HiScreen™ Ni FF

HiScreen Ni FF is a ready-to-use column for purification of histidine-tagged recombinant proteins by immobilized metal ion affinity chromatography (IMAC).

The column has a volume of 4.7 ml chromatography medium and is ideal for screening of purification conditions before scaling up, as well as for small scale purifications. Ni Sepharose™ 6 Fast Flow has low nickel ion (Ni^{2+}) leakage and is compatible with a wide range of additives used in protein purification.

The columns are used in an optimal way with liquid chromatography systems such as ÄKTA™.

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Please read these instructions carefully before using the products.

Intended use

The products are intended for research use only, and shall not be used in any clinical or *in vitro* procedures for diagnostic purposes.

Safety

For use and handling of the products in a safe way, please refer to the Safety Data Sheets.

1 Product description

HiScreen column characteristics

HiScreen columns are made of biocompatible polypropylene that does not interact with biomolecules. The columns are delivered with stoppers at the inlet and outlet. The arrow on the column label indicates the column orientation and the recommended flow direction, see Figure 1.

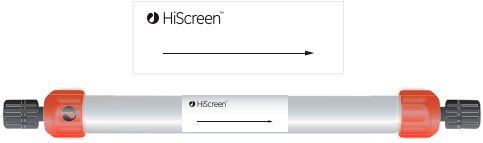

Fig 1. HiScreen column

For easy scaling-up and when a higher bed height is required, two columns can easily be connected in series using a union to give 20 cm bed height (see Section *Scaling up*).

Note: *HiScreen columns cannot be opened or refilled*

Note: *Make sure that the connector is tight to prevent leakage.*

Table 1. Characteristics of HiScreen column

Column volume (CV)	4.7 ml
Column dimensions	0.77 × 10 cm
Column hardware pressure limit	8 bar (0.8 MPa)

Note: *The pressure over the packed bed varies depending on a range of parameters such as the characteristics of the chromatography medium, sample/liquid viscosity and the column tubing used.*

Properties of Ni Sepharose 6 Fast Flow

HiScreen Ni FF columns are prepacked with Ni Sepharose 6 Fast Flow, which consists of 90 µm highly cross-linked agarose beads with an immobilized chelating group. The medium has been charged with Ni^{2+} ions.

Several amino acids, for example histidine, form complexes with many metal ions. Ni Sepharose 6 Fast Flow selectively binds proteins if suitable complex-forming amino acid residues are exposed on the protein surface.

Additional histidines, such as a histidine-tag, increase affinity for Ni^{2+} and generally make the histidine-tagged protein the strongest binder among other proteins in for example an *E. coli* extract.

The high flow rate properties of Ni Sepharose 6 Fast Flow make HiScreen Ni FF columns ideal for establishing optimal chromatographic conditions for scaling up.

Table 2. Characteristics of Ni Sepharose 6 FF

Matrix	Highly cross-linked 6% agarose
Average particle size (d_{50v}) [1]	90 µm
Metal ion capacity	Approx. 15 µmol Ni^{2+}/ml medium
Dynamic binding capacity [2]	Approx. 40 mg (histidine)6-tagged protein/ml medium (Ni^{2+}-charged).
Recommended flow velocity [3]	300 cm/h
Maximum flow velocity [3]	450 cm/h
pH stability [4] (for medium without metal ion)	
Working range	3 to 12
Cleaning-in-place	2 to 14
Compatibility during use	See *Table 3*.
Chemical stability (for medium without metal ion)	1 M NaOH, 70% acetic acid; tested for 12 h. 2% SDS; tested for 1 h. 30% 2-propanol.; tested for 30 min.
Avoid in buffers	Chelating agents, e.g., EDTA, EGTA, citrate (see *Table 3*).
Storage	4°C to 30°C in 20% ethanol

[1] d_{50v} is the average particle size of the cumulative volume distribution.

[2] Dynamic binding capacity conditions:

Samples:	1 mg/ml (histidine)6-tagged proteins (M_r 43 000) in binding buffer (QB 10% determination) or (histidine)6-tagged proteins (M_r 28 000) bound from *E. coli* extract.
Column volumes:	0.25 or 1 ml
Flow rates:	0.25 or 1 ml/min, respectively
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 7.4.

