

For life science research only.
Not for use in diagnostic procedures.



cOmplete His-Tag Purification Column

 **Version 02**

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Pre-charged, ready-to-use columns for small or large-scale purification of His-tagged proteins


Cat. No. 06 781 535 001

1 Column (5 ml Resin)

Cat. No. 06 781 543 001

5 Columns (1 ml Resin each)

Store at +2 to +8°C

 When properly stored, the product is stable until the expiration date printed on the label.

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1. What this Product Does

1.1 Introduction

Recombinant Protein Expression

Purifying a protein of interest is often essential for determining its function, structure, or interactions, for raising specific antibodies, or preparing enzymes for practical applications.

Isolation of naturally expressed proteins from their original source can be a complex process involving numerous chromatographic steps. Recombinant protein expression in dedicated host organisms can greatly simplify this task. Such expression systems generally ensure higher expression levels. Fusing the target protein to a tag also confers advantageous binding ability to an affinity matrix.

Protein Purification Using Immobilized Ni²⁺

The most common technique for efficiently obtaining large yields of highly purified protein in a short timeframe involves engineering a polyhistidine tag into the protein of interest, followed by purification using Immobilized Metal Ion Affinity Chromatography (IMAC). The most commonly used tag for large amounts of highly purified protein is a polyhistidine tag (His-tag). This tag has 6 to 14 histidines, typically fused to the N- or C-terminal end of a target protein. In some cases, the tag is also inserted into an exposed loop of the target protein. The imidazole side chains of a His-tag can form reversible coordinative bonds to divalent metal ions, such as Ni²⁺, Co²⁺, or Zn²⁺. This property can be used to separate polyhistidine-tagged target proteins from other proteins.

Ni²⁺ show highest affinity and selectivity for His-tags, and are therefore the preferred ions. Using a specific chelator covalently linked to a matrix, Ni²⁺ are immobilized to still permit interactions with histidine side chains. When His-tagged proteins are applied to such a Ni²⁺ resin, they specifically bind to the resin via Ni²⁺, while most untagged proteins do not.

Bound proteins are released from the resin using mild conditions. Imidazole competes for coordination sites on Ni²⁺ and therefore displaces His-tagged proteins from the resin. Alternatively, lowering the pH will protonate His-tags, decreasing their affinity for the resin and hence elute the His-tagged proteins.

His-Tags

Ideally, the His-tagged target protein binds much stronger to the Ni²⁺ chelate matrix than endogenous histidine-containing protein of the expression host. Relative binding strength depends on how many histidines can bind simultaneously to the matrix (avidity effect). Longer His-tags confer stronger binding and better separation of the target from potentially contaminating host proteins. The classic His-tag has six consecutive histidines. Tags with 10 to 14 histidines may produce a better purification.

Most importantly, His-tagged proteins can be purified using Ni²⁺ chelate matrices under both native and denaturing conditions. Due to their hydrophilic and flexible nature, these matrices increase the solubility of the target proteins and only rarely interfere with protein function.

This unique combination of features enables the His-tag to be a versatile tool for a wide range of protein purification applications.

1.2 Properties

cOmplete His-Tag Purification Columns include a sepharose-based, pre-charged, ready-to-use Ni²⁺ chelate resin for purification of His-tagged proteins. It allows for the production of highly pure proteins from crude lysates, using a one-step purification process.

cOmplete His-Tag Purification Columns are based on a chelator chemistry enabling an extremely tight binding of Ni²⁺ to the resin. In contrast to conventional nitrilotriacetic acid (NTA)-based resins and iminodiacetic acid (IDA)-based matrices, the chelator of cOmplete His-Tag Purification Columns protects Ni²⁺ effectively against reduction by thiols, resulting in minimal leaching of the ions.

Together, these features enable the researchers to adapt the buffer scheme to the specific needs of the target protein over a wide range of parameters. Specifically, cOmplete His-Tag Purification Columns allow for efficient protection of the target proteins from proteolytic degradation, oxidative damages and heavy metal contamination.

The reagent compatibility also allows the resin to be used with cOmplete ULTRA Tablets, Roche's protease inhibitor cocktail containing EDTA, and with PhosSTOP Tablets for inhibiting phosphatases, as well as with common reducing agents, such as DTT.

