instructions

HiTrap

HisTrap HP, 1 ml and 5 ml

Caution!
Contains nickel.
May produce an allergic reaction.

HiTrap affinity columns

HisTrap™ HP is a prepacked, ready-to-use column for the preparative purification of His-tagged recombinant proteins by immobilized metal affinity chromatography (IMAC) using precharged Ni Sepharose™ High Performance.

The special design of the column, together with the high performance matrix, provides fast, simple and easy separations in a convenient format.

Ni Sepharose High Performance has a low Ni^{2+} leakage and is compatible with a wide range of additives used in protein purification.

HisTrap HP columns can be operated with a syringe, peristaltic pump or liquid chromatography system such as ÄKTA™design or FPLC™ System.

ⓘ 71-5027-68 Edition AB

Amersham Biosciences

Code No.	Designation	No. supplied
17-5247-01	HisTrap HP 1 ml	5 x 1 ml
17-5247-05	HisTrap HP 1 ml	100 x 1 ml*
17-5248-01	HisTrap HP 5 ml	1 x 5 ml
17-5248-02	HisTrap HP 5 ml	5 x 5 ml
17-5248-05	HisTrap HP 5 ml	100 x 5 ml*
	Connectors	
	Luerlock female/M6 male	1
	Luerlock female/M6 female	1
	Tubing connector flangeless/M6 male	1
	Tubing connector flangeless/M6 female	1
	Union M6 female/1/16" male	1
	Union 1/16" female/M6 male	1
	Domed nut	2 or 5
	Instruction manual	1

* Special pack delivered on specific customer order.

Description

Medium properties

HisTrap HP 1 ml and 5 ml columns are packed with 1 ml and 5 ml of Ni Sepharose High Performance, respectively. Ni Sepharose High Performance consists of highly cross-linked agarose beads to which a chelating group has been immobilized. The metal ion nickel (Ni^{2+}) has then been coupled to the chelating matrix.

Several amino acids, for example histidine, form complexes with many metal ions. Ni Sepharose High Performance selectively binds proteins if suitable complex-forming amino acid residues are exposed on the protein surface. Tagging proteins with additional histidines, like $(His)_6$, 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.

Table 1 summarizes product characteristics.

Table 1. HisTrap HP characteristics.

Matrix	Highly cross-linked spherical agarose, 6%
Mean particle size	34 µm
Metal ion capacity	~15 µmol Ni^{2+}/ml medium
Dynamic binding capacity*	At least 40 mg $(His)_6$-tagged protein/ml medium
Column volumes	1 ml or 5 ml
Column dimensions	i.d. × h: 0.7 x 2.5 cm (1 ml) 1.6 x 2.5 cm (5 ml)
Recommended flow rate	1 and 5 ml/min for 1 and 5 ml column respectively
Max. flow rates	4 and 20 ml/min for 1 and 5 ml column respectively
Max back pressure**	0.3 MPa, 3 bar
Compatibility during use	Stable in all commonly used buffers, reducing agents, denaturants and detergents. See Table 2 for details.
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 min.
Avoid in buffers	Chelating agents, *e.g.* EDTA, EGTA, citrate (see Table 2)
pH stability***	short term (≤ 2 hours): 2-14 long term (≤ 1 week): 3-12
Storage	20% ethanol
Storage temperature	+4 to +8 °C

* Dynamic bindning capacity conditions:

Sample:	1 mg/ml $(His)_6$-tagged pure protein (M_r 28 000 or 43 000) in binding buffer ($Q_{B,\,10\%}$ determination) or $(His)_6$-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, 0.5 M imidazole, pH 7.4

Note: Dynamic binding capacity is protein dependent.

** H_2O at room temperature
*** Ni^{2+}-stripped medium

The coupling technique used gives both high capacity and high performance. The medium is stable over the pH range 3–12.

The Ni^{2+} charged medium tolerates all commonly used aqueous buffers, reducing agents, denaturants such as 6 M guanidine hydrochloride and 8 M urea, and a range of other additives (see Table 2).

Table 2. 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)
Other additives	500 mM imidazole
	20% ethanol
	50% glycerol
	100 mM Na_2SO_4
	0.5–1.5 M NaCl
	1 mM EDTA**
	60 mM citrate**
Buffer substances	50 mM sodium phosphate, pH 7.4
	50 mM Tris-HCl, 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.

** 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 an excess of $MgCl_2$. Note that stripping effects may vary with applied sample volume.

