Instructions 28-9574-96 AD

HiTrap™ TALON® crude, 1 ml and 5 ml TALON Superflow™

HiTrap TALON crude and TALON Superflow are intended for purification of histidine-tagged recombinant proteins by immobilized metal affinity chromatography (IMAC).

TALON Superflow chromatography medium is recommended for batch/gravity-flow purification. The medium can also be used for packing into liquid chromatography columns such as Tricorn™ or XK-columns.

HiTrap TALON crude, 1 ml and 5 ml, are columns prepacked with TALON Superflow. The columns allow fast, simple and convenient purification of proteins from unclarified lysates. HiTrap TALON crude columns can be operated with a syringe, peristaltic pump, or liquid chromatography systems such as ÄKTA™.

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Please read these instructions carefully before using HiTrap columns.

Intended use

HiTrap columns 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 product in a safe way, please refer to the Safety Data Sheet.

1 Product description

HiTrap column characteristics

The columns are made of biocompatible polypropylene that does not interact with biomolecules.

The columns are delivered with a stopper at the inlet and a snap-off end at the outlet. Table 1 lists the characteristics of HiTrap columns.

Fig 1. HiTrap, 1 ml column.

Fig 2. HiTrap, 5 ml column.

Note: *HiTrap columns cannot be opened or refilled.*

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

Table 1. Characteristics of HiTrap columns.

Column volume (CV)	1 ml	5 ml
Column dimensions	0.7 × 2.5 cm	1.6 × 2.5 cm
Column hardware pressure limit	5 bar (0.5 MPa)	5 bar (0.5 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.*

Supplied Connector kit with HiTrap column

Connectors supplied	Usage	No. supplied
Union 1/16" male/ luer female	For connection of syringe to HiTrap column	1
Stop plug female, 1/16"	For sealing bottom of HiTrap column	2, 5 or 7

HiTrap TALON crude

HiTrap TALON crude prepacked with TALON Superflow is intended for preparative purification of histidine-tagged recombinant proteins. After thorough cell disruption, it is possible to load the unclarified lysate on the specially designed column without precentrifugation and filtration. The filters in the top and bottom of the column make it possible to directly load unclarified lysates on the column without causing backpressure problems or leakage of the TALON Superflow beads. The HiTrap TALON crude column provides rapid and easy processing in a convenient format.

HiTrap TALON crude columns are made of biocompatible polypropylene that does not interact with biomolecules. Columns are delivered with a stopper on the inlet and a snap-off end on the outlet.

Columns can be operated with either a syringe, a peristaltic pump or a chromatography system such as ÄKTA.

TALON Superflow

The TALON Superflow medium consists of highly cross-linked agarose beads with an immobilized chelating group. The TALON ligand is a tetra-dentate chelator charged with cobalt. This medium is suitable also for purification of low expressed proteins. The medium binds polyhistidine-tagged proteins with high selectivity and exhibits a reduced affinity for host proteins.

- The medium is compatible with many commonly used reagents.
- TALON Superflow has low cobalt ion (Co^{2+}) leakage.
- TALON Superflow allows protein purification under native or denaturing conditions.

- The medium can be used with all prokaryotic and eukaryotic expression systems in a variety of formats, including prepacked columns, small- (or mini-) scale batch screening, large-scale batch preparations, and methods using gravity-flow columns and spin columns.
- The medium usually works well with protocols designed for Ni^{2+}-based IMAC columns.

The characteristics of TALON Superflow are summarized in Table 2.

Table 2. TALON Superflow characteristics.

Matrix	Cross-linked agarose, 6%
Particle size distribution	60-160 µm
Binding capacity[1]	up to 20 mg histidine-tagged protein/ml medium
Maximum linear flow rate[2]	2000 cm/h
pH stability[3]	
- short term (2 hours)	2 to 14
- long term (one week)	3 to 12
Storage	20% ethanol at 4°C to 8°C
Compatibility during use	Stable in all commonly used buffers, denaturants and detergents (see Table 3).

