sample may require further filtration after thawing and prior to use.

4. Protein Purification Protocols

Column preparation

1. Fill the syringe with distilled water. Remove the stopper and connect the column to the syringe with the provided luer adapter "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 distilled water until the air disappears.

Note: Leakage of nickel ions from HisTrap HP 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 page 7.

Note: Ni Sepharose High Performance is compatible with reducing agents (see Appendix A). However, removal of any weakly bound Ni^{2+} ions by performing a blank run without reducing agents (as described on page 7), *before* applying buffer/sample including reducing agents,

is recommended. Do not store HisTrap HP columns with buffers including reducing agents.

Blank run: Use binding and elution buffers *without* reducing agent.

1. Wash the column with 5 ml of distilled water (to remove the 20% ethanol).
2. Wash with 5 ml of binding buffer.
3. Wash with 5 ml of elution buffer.
4. 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.

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.
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 sample 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.
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 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 HP depends on the nature of the sample and should only be performed with identical His-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 Protocol with the optimal imidazole concentrations selected here.

1. Prepare buffers according to Table 1.

Table 1. Mixing table for one purification

Imidazole concentration in buffer	Phosphate buffer 8x stock solution pH 7.4	2 M Imidazole pH 7.4	Distilled 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 Appendix B on page 23.

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, e.g. 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.
9. Check the different fractions, e.g. 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 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 HP depends on the nature of the sample and should only be performed with identical His-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 His-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 His-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

Two or three HisTrap HP columns can be connected in series for quick scale-up of purifications (backpressure will increase). HisTrap HP is also available in a 5-ml format, see Ordering Information on page 17.

Pump operation

The purification process can also be performed using different chromatography systems, such as ÄKTAdesign™, FPLC™ System or a low-pressure laboratory pump (e.g. Peristaltic Pump P-1). This would be an alternative in some situations, e.g. 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.

Note: If a Peristaltic Pump P-1 is used, a maximum flow rate of 3 ml/min can be run on a HisTrap HP 1 ml column.

System/ equipment	Connector
Syringe	Union luer female/1/16″ male
FPLC Systems	Union 1/16″ female/M6 male and Union M6 female/1/16” male
ÄKTAdesign 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 HP column with 20% ethanol. Seal the column with the provided stop plugs to avoid dehydration The recommended storage temperature for the kit is +4 to +8 °C.

6. Troubleshooting

The following tips may be of assistance. If you have any further questions about the HisTrap HP column or the HisTrap HP Kit, please visit *www.hitrap.com,* 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.

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

Tips: 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.

Column has clogged:

- Cell debris in the sample may clog the column. Clean the column according to section 5, Column Cleaning and Storage.
- It is important to centrifuge and/or filter the sample through a 0.22 µm or a 0.45 µm filter, see Sample preparation on page 4.

Sample is too viscous:

- If the lysate is very viscous due to a high concentration of host nucleic acid, continue sonication until viscosity is reduced, and/or add DNase I to 5 µg/ml, Mg^{2+} to 1 mM, and incubate on ice for 10–15 minutes. Avoid over-heating of the sample. Alternatively, draw the lysate through a syringe needle several times.

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 ß-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 minutes to aid in 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, detergent cannot be easily removed by buffer exchange.

No His-tagged protein in the purified fractions:

- **Elution conditions are too mild (His-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:** For the next experiment, decrease protein concentration by eluting with linear imidazole gradient instead of imidazole step. Try detergents or changed NaCl concentration or elute under denaturing (unfolding) conditions (add 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% Triton X-100) or increase the NaCl concentration.
- **Concentration of imidazole in the sample and/or binding buffer is too high:** The protein is found in the flowthrough material. Decrease the imidazole concentration.

- **His-tag may be insufficiently exposed:** The protein is found in the flowthrough material; perform purification of unfolded protein in urea or Gua-HCl as for inclusion bodies.
- **Buffer/sample composition is incorrect:** The protein is found in the flowthrough material. Check pH and composition of the sample and binding buffer according to the mixing table, see Appendix B. Ensure that the concentration of chelating or strong reducing agents in the sample, as well as the imidazole concentration in the buffer, are not too high.

SDS-PAGE of samples collected during the preparation of bacterial sonicate may indicate that most of the His-tagged protein is located in the centrifugation pellet. Possible causes and solutions are:

- **Sonication may be insufficient:** Cell disruption may be checked by microscopic examination or monitored by measuring the release of nucleic acids at A_{260}. Addition of lysozyme (up to 0.1 volume of a 10 mg/ml lysozyme solution in 25 mM Tris-HCl, pH 8.0) prior to sonication may improve results. 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.
- **Protein may 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 denaturing buffers containing 20 mM sodium phosphate, 8 M urea or 6 M Gua-HCl, and different imidazole concentrations ranging from 10 mM to 500 mM, pH 7.4–7.6. Buffers with urea should also contain 500 mM NaCl. Use these buffers for sample preparation, as binding buffer and as elution buffer (and for optimization). For sample preparation and binding buffer, use 10 mM imidazole or the concentration selected in the Optimization Protocol.