Column properties

HisTrap HP columns are made of biocompatible polypropylene that does not interact with biomolecules. Columns are delivered with a stopper on the inlet and a twist-off end on the outlet. The columns have porous top and bottom frits that allow high flow rates. They cannot be opened or refilled.

Columns can be operated with either a syringe and the supplied luer adaptor, a peristaltic pump, or a chromatography system such as ÄKTAdesign or FPLC System.

Note: To prevent leakage, ensure that the adaptor is tight.

General considerations

HisTrap HP is supplied precharged with Ni^{2+} ions. In general, Ni^{2+} is the preferred metal ion for purification of recombinant His-tagged proteins. Note, however, that it may be wise to test other metal ions, e.g. Zn^{2+} and Co^{2+}, as the strength of binding depends on the nature of the His-tagged protein as well as the metal ion (see Optimization, page 13).

Choosing binding buffer also depends on the properties of the chelated metal ion and the binding properties of the sample molecules. We recommend binding at neutral to slightly alkaline pH (pH 7–8) in the presence of 0.5–1.0 M NaCl. Sodium phosphate buffers are often used. Tris-HCl can generally be used, but should be avoided in cases where the metal-protein affinity is very weak, since it may reduce binding strength. Avoid chelating agents such as EDTA or citrate in buffers, see Table 2.

Including salt, *e.g.* 0.5–1.0 M NaCl, in the buffers and samples eliminates any ion exchange effects. It can also have a marginal effect on the retention of proteins.

Imidazole is commonly used for elution and, at lower concentrations, to increase the selectivity for His-tagged proteins in IMAC. The binding of non-tagged host cell proteins is discouraged with imidazole, but a too high concentration of imidazole will also prevent binding of His-tagged proteins. 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. We recommend a highly pure imidazole. Such imidazole gives essentially no absorbance at 280 nm.

Bound proteins can also be eluted from the medium by several other methods or combinations of methods. A lowering of pH within the range of 2.5–7.5 can be used, for example. At pH values below 4, metal ions will start to be stripped off the medium.

Note: If the proteins are sensitive to low pH, we recommend collecting the eluted fractions in tubes containing 1 M Tris-HCl, pH 9.0 (60–200 µl/ml fraction) to restore the pH to neutral.

Chelating agents such as EGTA or EDTA will also strip the metal ions from the medium and thereby cause protein elution, but the target protein pool will then contain Ni^{2+} ions. In this case, the Ni^{2+} ions can be removed by desalting on a HiTrap Desalting, a PD-10, or a HiPrep™ 26/10 Desalting column.

Leakage of Ni^{2+} from Ni Sepharose High Performance is very low under all normal conditions, lower than for other IMAC media tested. For applications where extremely low leakage during purification is critical, leakage can be even further reduced by performing a blank run (see page 11). Likewise, a

blank run should also be performed **before** applying buffers/ samples with reducing agents (see page 11).

Whatever conditions are chosen, HisTrap HP columns can be operated with a syringe, peristaltic pump or a chromatography system.

Note: If a P-1 pump is used, the maximum flow rate that can be run on a HisTrap HP 1 ml column is 1–3 ml/min.

Operation

Buffer preparation

Water and chemicals used for buffer preparation should be of high purity. Filter buffers through a 0.22 µm or a 0.45 µm filter before use.

We recommend the following binding and elution buffers. If the recombinant His-tagged proteins are expressed as inclusion bodies, include 6 M guanidine hydrochloride or 8 M urea in all buffers and sample. On-column refolding of the denatured protein may be possible.

Use a high purity imidazole as this will give very low or no absorbance at 280 nm.

Recommended starting conditions

Binding buffer:	20 mM sodium phosphate, 0.5 M NaCl, 20–40 mM imidazole, pH 7.4 (The imidazole concentration is protein dependent)
Elution buffer:	20 mM sodium phosphate, 0.5 M NaCl, 500 mM imidazole, pH 7.4

Sample preparation

For optimal growth, induction and cell lysis conditions for your recombinant His-tagged clones, please refer to recommended protocols.

Adjust the sample to the composition of the binding buffer by adding buffer, salt and additives from concentrated stock solutions, by diluting it with binding buffer or by buffer exchange using HiTrap Desalting, a PD-10 column, or HiPrep 26/10 Desalting (see Table 4). Filter the sample through a 0.22 µm or a 0.45 µm filter and/or centrifuge it immediately before applying it to the column. To prevent the binding of cellular host proteins with exposed histidine, it is essential to include imidazole at a low concentration in sample and binding buffer (see Optimization).