[1] The binding capacity for individual proteins may vary.
[2] H_2O in a 0.75 x 10 cm column
[3] Co^{2+}-stripped medium.

Compatibility

TALON Superflow chromatography medium is compatible with all commonly used aqueous buffers, denaturants such as 6 M Gua-HCl and 8 M urea, and a range of other additives (see Table 3).

Table 3. Compatible reagents for TALON Superflow[1].

Reagent	Acceptable Concentration
β-Mercaptoethanol[2]	10 mM (with caution)
CHAPS, SDS, sarcosyl[3]	1 % (with caution)
Ethanol[4]	30 %
Ethylene glycol	30 %
HEPES	50 mM
Glycerol	20 %
Guanidinium hydrochloride	6 M
Imidazole[5]	≤ 500 mM at pH 7.0 to 8.0, for elution
KCl	500 mM
MES	20 mM
MOPS	50 mM
NaCl	1.0 M
NP-40	1 %
TRIS[6]	50 mM
Triton™-X 100	< 1 %
Urea	8 M

1 Data provided by Clontech Laboratories, Inc.

2 Use TALON Superflow immediately after equilibrating with buffers containing β-Mercaptoethanol. Otherwise, the medium will change color. Do not store the medium in buffers containing β-Mercaptoethanol.

3 Ionic detergents like CHAPS (3-[(30Cholamidopropyl)-dimethylammonio]-1-propane-sulfonate), SDS (sodium dodecyl sulfate), and sarkosyl are compatible up to 1%. However, due to their charged nature, you should anticipate interference with binding.

4 Ethanol may precipitate proteins, causing low yields and column clogging.

5 Imidazole at concentrations higher than 5-10 mM may cause lower yields of histidine-tagged proteins, because it competes with the histidine side chains (imidazole groups) for binding to the immobilized metal ions.

6 TRIS coordinates weakly with metal ions, causing a decrease in capacity.

Avoid using the following reagents

- DTT (dithiothreitol), DTE (dithioerythritol) and TCEP (TRIS (2-carboxyethyl)phosphine). Protein binding capacity will decrease rapidly.

  Note: *Use of strong reducing agents will cause discoloring of the medium and will interfere with the binding of the cobalt metal ions to the chromatography medium.*

- EDTA (ethylenediaminetetraacetic acid) and EGTA (ethylene glycolbis([β-amino-ethyl ether]). These chelators will strip off the cobalt ions from the medium.

  Note: *Although EDTA can be used at indicated points, it must be removed from the sample by buffer exchange prior to application to TALON Superflow.*

2 Buffer recommendations

General

- We recommend binding at neutral to slightly alkaline pH (pH 7 to 8) in the presence of 0.3 M to 0.5 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. In general, imidazole is used for elution of histidine-tagged proteins.
- Including salt in the buffers and samples, for example 0.3 M to 0.5 M NaCl, eliminates ion-exchange effects but can also have a marginal effect on the retention of proteins.
- Below pH 4, metal ions will be stripped off the medium.
- EGTA and EDTA will strip metal ions from the medium and thereby cause protein elution, but the target pool will then contain chelated Co^{2+} ions. In this case, the Co^{2+} ions can be removed by desalting on HiTrap Desalting, PD-10 Desalting, or HiPrep™ 26/10 Desalting columns, see Ordering information.

Native protein purification

Binding buffer: 50 mM sodium phosphate, 300 mM NaCl [1], pH 7.4

Wash buffer: 50 mM sodium phosphate, 300 mM NaCl [1], 5 mM imidazole [2], pH 7.4

Elution buffer: 50 mM sodium phosphate, 300 mM NaCl [1], 150 mM imidazole [2], pH 7.4

[1] Unspecific binding of proteins due to electrostatic interactions can be decreased by increasing the NaCl concentration up to 500 mM.

[2] The imidazole concentration required for wash and elution is protein-dependent. Higher or lower concentrations might be needed.

For buffer preparation, please refer to established protocols.