Technical Specifications

Matrix Sepharose-CL 6B

Bead Size 45 to 165 μM

Binding Capacity ≥ 40 mg protein per ml bed volume of resin

Ⓢ The binding capacity of the resin to various types of proteins may vary according to the protein characteristics such as the size of the protein.

cOmplete His-Tag Purification Columns bind with a high specificity to the polyhistidine-tagged protein. As a consequence, the binding kinetics may appear to be different when compared to conventional metal chelate matrices. Full capacity of cOmplete His-Tag Purification Columns can be achieved by allowing more time for the protein to bind to the resin by lowering the flow rate during the chromatography purification procedure.

Maximal Linear Flow Rate 1,420 cm/hour

Recommended Volumetric Flow Rate	<p>5 ml column (06 781 535 001): 2.5 to 10 ml/min. 1 ml column (06 781 543 001): 0.5 to 2.0 ml/min. The volumetric flow rate is a function of the cross section of the column.</p> <p>Ⓢ Using the following formula, a linear flow rate can be converted to a volumetric flow (ml/min):</p> $\text{Linear flow rate (cm/hour)} \times \text{column cross sectional area (cm}^2\text{)} / 60$ <p>Ⓢ The column cross sectional area is defined as $\pi \times r^2$, whereas π is the constant pi and r is the inner radius of the column.</p>
Recommended Imidazole Concentration for Load/Wash	<p>Nonspecific binding of proteins without a His-tag to cOmpete His-Tag Purification Resin is low. Use up to 5 mM imidazole in load and/or wash buffers.</p> <p>⚠ If establishing a new assay for purification of His-tag proteins using cOmpete His-Tag Purification Columns, do not use imidazole for loading and washing.</p> <p>If, <i>e.g.</i>, the purity of the His-tag protein needs to be improved following this first step, use imidazole in a final concentration of up to 5 mM in a second step for loading and/or washing.</p>
Recommended Imidazole Concentration for Elution	<p>Up to 500 mM</p> <p>Ⓢ In contrast to other available resins, bound His-tagged protein typically elutes from cOmpete His-Tag Purification Columns with a lower imidazole concentration, <i>e.g.</i>, 25 to 45 mM.</p>
Compatibility for Long Term Storage	<p>20% ethanol, pH 4.0 to pH 9.0</p>
Compatibility During Chromatography	<p>The resin is compatible with 10 mM EDTA, 10 mM DTT during the purification (1 hour incubation), 6 M guanidinium-HCl, 8 M urea, pH 2.0 to pH 14.0.</p>
Compatibility During Cleaning	<p>4% SDS</p>
Form	<p>cOmpete His-Tag Purification Resin filled in columns, pre-charged with Ni²⁺, stored in 20% ethanol.</p>

1.3 Content

Pre-charged, ready-to-use columns for small or large scale purification of His-tagged proteins:

Component	Content
5 ml Column	06 781 535 001 <ul style="list-style-type: none">• 1 column• 5 ml bed volume of resin within the column• Inner diameter: 16.2 mm• Height: 25 mm
1 ml Column	06 781 543 001 <ul style="list-style-type: none">• 5 columns• 1 ml bed volume of resin within the column• Inner diameter: 7.2 mm• Height: 25 mm

1.4 Storage and Stability

The columns are stable at +2 to +8°C until the expiration date printed on the label.

🕒 The product is shipped at +15 to +25°C.

1.5 Application

cOmplete His-Tag Purification Columns can be used for purification of His-tagged proteins, yielding highly purified proteins from crude lysates.

This product can be used for target protein purification using both native and denaturing conditions.

cOmplete His-Tag Purification Columns are compatible with common reducing agents and chelators while preventing contamination of the protein preparation with heavy metals.

2. How to Use this Product

2.1 General Considerations

The purification of a single type of protein from a complex mixture includes a series of procedures. The following aspects should be considered.

Choice of an Appropriate Host Organism

The choice of an appropriate expression system is crucial to achieve successful protein purification. Most proteins are produced in *E. coli*.

E. coli is often the host of choice because it combines easy strain construction, rapid growth, and inexpensive culture handling with high overexpression capabilities and low background binding of host proteins. *E. coli* features cytosolic and periplasmic expression and can be used to express recombinant proteins in both reducing and oxidizing environments.

In addition to *E. coli*, specialized mammalian-, insect- and yeast-based expression systems are available, and can be employed when folding problems are encountered in *E. coli* or if eukaryotic posttranslational modifications are a concern.

Choice Between Native Versus Denaturing Purifications

cOmplete His-Tag Purification Columns can be used to purify proteins using both native and denaturing conditions. In most cases, a native purification is preferred over a denaturing purification, as the vast majority of functional applications rely on a properly folded protein.