Table 4. Prepacked columns for desalting and buffer exchange.

Code No.	Column	Load	Elute	Comments	Notes
17-0851-01	PD-10	2.5 ml	3.5 ml	Prepacked with Sephadex™ G-25. Requires only gravity to run.	For desalting and buffer exchange of protein extracts (M_r>5000).
17-1408-01	HiTrap Desalting	0.1–1.5 ml	1.3–4.0 x applied volume	Prepacked with Sephadex G-25 Superfine. Requires a syringe or pump to run.	For desalting and buffer exchange of protein extracts (M_r>5000).
17-5087-01	HiPrep 26/10 Desalting	Up to 15 ml	15–20 ml	Prepacked with Sephadex G-25 Fine. Requires a pump to run.	For desalting and buffer exchange of protein extracts (M_r>5000).

17-0855-01	NICK™	0.1 ml	0.4 ml	Prepacked with Sephadex G-25. Requires only gravity to run.	For separation of proteins (M_r>5000) and nick-translated DNA from radio-labelled nucleotides not shorter than 120 mers, and similar separations.
17-0853-01 17-0854-01 17-0852-01	NAP™-5 NAP-10 NAP-25	0.5 ml 1.0 ml 2.5 ml	1.0 ml 1.5 ml 3.5 ml	Prepacked with Sephadex G-25 DNA grade. Require only gravity to run.	For purification of proteins (M_r>5000), DNA and oligonucleo-tides greater than 10 bases in length.

Purification

1. Fill the syringe or pump tubing with distilled water. Remove the stopper and connect the column to the syringe (use the adaptor provided), laboratory pump or chromatography system tubing "drop-to-drop" to avoid introducing air into the system.
2. Remove the twist-off end.
3. Wash the column with 3–5 column volumes of distilled water.
4. Equilibrate the column with at least 5 column volumes of binding buffer. Recommended flow rates are 1 ml/min or 5 ml/min for the 1 and 5 ml columns respectively.
5. Apply the pretreated sample using a syringe or a pump.
6. Wash with binding buffer until the absorbance reaches a steady baseline.

Note: The purity of recombinant His-tagged proteins can often be increased by washing with binding buffer containing as high concentration of imidazole as possible. However, care must be taken not to use a concentration of imidazole that causes elution of the His-tagged protein.

The concentration of imidazole that will give optimal purification results is protein-dependent, and is usually slightly higher for Ni Sepharose High Performance than for similar IMAC media on the market (see Optimization).

7. Elute with elution buffer using a step or linear gradient. 5 column volumes are usually sufficient if the protein of interest is eluted by a step gradient. Other volumes (or a different elution buffer) may be required if the protein binds strongly to the medium.

   A shallow gradient, *e.g.* a linear gradient over 20 column volumes or more, may separate proteins with similar binding strengths.

Note: If imidazole needs to be removed from the protein, use HiTrap Desalting, a PD-10 column, or HiPrep 26/10 Desalting depending on the sample volume.

Note: Resistance towards reducing agents is high for Ni Sepharose High Performance. However, it is recommended to remove any weakly bound Ni^{2+} ions by performing a blank run without reducing agents (as described on page 11) **before** applying buffer/sample including reducing agents. It is also recommended not to leave HisTrap HP columns with buffers including reducing agents when not in use.

Note: Leakage of Ni^{2+} from Ni Sepharose High Performance is low under all normal conditions. The leakage is lower than for other IMAC media tested. For very critical applications, leakage during purification can be even further diminished by performing a blank run (as described on page 11) before loading sample.

Blank run:

Use binding buffer and elution buffer **without** reducing agents.

1. Wash the column with 5 column volumes of distilled water (to remove the 20% ethanol).
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.

Stripping and recharging

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

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

Re-charge the water-washed column by loading 0.5 ml or 2.5 ml of 0.1 M $NiSO_4$ in distilled water on HisTrap HP 1 ml and 5 ml column, respectively. Salts of other metals, chlorides or sulfates, may also be used (see Optimization). Wash with 5 column volumes distilled water, and 5 column volumes binding buffer (important to wash with binding buffer for pH adjustment before storage in 20% ethanol).

Note: The column does not have to be stripped and re-charged between each purification if the same protein is going to be purified; it is sufficient to strip and re-charge it after 5–7 purifications, depending on the cell extract, extract volume, target protein, etc.