Denaturing protein purification

If the recombinant histidine-tagged protein is expressed as inclusion bodies, include 6 M Guanidine-HCl (Gua-HCl) or 8 M urea in all buffers and sample to promote protein unfolding. On-column refolding of the denatured protein may be possible, but depends on the protein. Advice for overcoming problems associated with inclusion bodies is described in Tips and hints, Section 8.

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.

3 Protocols

Sample preparation

For optimal growth and induction, please refer to established protocols.

Cell lysis protocol

1. Dilution of cell paste: Add 5 to 10 ml of binding buffer for each gram of cell paste.
2. Enzymatic lysis: 0.2 mg/ml lysozyme, 20 µg/ml DNAse, 1 mM $MgCl_2$, 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. If a commercial lysis kit is used—follow the recommended manufacturer's protocol. Note that the protocol may need to be optimized for optimal performance.
3. Mechanical lysis*:

   Sonication on ice, approx. 10 min

   or

   Homogenization with a French press or other homogenizer

   or

   Freeze/thaw, repeated at least five times

   * To prevent clogging of the column and back pressure problems, mechanical lysis time may have to be extended compared with standard protocols to secure an optimized lysate for sample loading. Different proteins have different sensitivity to cell lysis and caution has to be taken to avoid frothing and overheating of the sample.
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.

   Note: *If the sonicated or homogenized unclarified cell lysate is frozen before use, precipitation and aggregation may increase. New mechanical lysis of the lysate can then prevent increased back pressure problems when loading on the column.*

HiTrap TALON crude protocols

Purification protocol

1 Fill the pump tubing or syringe with distilled water. Remove the stopper and connect the column to the chromatography system tubing, syringe (use the adapter provided) or laboratory pump "drop-to-drop" to avoid introducing air into the system.

2 Remove the snap-off end at the column outlet.

3 Wash out the ethanol with 3 to 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 ml and 5 ml columns, respectively. In some cases, a blank run is recommended before final equilibration/ sample application.

Note: *Leakage of Co^{2+} from TALON Superflow is low under all normal conditions. For very critical applications, leakage during purification can be even further reduced by performing a blank run before loading sample.*
Blank run:
a) Wash the column with 5 column volumes of distilled water.
b) Wash with 5 column volumes of elution buffer.
c) Equilibrate with 10 column volumes of binding buffer.

5 Apply the unclarified lysate with a pump or a syringe.

Continuous stirring of the sample during sample loading may be necessary to prevent sedimentation.

Typical loading volumes of unclarified lysate (highly dependent on specific sample, sample pre-treatment, and temperature at sample loading):

HiTrap TALON crude 1 ml: Up to 100 ml

HiTrap TALON crude 5 ml: Up to 500 ml

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. Do not*

exceed the maximum back pressure of the column. Large volumes may increase back pressure, making the use of a syringe more difficult.

6 Wash with wash buffer until the absorbance reaches a steady baseline (generally at least 15 to 20 column volumes).

7 Elute with elution buffer using a one-step procedure or a linear gradient.

For step elution, 8 column volumes of elution buffer are usually sufficient. A shallow gradient, for example a linear gradient over 20 column volumes or more, can separate proteins with similar binding strengths.

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

TALON Superflow protocols

Column packing

TALON Superflow is supplied preswollen in 20% ethanol. Prepare a slurry by decanting the 20% ethanol solution and replacing it with distilled water in a ratio of 75% settled medium to 25% distilled water.

Packing a column

1 Assemble the column (and packing reservoir if necessary).

2 Remove air from the end-piece and adapter by flushing with water. Make sure no air has been trapped under the column bed support. Close the column outlet leaving the bed support covered with water.

3 Resuspend the medium and pour the slurry into the column in a single continuous motion. Pouring the slurry down a glass rod held against the column wall will minimize the introduction of air bubbles.

4 If using a packing reservoir, immediately fill the remainder of the column and reservoir with water. Mount the adapter or lid of the packing reservoir and connect the column to a pump. Avoid trapping air bubbles under the adapter or in the inlet tubing.