[3] Water at room temperature.

For viscous buffers and samples the flow velocity must be optimized. Starting with a low flow velocity is recommended.

[4] Working range: pH interval where the medium can be handled without significant change in function.

Cleaning-in-place: pH interval where the medium can be subjected to cleaning-in-place without significant change in function.

Buffers and compatibility

Table 3. Ni Sepharose 6 FF is compatible with the following compounds up to 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 [2]	8 M urea 6 M Gua-HCl
Detergents	2% Triton™ X-100 (nonionic) 2% Tween™ 20 (nonionic) 2% NP-40 (nonionic) 2% cholate (anionic) 1% CHAPS (zwitterionic)
Other additives	500 mM imidazole 20% ethanol 50% glycerol 100 mM Na_2SO_4 1.5 M NaCl 1 mM EDTA [3] 60 mM citrate
Buffer	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 Ni Sepharose 6 FF is compatible with reducing agents. However, for optimal performance, removal of any weakly bound Ni^{2+} ions by performing a blank run without reducing agents (as described in Section *Blank run, on page 13*.) before applying buffer/sample including reducing agents is recommended. Do not leave Hi Screen Ni FF columns with buffers including reducing agents when not in use.

2 Tested for one 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 in 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.

2 General considerations

Introduction

This section describes important information that should be considered when using Hi Screen Ni FF in order to achieve the best results. The actions for minimizing nickel leakage and discoloring are normally not needed but can be performed for sensitive applications.

Imidazole concentration

The recommended binding buffer is:

- 20 mM sodium phosphate, 500 mM NaCl, **20 to 40 mM imidazole**, pH 7.4

The imidazole concentration in sample and binding buffer can be further increased if there is a need for higher final purity. If, on the other hand, there is a need for higher yield the imidazole concentration can be lowered (this may result in lower final purity).

Minimize nickel-ion leakage

- Leakage of Ni-ions from Hi Screen Ni FF is very low under all normal conditions. For applications where extremely low leakage during purification is critical, leakage can be diminished by performing a blank run. See Section *Blank run, on page 13*.
- Use binding and elution buffers without reducing agents.

Reduce discoloring when reducing agents are used

Hi Screen Ni FF is compatible with reducing agents as listed in *Table 3*. Discoloring is always seen when using high concentrations of reducing agents. In most cases this does not affect the performance of the chromatography medium. To minimize the discoloring, perform a blank run using buffers without reducing agents before the purification. See Section *Blank run, on page 13*.

Table 4. Prepacked columns for desalting

Column	Code No.	Loading volume	Elution volume	Comments	Application
HiPrep™ 26/10 Desalting	17-5087-01	2.5 to 15 ml	7.5 to 20 ml	Prepacked with Sephadex™ G-25 Fine. Requires a laboratory pump or a chromatography system to run.	For desalting and buffer exchange of protein extracts (M_r > 5000).
HiTrap Desalting	17-1408-01	0.25 to 1.5 ml	1.0 to 2.0 ml	Prepacked with Sephadex G-25 Superfine. Requires a syringe or pump to run.	
PD-10 Desalting	17-0851-01	1.0 to 2.5 ml[1] 1.75 to 2.5 ml[2]	3.5 ml[1] up to 2.5 ml[2]	Prepacked with Sephadex G-25 Medium. Runs by gravity flow or centrifugation	For desalting, buffer exchange, and cleanup of proteins and other large biomolecules (M_r > 5000).
PD MiniTrap™ G-25	28-9180-07	0.1 to 0.5 ml[1] 0.2 to 0.5 ml[2]	1.0 ml[1] up to 0.5 ml[2]		
PD MidiTrap™ G-25	28-9180-08	0.5 to 1.0 ml[1] 0.75 to 1.0 ml[2]	1.5 ml[1] up to 1.0 ml[2]		

[1] Volumes with gravity elution
[2] Volumes with centrifugation

3 General process development

HiScreen column format is ideal to use for parameter and method optimization and for robustness testing when developing a new purification process. The small column volume, 4.7 ml, and the 10 cm bed height makes it possible to perform scalable experiments at relevant process flow rates. If necessary, two columns can easily be connected in series with a union to give 20 cm bed height (see *Section 9 Scaling up, on page 20*).