Cleaning-in-place

When an increase in back-pressure is seen, the column can be cleaned. Before cleaning, strip off the Ni^{2+} ions by using the recommended procedure (see page 11).

After cleaning, store in 20% ethanol (wash with 5 column volumes) or recharge with Ni^{2+} prior to storage in ethanol.

The Ni^{2+}-stripped column can be cleaned by the following methods.

- Remove ionically bound proteins by washing the column with several column volumes of 1.5 M NaCl. Then wash the column with approx. 10 column volumes of distilled water.
- 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 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.

  Alternatively, wash the column with 2 column volumes of detergent in a basic or acidic solution. Use, for example, 0.1–0.5% non-ionic detergent in 0.1 M acetic acid, contact time 1–2 hours. After treatment, always remove residual detergent by washing with at least 5 column volumes of 70% ethanol. Then wash the column with approx. 10 column volumes of distilled water.

Scaling up

Two or three HisTrap HP 1 ml or 5 ml columns can be connected in series for quick scale up (note that the back-pressure will increase).

Ni Sepharose High Performance, the medium prepacked in HisTrap HP columns, is supplied pre-swollen in 25 and 100 ml lab packs (see Ordering information). An alternative scale-up strategy is thus to pack the medium in empty columns. Columns from the Tricorn™ and XK series are suitable.

Storage

Store HisTrap HP columns in 20% ethanol.

Optimization

Imidazole is commonly used in sample and in wash buffer to increase the selectivity for His-tagged proteins in IMAC. The binding of non-tagged host cell proteins is thus reduced by imidazole, but a too high concentration of imidazole will also prevent binding of His-tagged proteins. 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. We recommend the use of high purity imidazole, such imidazole gives essentially no absorbance at 280 nm. Finding the optimal imidazole concentration for a specific His-tagged protein is a trial-and-error effort, but 20–40 mM is a good starting point for many proteins.

Ni^{2+} is usually the first choice metal ion for purifying most $(His)_6$-tagged recombinant proteins from non-tagged host cell proteins, and also the one most generally used.

Nevertheless, it is not always possible to predict which metal ion will be best for a given protein. The strength of binding between a protein and a metal ion is affected by several factors, including the length, position and exposure of the affinity tag on the protein, the type of ion used, and the pH of buffers, so some proteins may be easier to purify with ions other than Ni^{2+}.

A quick and effective way to test this possibility and optimize separation conditions is to use HiTrap Chelating HP 1 ml columns, which are packed with Chelating Sepharose High Performance (not metal ion charged). Each column can be charged with different metal ions, *e.g.* Cu^{2+}, Co^{2+}, Zn^{2+}, Ca^{2+} or Fe^{2+}, allowing rapid and convenient comparison of the protein purification. Instructions are included with each column.

A study to compare the purification of six $(His)_6$-tagged recombinant proteins, including three variants of maltose binding protein, with different metal ions has indicated that Ni^{2+} generally gives best selectivity between His-tagged and non-tagged host cell proteins. (Data available in Application Note 18-1145-18).

Troubleshooting

The following tips may be of assistance. If you have any further questions about your HisTrap HP column, please visit *www.hitrap.com* or contact our technical support or your local Amersham Biosciences representative.

Note: When using high concentrations of urea or guanidine hydrochloride, protein unfolding generally takes place. Refolding on-column (or after elution) is protein dependent.

Tips: Samples containing urea can be analyzed directly by SDS-PAGE whereas samples containing guanidine hydrochloride must be buffer-exchanged to a buffer with urea before SDS-PAGE.

The column has clogged:

- Cell debris in the sample may clog the column. Clean the column according to the section Cleaning-in-place.
- It is important to filter the sample through a 0.22 µm or a 0.45 µm filter, see Sample preparation.

The sample is too viscous:

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

The 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 possible but is protein dependent), 5 mM TCEP, 10 mM reduced glutathione, 8 M urea or 6 M guanidine hydrochloride. Mix gently for 30 minutes to aid solubilization of the fusion protein. Note that Triton X-100 and NP-40 have a high A_{280} value but the A_{280} value for Tween is low. Furthermore detergents cannot be easily removed by buffer exchange.