5 Open the bottom outlet of the column and set the pump to run at the desired flow rate. This should be at least 133% of the flow rate to be used during subsequent chromatographic procedures. However, the maximum flow rate, see Table 2, is typically employed during packing.

Note: *For subsequent chromatography procedures, do not exceed 75% of the packing flow rate.*

6 Maintain packing flow rate for at least 3 bed volumes after a constant bed height is reached. Mark the bed height on the column.

7 Stop the pump and close the column outlet.

8 If using a packing reservoir, disconnect the reservoir and fit the adapter to the column.

9 With the adapter inlet disconnected, push the adapter down into the column until it reaches the mark. Allow the packing solution to flush the adapter inlet. Lock the adapter in position.

10 Connect the column to a pump or a chromatography system and start equilibration. Re-adjust the adapter if necessary.

Purification procedure for a packed column

1 If the column contains 20% ethanol, wash it with 5 bed volumes of distilled water. Use a linear flow rate of 50 cm/h to 100 cm/h. See Section 9 how to convert linear flow (cm/h) to volumetric flow rate (ml/min).

2 Equilibrate the column with 5 to 10 bed volumes of binding buffer. Recommended linear flow rate: 150 cm/h. In some cases, a blank run is recommended before final equilibration/ sample application (see step 4 on page 10).

3 Apply the pretreated, filtered or centrifuged sample.

4 Wash with wash buffer until the absorbance reaches the baseline.

5 Elute with elution buffer using a step or linear gradient. For step elution, 8 bed volumes of elution buffer are usually sufficient. A shallow gradient, for example a linear gradient over 20 bed volumes, may separate proteins with similar binding strengths.

Batch/gravity-flow purification

1 Prepare the empty Disposable PD-10 Column.
   - **a** Wash the filter with 20% ethanol.
   - **b** Rinse the filter with distilled water.
   - **c** Insert the filter into the empty Disposable PD-10 Column. (Other empty gravity-flow columns can also be used.)

2 Prepare the TALON Superflow medium.
   - **a** Gently shake the bottle until the slurry is homogeneous.
   - **b** Remove a sufficient amount of slurry from the bottle and transfer to a centrifuge tube.
   - **c** Sediment the medium by centrifugation at 500 × g for 5 min.
   - **d** Discard the supernatant and replace with 5 bed volumes of distilled water.
   - **e** Gently shake the slurry for 3 min and resediment by centrifugation at 500 × g for 5 min.
   - **f** Repeat steps d and e using binding buffer instead of distilled water.
   - **g** Transfer the slurry to a measuring cylinder.
   - **h** Add an appropriate volume of binding buffer to make a 50% slurry.
   - **i** Transfer the 50% slurry to an appropriate container such as a tube, beaker or flask.

3 Perform purification.
   - **a** Add sample to the 50% slurry. Maximum binding capacity of TALON Superflow is approximately 20 mg/ml medium. This means that 1 ml of the 50% slurry can bind maximum 10 mg of histidine-tagged protein.
   - **b** Incubate sample and the TALON Superflow slurry on a shaker at low speed for 20-60 min.
   - **c** Load sample/TALON Superflow mix onto the PD-10 column and collect the flowthrough.
   - **d** Wash with 2 to 5 bed volumes of wash buffer and collect the flowthrough. For example, if 0.5 ml of TALON Superflow is

used (1 ml of 50% slurry), wash with 1 to 2.5 ml of wash buffer.

e Elute with 4 bed volumes of elution buffer and collect the eluted fractions in four separate tubes.

f Measure absorbance at 280 nm using a spectrophotometer and confirm purity of the pooled fractions by SDS-PAGE. Use elution buffer as the blank.

4 Optimization

Concentration of imidazole

Imidazole at low concentrations is commonly used in the binding and the wash buffers to minimize binding of host cell proteins. Imidazole can also be included in the sample (generally at the same concentration as in the wash buffer) to further minimize binding of host cell proteins. Too high concentration of imidazole in sample and binding buffer may 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 (strong binding of histidine-tagged target protein). This optimal concentration is different for different histidine-tagged proteins. Finding the optimal imidazole concentration for a specific histidine-tagged protein is a trial-and error effort, but 0 to 10 mM in the binding and wash buffers is a good starting point for many proteins. Use high purity imidazole, which gives essentially no absorbance at 280 nm. Another approach is to perform gradient elution from 0 to 150 mM imidazole over 20 column volumes.