Purification under denaturing conditions may also be preferred under specific circumstances, such as when proteolytic degradation occurs during purification, contamination is likely from host proteins, and when low target protein solubility is present. Typical denaturing agents for such situations are urea and guanidinium-HCl.

Ⓢ The binding capacity and binding kinetics of the affinity matrix could differ in native and denaturing purification protocols.

Choice of the Appropriate Length of the His-Tag

As described in section *Introduction*, the length of the His-tag fused to the target protein determines the binding specificity. When using hexahistidine tags, there may be occasions where the histidine amino acids from the tag cannot be readily distinguished from endogenous histidine amino acids. His10- to His14-tagged proteins can be more efficiently separated from histidine-rich host proteins.

Ⓢ cOmplete His-Tag Purification Columns are compatible with a polyhistidine tag length from 6 to 14 histidines.

Choice of the Appropriate Buffer System

cOmplete His-Tag Purification Columns are compatible with a wide range of buffers. To maximize purification effectiveness, it is important to select the optimal buffer for the stability and solubility of the target protein.

Due to the high binding strength of Ni^{2+} to the resin within cOmplete His-Tag Purification Columns, the optimal buffer can be selected for protein purification without having to compromise between protein stability and resin stability. Buffer composition can also be adjusted according to the needs of the target protein.

Buffers containing EDTA and DTT are compatible with cOmplete His-Tag Purification Columns. These features effectively inhibit metalloproteases and facilitate the purification of proteins prone to oxidation.

To achieve optimal protein purity, the stringency during the binding and washing steps, as well as the conditions for elution, can be fine-tuned by adjusting either the concentration of imidazole or the pH value.

Imidazole competes with His-tagged proteins to bind Ni^{2+} immobilized on the resin of cOmplete His-Tag Purification Columns. Addition of low concentrations of imidazole may help to revert undesired binding of host proteins to the resin.

The binding of the His-tagged target protein to the resin of cOmplete His-Tag Purification Columns is also pH dependent.

- Ⓢ While it is most convenient to adjust the pH of buffers at +15 to +25°C, the pK_a value of commonly used buffers changes with the temperature. For this reason, the pH value of buffers should be adjusted at the same temperature as the temperature of the purification experiment.
- Ⓢ *E. coli* grown with a fermentable carbon source (such as glucose) may produce organic acids that lower the pH. For this reason, buffer the media with, *e.g.*, K_2HPO_4 , and resuspend cells in an alkaline buffer with high buffering capacity. For best results, recheck the pH value after cell resuspension and readjust the pH if necessary.

Lysate Preparation

Prior to lysate preparation, the target protein should be recombinantly expressed in a host organism. Here again, *E. coli* is, in most cases, the host of choice.

The choice of an *E. coli* strain, expression conditions (temperature, media composition, induction strength, duration of induction), and the choice of lysis buffer can have a significant impact on target protein yield and purity.

Typically, these parameters must be optimized on a case-to-case basis.

- Ⓢ Optimal methods for lysate preparation may significantly differ between different host organisms.
- ⚠ After harvesting, cells and lysates should be handled on ice or at +2 to +8°C.

2.2 Before You Begin

Read each protocol completely before starting. Successful results depend on performing the protocol steps correctly.

Handling Requirements

The resin within cOComplete His-Tag Purification Columns must always be maintained in buffer and never be allowed to dry.

Laboratory Procedures

- Do not eat, drink or smoke in the laboratory area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats, and eye protection, when handling the resin and samples containing the proteins.

2.3 Purification Protocols

cOComplete His-Tag Purification Columns are compatible with automated chromatography systems such as ÄKTAexplorer.

Purification Under Native Conditions

Purification of native proteins should be performed using optimal buffer conditions for the target protein. Buffers recommended in this document are well established examples and can be adapted to achieve optimal conditions for a specific target protein.

cOComplete His-Tag Purification Columns offer flexibility in selecting the optimal buffer conditions.

⚠ The binding capacity may drop significantly if the buffer composition is suboptimal.

🕒 For best results, load with a low flow rate to bind the target proteins more efficiently to the resin.

⚠ cOComplete His-Tag Purification Columns have been optimized using **Buffer A** and **Buffer B** specified in the table below. Other buffers might function as well, but need to be tested prior to use with cOComplete His-Tag Purification Columns.

Component	Composition
Buffer A	<ul style="list-style-type: none"> • 50 mM NaH₂PO₄ pH 8.0 • 300 mM NaCl
Buffer B	<ul style="list-style-type: none"> • 50 mM NaH₂PO₄ pH 8.0 • 300 mM NaCl • 250 mM imidazole

- ③ The following protocol describes the experimental procedures when using an ÄKTAexplorer 100 System (GE Healthcare Life Sciences) for FPLC purification.