No His-tagged protein in the purified fractions:

- **The 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.
- **The protein has precipitated in the column:** Decrease amount of sample or decrease protein concentration by eluting with linear imidazole gradient instead of imidazole steps. Elute under denaturing (unfolding) conditions (add 4–8 M urea or 4–6 M guanidine hydrochloride).
- **Non-specific hydrophobic or other interaction:** Add a non-ionic detergent to the elution buffer (e.g. 0.2% Triton X-100) or increase the NaCl concentration.
- **The concentration of imidazole in the binding buffer is too high:** The protein is found in the flow-through material. Decrease the imidazole concentration in the binding buffer.
- **His-tag may be insufficiently exposed:** The protein is found in the flow-through material. Perform purification of unfolded protein in urea or guanidine hydrochloride as for inclusion bodies. To minimize dilution solid urea or guanidine hydrochloride can be added to the sample.
- **The buffer/sample composition is incorrect:** The protein is found in the flow-through material. Check pH and composition of sample and binding buffer. Ensure that chelating or strong reducing agents are not present in the sample at too high concentration, and that the concentration of imidazole is not too high.

SDS-PAGE analysis of samples collected during the preparation of the bacterial lysate may indicate that the majority of the

His-tagged protein is located in the post-lysis 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 as this may denature the fusion protein. Over-sonication can also lead to co-purification of host proteins with the fusion protein.
- **The 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 guanidine hydrochloride, 4–8 M urea or strong detergents.

  Prepare buffers containing 20 mM sodium phosphate, 8 M urea or 6 M guanidine hydrochloride, and suitable imidazole concentrations, pH 7.4–7.6. Use these buffers for sample preparation, as binding buffer and as elution buffer. For sample preparation and binding buffer use 20 mM imidazole or the concentration selected during the optimization trials (including urea or guanidine hydrochloride).

The protein is collected but is not pure (multiple bands on SDS-PAGE):

- **Partial degradation of tagged protein by proteases:** Add protease inhibitors (use EDTA with caution, see Table 2).
- **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 as high concentration of imidazole as possible,

without causing elution of the 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 chromotography (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 or/and reducing agents before sonicating 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 non-specific interactions.

The His-tagged protein is eluted during the sample application/wash:

- **The buffer/sample composition is incorrect:** Check pH and composition of sample and binding buffer. Ensure that chelating or strong reducing agents are not present in the sample at a too high concentration, and that the concentration of imidazole is not too high.
- **The His-tag is partially hidden:** Purify under denaturing conditions (use 4–8 M urea or 4–6 M guanidine hydrochloride).
- **The column capacity is exceeded:** Join two or three HisTrap HP 1 ml columns together or change to a HisTrap HP 5 ml column.

Further information

Check *www.hitrap.com* and *www.chromatography.amershambiosciences.com* for further information. Several handbooks also contain useful information, see Ordering information.

Ordering Information

Designation	No. supplied	Code no.
HisTrap HP	5 x 1 ml	17-5247-01
HisTrap HP	100 x 1 ml*	17-5247-05
HisTrap HP	1 x 5 ml	17-5248-01
HisTrap HP	5 x 5 ml	17-5248-02
HisTrap HP	100 x 5 ml*	17-5248-05
HisTrap HP Kit	3 x 1 ml	17-5249-01
Ni Sepharose High Performance	25 ml	17-5268-01
Ni Sepharose High Performance	100 ml	17-5268-02
HiTrap Desalting	5 x 5 ml	17-1408-01
HiTrap Desalting	100 x 5 ml*	11-0003-29
PD-10 Column	30	17-0851-01
HiPrep 26/10 Desalting	1 x 53 ml	17-5087-01
HiPrep 26/10 Desalting	4 x 53 ml	17-5087-02

* Special pack delivered on specific customer order.

Accessories

Designation	No. supplied	Code no.
Domed nut*	4	18-2450-01
Union Luerlock		
female/M6 female*	2	18-1027-12
female/M6 male*	2	18-1027-62
Tubing connector		
flangeless/M6 female*	2	18-1003-68
flangeless/M6 male*	2	18-1017-98
To connect columns with M6 connections to ÄKTAdesign:		
Union M6 female /1/16" male*	5	18-3858-01
Union 1/16" female/M6 male*	6	18-1112-57
Literature		
Recombinant Protein Handbook,		
Protein Amplification and Simple Purification	1	18-1142-75
Affinity Chromatography Handbook, Principle and Methods	1	18-1022-29
Affinity Chromatography Columns and Media Product Profile	1	18-1121-86
Convenient Protein Purification, HiTrap Column Guide	1	18-1129-81

* Included in HisTrap HP column packages.

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