The figure below outlines typical steps during general process development.

Already from start in process development it is necessary to consider process cost, cleaning of the medium and environmental constraints.

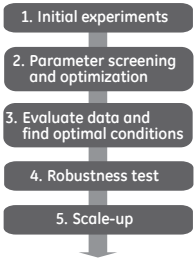

Fig 2. Typical steps during process development.

Design of Experiments (DoE) is an effective tool for investigating the effect of several parameters on protein recovery in order to establish the optimal purification protocol. See handbook *Design of Experiments in Protein Production and Purification*. A common approach in DoE is to define a reference experiment (center point) and perform representative experiments around that point. Some initial experiments are required in order to define the center point and the variable ranges. DoE may be used for parameter screening and optimization as well as robustness testing.

The robustness of a process is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters, and provides an indication of its reliability during normal usage. An objective of a robustness test is the evaluation of factors potentially causing variability in the responses of the method, for example, purity and yield. For this purpose, small variations in method parameters are introduced.

For scale-up, see *Section 9 Scaling up, on page 20*.

4 Optimization

General

IMAC is based on the specific interaction between immobilized metal ions and certain amino acid side chains exposed on the surface of proteins (mainly histidine and to a lesser extent cysteine and tryptophan). The strength of interaction with immobilized metal ions is dependent on the type, number and spatial distribution of the amino acid side chains, and on the nature of the metal ion used. The chromatographic operating conditions (pH, type and concentration of salt, additives, etc.) also contribute to the interaction observed.

Concentration of imidazole in binding/wash buffer

Imidazole at low concentrations is commonly used in binding/wash buffers to minimize the 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 binding/wash buffer). At somewhat higher concentrations, imidazole may decrease the binding of histidine-tagged proteins, leading to a lower yield. The concentration of imidazole must therefore be optimized to ensure the best balance of high purity (low binding of unwanted host cell proteins), and high yield (binding all of the histidine-tagged protein).

This optimal concentration is different for different histidine-tagged proteins/target proteins, and is usually slightly higher for Ni Sepharose 6 Fast Flow than for similar nickel precharged chromatography media on the market.

Finding the optimal imidazole concentration for a specific target protein is a trial-and-error effort, but 20 to 40 mM in the sample as well as in the binding/wash buffer is a good starting point for many histidine-tagged proteins when using Ni^{2+} or Co^{2+}.

When maximum binding and yield of the histidine-tagged protein (rather than purity) is the main objective, choose a low imidazole concentration for binding and wash, even if that concentration in some cases may lead to suboptimal purity.

Use high purity imidazole, which has essentially no absorbance at 280 nm.

As alternatives to imidazole elution, histidine-tagged proteins can be eluted from the chromatography medium by several other methods or combinations of methods, for example lowering pH within the range of 2.5 to 7.5. At pH values below 4, metal ions will be stripped off the medium.

Note: *If the proteins are sensitive to low pH, it is recommended to collect the eluted fractions in tubes containing 1 M Tris-HCl, pH 9.0 (60 to 200 µl/ml fraction) to restore the pH to neutral.*

EGTA and EDTA will strip the metal ions from the medium and thereby cause protein elution, but the target pool will then contain Ni^{2+} ions. In this case the Ni^{2+} ions can be removed by desalting on HiTrap™ Desalting, or HiPrep 26/10 Desalting columns, see *Table 4*.

Choice of metal ion

Hi Screen Ni FF is supplied precharged with Ni^{2+} ions. In general, Ni^{2+} is the preferred metal ion for purification of recombinant histidine-tagged proteins. Note, however, that in some cases 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 histidine-tagged protein as well as the metal ion.

We recommend binding at neutral to slightly alkaline pH (pH 7 to 8) in the presence of 0.5 to 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 3*. In general, imidazole is used for elution of histidine-tagged proteins.

Including salt (e.g., 0.5 to 1.0 M NaCl) in the buffers and samples, eliminates ion-exchange effects, but can also have a marginal effect on the retention of proteins.