5 Regeneration

Purification of histidine-tagged proteins using imidazole gradients will cause TALON Superflow to take on a dark purplish color. Washing the medium with 5 to 10 bed/column volumes of 20 mM MES Buffer (pH 5.0) will restore the normal pink color and bring the absorbance at 280 nm back to the original baseline level. After equilibrating with binding buffer, the medium/column is ready for reuse.

Stripping and re-charging

1. Strip the TALON Superflow/HiTrap TALON crude of cobalt ions by washing with 10 bed/column volumes of 0.2 M EDTA, pH 7.0.
2. Wash excess EDTA from the medium with an additional 10 bed/column volumes distilled water
3. Charge the chromatography medium with 10 bed/column volumes of 50 mM $CoCl_2$ solution.
4. Wash with 7 bed/column volumes of distilled water followed by 3 bed/column volumes of 300 mM NaCl and by 3 bed/column volumes of distilled water to remove excess cobalt metal ions.
5. Equilibrate with 10 bed/column volumes of binding buffer.

Note: *TALON Superflow/HiTrap TALON crude does not have to be stripped and recharged between each purification if the same protein is to be purified. It may be sufficient to strip and recharge it after approximately 2 to 5 purifications, depending on the specific sample, sample pre-treatment, sample volume, etc.*

6 Adjusting pressure limits in chromatography system software

Pressure generated by the flow through a column affects the packed bed and the column hardware, see Fig 3. 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.*

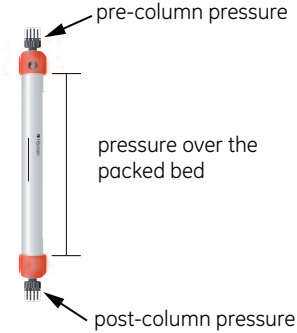

Fig 3. Pre-column and post-column measurements.

ÄKTA avant

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:

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.*

7 Storage

Store at 4°C to 8°C in 20% ethanol.

8 Tips and hints

The following tips may be of assistance. If you have further questions about HiTrap TALON crude columns or TALON Superflow,

Event	Possible cause: Action
Increased backpressure	Increase the efficiency of the mechanical cell disruption. Keep the sample on ice to avoid frothing and overheating as this may denature the target protein.
	Increase dilution of the cell paste before cell disruption or dilute after the lysis to reduce viscosity.
	If the lysate is very viscous due to a high concentration of host nucleic acid, continue cell disruption until the viscosity is reduced, and/or add an additional dose of DNAse and Mg^{2+} (see Sample preparation, section 3). Alternatively, draw the lysate through a syringe needle several times.
	Freeze/thaw of the unclarified lysate may increase precipitation and aggregation. Mechanical lysis 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 regeneration 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: up to 1% Triton X-100, 1% NP-40, 1% CHAPS, 1.0 M NaCl, 20% glycerol, 10 mM β-mercaptoethanol, 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 have a high absorbance at 280 nm. Furthermore, detergents cannot be easily removed by buffer exchange.

Event	Possible cause: Action
Protein is difficult to dissolve or precipitates during purification	Insoluble protein (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/wash and elution buffers. 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 add EDTA to the elution buffer (will cause stripping of cobalt ions).
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% Triton X-100) 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.

Event	Possible cause: Action
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).
	Contaminants have high affinity for cobalt 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 wash 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, adding a second chromatography step such as gel filtration may be necessary.
	Contaminants are associated with tagged proteins: Add detergent and/or reducing agents before disrupting cells. Increase detergent levels (e.g. up to 1% Triton X-100), or add glycerol (up to 20%) to the wash buffer to disrupt nonspecific interactions.
	Electrostatic interactions of contaminats with the tagged proteins or the chromatography medium: Add NaCl up to 500 mM in the buffers. Above 500 mM hydrophobic interactions might occur.
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 HiTrap TALON crude 1 ml columns have been used, change to a larger column, HiTrap TALON crude 5 ml.