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

- **Partial degradation of tagged proteins by proteases:** Add protease inhibitors (use EDTA with caution see Table 2).
- **Contaminants are associated with tagged proteins:** Add detergent and/or reducing agents before sonication of the cells. Increase the detergent levels (e.g. up to 2% Triton X-100 or 2% Tween 20), or add glycerol (up to 50%) to the wash buffer to disrupt nonspecific interactions.
- **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 before elution with binding buffer containing the highest possible concentration of imidazole that does not allow elution of the target 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.

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

- **Buffer/sample composition is incorrect:** Check pH and composition of sample and of binding buffer according to the mixing table, see Appendix B. Ensure that the concentration of chelating or strong reducing agents in the sample, as well as the imidazole concentration in the buffer, are not too high.
- **His-tag is partially obstructed:** Purify under denaturing conditions (use 4–8 M urea or 4–6 M Gua-HCl).
- **Column capacity is exceeded:** Join two or three HisTrap HP 1 ml columns together or use a HisTrap HP 5 ml column.

7. Ordering Information

Designation	No. Supplied	Code No.
HisTrap HP Kit	1 kit	17-5249-01
HisTrap HP	5 × 1 ml	17-5247-01
HisTrap HP	1 × 5 ml	17-5248-01
HisTrap HP	5 × 5 ml	17-5248-02
HiTrap Desalting	5 × 5 ml	17-1408-01
PD-10 Desalting Column	30	17-0851-01

Accessories

Designation	No. Supplied	Code No.
1/16" male/luer female	2	18-1112-51
Union luerlock female/M6 female	2	18-1027-12
Union 1/16" female/M6 male	6	18-1112-57
Tubing connector flangeless/M6 male	2	18-1017-98
Tubing connector flangeless/M6 female	2	18-1003-68
Union M6 female /1/16" male	5	18-3858-01
Stop plug female, 1/16"	5	11-0004-64
Fingertight stop plug, 1/16"	5	11-0003-55

Literature

Recombinant Protein Handbook, Protein Amplification and Simple Purification	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 Product Profile	18-1121-86

8. References

1. Colangeli R., *et al.* Three-step purification of lipopolysaccaride-free poly-histidine-tagged recombinant antigens of Myobacterium tuberculosis, *J. Chromatography B*, **714**, 223–235 (1998).
2. Application Note: Rapid and efficient purification and refolding of a $(His)_6$-tagged recombinant protein produced in *E.coli* as inclusion bodies, 18-1134-37, Amersham Biosciences.

Appendix A
Description & Characteristics

Column

HisTrap HP is packed with Ni Sepharose™ High Performance. 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, e.g. Peristaltic Pump P-1 or an ÄKTAdesign chromatography system.

The column cannot be opened or refilled.

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

Medium

Ni Sepharose High Performance consists of 34 µm particles of highly cross-linked 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). The medium is optimized for purification of His-tagged proteins.

Table 2. Characteristics of HisTrap HP 1 ml

Matrix	Highly cross-linked spherical agrose, 6%
Column volume	1 ml
Column dimensions	0.7 × 2.5 cm
Dynamic binding capacity*	At least 40 mg $(His)_6$-tagged protein per column
Average particle size	34 µm
Pre-charged metal ion	Ni^{2+}
Maximum flow rate	4 ml/min
Recommended flow rate	1–4 ml/min
Maximum back pressure†	0.3 MPa, 3 bar
Compatibility during use	Stable in all commonly used aqueous buffers, reducing agents and denaturants. See Table 2.
Chemical stability‡	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‡	
Short term (≤ 2 hours)	2–14
Long term (≤ 1 week)	3–12
Storage	20% ethanol (antimicrobial agent)
Avoid	Chelating agents e.g. EDTA, EGTA, citrate

* Dynamic binding capacity conditions:

Sample:	1 mg/ml $(His)_6$-tagged pure protein (M_r 28 000 or 43 000) in binding buffer ($Q_{B10\%}$-determination) or protein bound from *E. coli* extracts
Column volume:	0.25 ml or 1 ml
Flow rate:	0.25 ml/min or 1 ml/min
Binding buffer:	20 mM sodium phosphate, 500 mM NaCl, 500 mM imidazole, pH 7.4
Elution buffer:	20 mM sodium phosphate, 500 mM NaCl, 500 mM imidazole, pH 7.4
Note:	Dynamic binding capacity is protein-dependent

† H_2O at room temperature.

‡ Ni^{2+}-stripped medium.

Buffers

The phosphate buffer and the imidazole solution included in HisTrap HP 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 $Na_2HPO_4 \cdot 2H_2O$ (177.99 g/mol), 1.11 g $NaH_2PO_4 \cdot H_2O$ (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 High Performance is compatible with the following compounds, at least 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 (non-ionic)
	2% Tween 20 (non-ionic)
	2% NP-40 (non-ionic)
	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‡
	60 mM citrate‡
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†

* See notes and blank run, pages 6–7.

† 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 buffers). Any metal ion stripping may be counteracted by addition of a small excess of $MgCl_2$ before centrifugation/filtration of the sample. Note that stripping effects may vary with applied sample volume.

Appendix B
Buffers, Mixing Table for 50 ml Buffer

Imidazole concentration in buffer mM	Phosphate buffer 8x stock solution pH 7.4 ml	2 M Imidazole pH 7.4 ml	Distilled 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
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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