Leakage of Ni^{2+} from Ni Sepharose 6 Fast Flow is very low under all normal conditions, **lower than for other IMAC media tested** (see Data File *Ni Sepharose 6 Fast Flow*, 11-0008-86). For applications where extremely low leakage during purification is critical, leakage can be even further reduced by performing a blank run (see *Blank run, on page 13*). Likewise, a blank run should also be performed before applying buffers/samples containing reducing agents.

5 Preparation

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.

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

Recommended buffers

Binding buffer:	20 mM sodium phosphate, 0.5 M NaCl, 20 to 40 mM imidazole, pH 7.4 (The optimal imidazole concentration is protein dependent; 20 to 40 mM is suitable for many histidine-tagged proteins.)
Elution buffer:	20 mM sodium phosphate, 0.5 M NaCl, 500 mM imidazole, pH 7.4 (The imidazole concentration required for elution is protein dependent).

If the recombinant histidine-tagged protein is expressed as inclusion bodies, include 6 M Gua-HCl or 8 M urea in all buffers and sample. On-column refolding of the denatured protein may be possible.

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

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. See Table 4 for column selection.*

Blank run

Note: *Perform a blank run without reducing agents before applying buffers/samples containing reducing agents. Likewise, a blank run is recommended for critical purifications where metal ion leakage during purification must be minimized.*

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

Step	Action
1	If the column has been stored in 20% ethanol, wash it with 25 ml of distilled water.
2	Wash with 25 ml of the buffer that has been chosen for protein elution, e.g., imidazole elution buffer or low-pH elution buffer.
3	Equilibrate with 25 to 50 ml of binding buffer. Imidazole equilibration can be monitored by absorbance, e.g., at 220 nm.

Sample preparation

Step	Action
1	Adjust the sample to the composition of the binding buffer, using one of these methods: • Dilute the sample with binding buffer. • Exchange buffer using a desalting column, see *Table 4*.
2	Filter the sample through a 0.45 µm filter or centrifuge immediately before loading it to the column. This prevents clogging and increases the life time of the column when loading large sample volumes.

Note: *To minimize the co-adsorption of unwanted host cell proteins, it is essential to include imidazole at a low concentration in the sample and binding buffer, see* *Section 4 Optimization, on page 10**.*

6 Purification

Recommended flow rates

Flow rate: 2.3 ml/min (300 cm/h)

Column tubing

Choose the optimal tubing kit for the column and the application you intend to run. (i.d.: 0.25, 0.50 or 0.75 (mm)). A tubing with wider inner diameter gives broader peaks whereas a tubing with a smaller inner diameter gives higher back pressure.

Procedure

Step	Action
1	Equilibrate with at least 5 column volumes (CV) of binding buffer. Avoid introducing air into the column. ***Note:*** *To prevent leakage, make sure that the connectors are tight. Use fingertight 1/16" connector (28-4010-81).* ***Note:*** *In some cases, a blank run is recommended before final equilibration/sample application, see Section* *Blank run, on page 13**.*
2	Adjust the sample to the chosen starting conditions and load on the column.
3	Wash with 5 to 10 CV binding buffer until the UV trace of the effluent returns to near baseline.

Step	Action
4	Elute either by linear gradient elution or a step elution at recommended flow rates. • Linear gradient elution: Elute with 0% to 100% elution buffer (up to 500 mM imidazole) in 10 to 20 CV. • Step elution: Elute with 5 CV elution buffer including imidazole at chosen concentration. If necessary, repeat at higher imidazole concentrations until the target protein has been eluted.
5	Re-equilibrate the column with 5 to 10 CV binding buffer or until the UV baseline, eluent pH, and conductivity reach the required values. ***Note:*** *Do not exceed the maximum recommended flow and/or back pressure for the column.* ***Note:*** *If imidazole needs to be removed from the eluted protein, use a desalting column according to Table 4.*

7 Stripping and recharging

The column does not have to be stripped and recharged between each purification cycle if the same protein is to be purified. It may be sufficient to strip and recharge it after five to seven purifications, or when the medium starts to change color to more white. The interval depends on parameters such as the metal ion used, sample properties, sample volume, target protein, etc.

Stripping

Stripping buffer

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

Step	Action
1	Wash with at least 5 to 10 column volumes (CV) of stripping buffer
2	Wash with at least 5 to10 CV binding buffer
3	Wash with at least 5 to 10 CV distilled water
4	Clean the column, see *Section 8 Cleaning-in-place (CIP), on page 18* and/or recharge with metal ions according to the method below.