Event	Possible cause: Action
Unwanted air bubble formation	Unclarified lysates may cause increased air bubble formation during purification. An attached flow restrictor in the chromatography system can prevent this. If a flow restrictor is attached, it is important to change the pressure limit to 0.5 MPa (5 bar) on the ÄKTA design system (where the column and the flow restrictor give a pressure of 0.3 MPa and 0.2 MPa, respectively).

9 Linear flow and volumetric flow rate

It is convenient when comparing results for columns of different sizes to express flow as linear flow (cm/h). However, flow is usually measured in volumetric flow rate (ml/min). To convert between linear flow and volumetric flow rate use one of the formulas below.

From linear flow rate (cm/h) to volumetric flow rate (ml/min)

$$\text{Volumetric flow rate (ml/min)} = \frac{\text{Linear flow (cm/h)}}{60} \times \text{column cross sectional area } (\text{cm}^2)$$

$$= \frac{Y}{60} \times \frac{\pi \times d^2}{4}$$

where
Y = linear flow in cm/h
d = column inner diameter in cm

From volumetric flow rate (ml/min) to linear flow rate (cm/hour)

$$\text{Linear flow (cm/h)} = \frac{\text{Volumetric flow rate (ml/min)} \times 60}{\text{column cross sectional area } (\text{cm}^2)}$$

$$= Z \times 60 \times \frac{4}{\pi \times d^2}$$

where
Z = volumetric flow rate in ml/min
d = column inner diameter in cm

Conversion using syringe

1 ml/min = approximately 30 drops/min on a HiTrap 1 ml column

5 ml/min = approximately 120 drops/min on a HiTrap 5 ml column

10 Ordering information

Products	Quantity	Code No.
HiTrap TALON crude	1 × 1 ml	29-0485-65
	5 × 1 ml	28-9537-66
	100 × 1 ml [1]	28-9538-05
HiTrap TALON crude	5 × 5 ml	28-9537-67
	100 × 5 ml [1]	28-9538-09
TALON Superflow	10 ml	28-9574-99
	50 ml	28-9575-02

[1] Pack size available by special order.

Related products	Quantity	Code No.
HiTrap Desalting	1 × 5 ml	29-0486-84
	5 × 5 ml	17-1408-01
	100 × 5 ml [1]	11-0003-29
PD-10 Desalting Column	30	17-0851-01
HiPrep 26/10 Desalting	1 x 53 ml	17-5087-01
	4 x 53 ml	17-5087-02

[1] Pack size available by special order.

Empty lab-scale columns	Quantity	Code No.
Tricorn 5/20 column, 5 mm i.d.	1	28-4064-08
Tricorn 5/50 column, 5 mm i.d.	1	28-4064-09
Tricorn 10/20 column, 10 mm i.d.	1	28-4064-13
Tricorn 10/50 column, 10 mm i.d.	1	28-4064-14
Tricorn 10/100 column, 10 mm i.d.	1	28-4064-15
XK 16/20 column, 16 mm i.d.	1	28-9889-37

HiTrap Accessories	Quantity	Code No.
1/16" male/luer female *(For connection of syringe to top of HiTrap column)*	2	18-1112-51
Tubing connector flangeless/M6 female *(For connection of tubing to bottom of HiTrap column)*	2	18-1003-68
Tubing connector flangeless/M6 male *(For connection of tubing to top of HiTrap column)*	2	18-1017-98
Union 1/16" female/M6 male *(For connection to original FPLC System through bottom of HiTrap column)*	6	18-1112-57
Union M6 female /1/16" male *(For connection to original FPLC System through top of HiTrap column)*	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" *(For sealing bottom of HiTrap column)*	5	11-0004-64
Fingertight stop plug, 1/16"	5	11-0003-55

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