Recharging

Step	Action
1	Make sure that the column is equilibrated with distilled water. If not, equilibrate with 5 CV distilled water.
2	Load 0.5 CV 0.1 M $NiSO_4$ in distilled water. ***Note:*** *Salts of other metals, chlorides or sulfates, may also be used, see* *Section 4 Optimization, on page 10*
3	Wash with 5 CV distilled water.
4	Wash with 5 CV binding buffer (to adjust pH).
5	For future use, equilibrate with 5 to 10 CV 20% ethanol and store.

8 Cleaning-in-place (CIP)

General description

Correct preparation of samples and buffers should maintain columns in good condition. However, reduced performance, increased back pressure or blockage indicates that the column needs cleaning.

CIP removes very tightly bound, precipitated or denatured substances from the medium. If such contaminants are allowed to accumulate, they may affect the chromatographic properties of the prepacked column, reduce the capacity of the medium and, potentially, come off in subsequent runs. If the fouling is severe, it may block the column, increase back pressure and affect the flow properties.

CIP should be performed regularly to prevent the build-up of contaminants and to maintain the capacity, flow properties and general performance of prepacked columns.

It is recommended to perform a CIP:

- When an increase in back pressure is seen.
- If reduced column performance is observed.
- Between runs when the same column is used for purification of different proteins to prevent possible cross-contamination.

CIP protocol

The **metal ion stripped** column can be cleaned by the following procedures.

To remove...	Then...
Ionically bound proteins	1 Wash with several column volumes (CV) of 1.5 M NaCl. 2 Wash with at least 3 CV distilled water.
Precipitated proteins, hydrophobically bound proteins, and lipoproteins	1 Wash the column with 1 M NaOH, contact time usually 1 to 2 h (longer time may be required to inactivate endotoxins). 2 Wash with approximately 3 to 10 CV binding buffer. 3 Wash with 5 to 10 CV distilled water.
Hydrophobically bound proteins, lipoproteins, and lipids.	1 Wash with 5 to 10 CV 30% isopropanol for at least 15 to 20 min. 2 Wash with approximately 10 CV distilled water. or 1 Wash with 2 CV detergent in a basic or acidic solution. Use, for example, 0.1 to 0.5% nonionic detergent in 0.1 M acetic acid, contact time 1 to 2 h. 2 Remove residual detergent by washing with at least 5 CV 70% ethanol [1]. 3 Wash with 3 to 10 CV binding buffer.

[1] Specific regulations may apply when using 70% ethanol since the use of explosion proof areas and equipment may be required.

9 Scaling up

After optimizing the method at laboratory scale, the process is ready for scaling up.

For quick scale-up of purification, two HiScreen columns can easily be connected in series with a union (18-1120-93) to give 20 cm bed height. Note that the back pressure will increase. This can easily be addressed by lowering the flow rate.

Factors, such as clearance of critical impurities, may change when column bed height is modified and should be validated using the final bed height.

Scale-up to a larger column is typically performed by keeping bed height and flow velocity (cm/h) constant while increasing bed diameter and flow rate (ml/min or l/h).

Bulk media is available for further scale-up, see *Section 13 Ordering information, on page 24*.

10 Adjusting pressure limits in chromatography system software

Pressure generated by the flow through a column affects the packed bed and the column hardware, see Figure below. Increased pressure is generated when running/using one or a combination of the following conditions:

- High flow rates
- Buffers or sample with high viscosity
- Low temperature
- A flow restrictor

Note: *Exceeding the flow limit (see Table 2) may damage the column.*

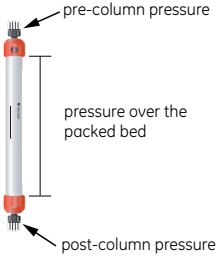

Fig 3. Pre-column and post-column measurements.

ÄKTA avant and ÄKTA pure

The system will automatically monitor the pressures (pre-column pressure and pressure over the packed bed, Δp). The pre-column pressure limit is the column hardware pressure limit (see Table 1).

The maximum pressure the packed bed can withstand depends on media characteristics and sample/liquid viscosity. The measured value also depends on the tubing used to connect the column to the instrument.

ÄKTAexplorer, ÄKTApurifier, ÄKTAFPLC and other systems with pressure sensor in the pump

To obtain optimal functionality, the pressure limit in the software may be adjusted according to the following procedure:

Step	Action
1	Replace the column with a piece of tubing. Run the pump at the maximum intended flow rate. Note the pressure as *total system pressure*, P1.
2	Disconnect the tubing and run the pump at the same flow rate used in step 1. Note that there will be a drip from the column valve. Note this pressure as P2.
3	Calculate the new pressure limit as a sum of P2 and the column hardware pressure limit (see Table 1). Replace the pressure limit in the software with the calculated value. The actual pressure over the packed bed (Δp) will during run be equal to actual measured pressure - *total system pressure* (P1).

Note: *Repeat the procedure each time the parameters are changed.*

11 Storage

Store Hi Screen Ni FF columns equilibrated with 5 to 10 CV 20% ethanol at 4°C to 30°C. Do not freeze.

Make sure that the column is tightly sealed to avoid drying out.

12 Troubleshooting

Problem	Possible cause/*corrective action*
High back pressure during the run	The column is clogged. *Clean the column, see Section 8 Cleaning-in-place (CIP), on page 18.* High viscosity of solutions. *Use lower flow rate.*
Unstable pressure curve during sample loading	Air bubbles trapped in the sample pump. *If possible, degas the sample using a vacuum degasser.*
Gradual broadening of the eluate peak	Insufficient elution and CIP, caused by contaminants accumulating in the column. *Optimize the elution conditions, the CIP protocol and/or perform CIP more frequently.*
Gradual decrease in yield	Insufficient elution and CIP. *Optimize the elution conditions, the CIP protocol and/or perform CIP more frequently.*
Precipitation during elution	Sub-optimal elution conditions and CIP. *Optimize the elution conditions, the CIP protocol and/or perform CIP more frequently.*
Gradual increase in CIP peaks	Sub-optimal elution conditions and CIP. *Optimize the elution conditions, the CIP protocol and/or perform CIP more frequently.*
High back pressure during CIP	Proteins precipitated in column. *Optimize elution conditions and/or run high salt wash before CIP or use lower flow rate.*
Reduced column performance despite optimized elution and CIP	*Change to a new column. The longevity of the column depends mainly on the sample and sample preparation.*

13 Ordering information

Product	Quantity	Code No.
Hi Screen Ni FF	1 × 4.7 ml	28-9782-44

Related products	Quantity	Code No.
HisTrap™ FF	5 × 1 ml	17-5319-01
	5 × 5 ml	17-5255-01
HisTrap FF crude	5 × 1 ml	11-0004-58
	5 × 5 ml	17-5286-01
HisPrep™ FF 16/10	1 × 20 ml	28-9365-51
Ni Sepharose 6 Fast Flow	25 ml	17-5318-01
	100 ml	17-5318-02
	500 ml [1]	17-5318-03
HisTrap excel	5 × 1 ml	17-3712-05
	5 × 5 ml	17-3712-06
Ni Sepharose excel	100 ml	17-3712-02
	500 ml	17-3712-03

[1] Process-scale quantities are available. Please contact your local representative.

Accessories HiScreen	Quantity	Code No.
HiTrap/HiPrep, 1/16" male connector for ÄKTA *(For connection of columns with 1/16" fittings to ÄKTA)*	8	28-4010-81
Union 1/16" male/1/16" male with 0.5 mm i.d. *(For connecting two columns with 1/16" fittings in series)*	2	18-1120-93
Fingertight stop plug, 1/16" [1] *(For sealing a HiScreen column)*	5	11-0003-55

[1] One fingertight stop plug is connected to the inlet and the outlet of each HiScreen column at delivery.

Related literature	Code No.
Affinity Chromatography Handbook, Principles and Methods	18-1022-29
Recombinant Protein Purification Handbook, Principles and Methods	18-1142-75
Affinity Chromatography Column and Media, Selection Guide	18-1121-86
Ni Sepharose and IMAC Sepharose, Selection Guide	28-4070-92
Prepacked chromatography columns for ÄKTA systems, Selection Guide	28-9317-78

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