Step Protocol

- ① Wash the pump with 10 to 20 ml **Buffer A** using the *System Wash* function of the ÄKTAexplorer System at a flow rate of, *e.g.*, 10 ml/min. Ensure that all air is displaced from the pumps and tubings of the system.
 - ② Remove the plug at the column outlet and attach it to the outlet tubing of the ÄKTAexplorer System.
 - ③ Save the plug of the column outlet in case the column needs to be stored or is to be reused.
 - ③ As soon as **Buffer A** is running out of the inlet tubing of the ÄKTAexplorer System, remove the upper plug from the column and immediately attach it to the inlet tubing of the ÄKTAexplorer System. Continuously measure OD₂₈₀ values on the ÄKTAexplorer System.
 - ③ If a fluorescent protein is purified, continuously measure the OD values at the absorption maximum of the fluorescence dye; *e.g.*, OD₄₈₅ for GFP (Green Fluorescent Protein) or OD₄₃₅ for CFP (Cyan Fluorescent Protein).
 - ③ Save the plug of the column inlet in case the column needs to be stored or is to be reused.
 - ④ Define the flow rate as **10 ml/min for the 5 ml column** or **2 ml/min for the 1 ml column** and equilibrate the column with 10 column volumes of **Buffer A**.
 - ⑤ Pause the run. Load the cleared sample (*e.g.*, after an ultracentrifugation or filtration step) onto the column with a volumetric flow rate of **2.5 ml/min for the 5 ml column** or **0.5 to 1 ml/min for the 1 ml column**.
 - ⚠ **To prevent blockage of the column, remove insoluble material prior to loading the column.**
 - ⚠ Since the binding specificity of the resin is high, the kinetics of adhesion of the protein to the resin is slower than other available resins. If using high volumetric flow rates for loading, protein yield can decrease.
 - ⑥ Wash the column with **Buffer A** until the OD₂₈₀ value reaches the baseline level (approximately 10 column volumes).
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- 7 Elute the His-tagged protein with a gradient of **Buffer A** (without imidazole) and **Buffer B** (250 mM imidazole).
- ⚠ **Protein peaks can be expected between 25 to 45 mM imidazole.** Due to the specific characteristics of cComplete His-Tag Purification Columns, a protein can already be eluted with approximately 25 mM imidazole.
 - ⚠ The amount of imidazole required in the elution buffer for efficient release of the target protein from the resin depends on various parameters, *e.g.*
 - the length of the His-tag,
 - the accessibility of the His-tag.
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- 8 Wash and equilibrate for the next run. For details, refer to section *Cleaning Procedures*.
- ⚠ If the column is not immediately reused, clean the column with 2 column volumes of 2 M imidazole to remove nonspecific binding of proteins. Equilibrate the column in a 20% ethanol solution and tightly close the column at both threads with plugs. Store at +2 to +8°C to prevent cell growth.
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- 🔍 Refer to sections *Purification Process Optimization* and *Troubleshooting* for technical advice in optimizing the purification results.

Purification Under Denaturing Conditions

Purification of denatured proteins should be performed using optimal buffer conditions for the target protein. Buffers recommended in this document are well established examples and can be adapted to achieve optimal conditions for a specific target protein.

cComplete His-Tag Purification Columns offer flexibility in selecting optimal buffer conditions.

Denature the protein or dissolve the inclusion bodies in a buffer containing 6 M guanidinium-HCl or 8 M urea.

- ⚠ The addition of urea to buffered solutions will cause the pH to drop. It is essential to adjust the pH of the buffer with NaOH after urea addition.
- ⚠ **The binding capacity may also drop significantly if the buffer composition is suboptimal.**
- 🔍 For best results, load with a low flow rate to bind the target proteins more efficiently to the resin.
- ⚠ cComplete His-Tag Purification Columns have been optimized using **Buffer C, Buffer D, Buffer E, and Buffer F** specified in the table below. Other buffers might function as well, but need to be tested prior to use with cComplete His-Tag Purification Columns.

Component	Composition
Buffer C	<ul style="list-style-type: none"> • 100 mM NaH₂PO₄ • 10 mM Tris-HCl • 8 M urea • pH 8.0
Buffer D	<ul style="list-style-type: none"> • 100 mM NaH₂PO₄ • 10 mM Tris-HCl • 8 M urea • pH 6.3
Buffer E	<ul style="list-style-type: none"> • 100 mM NaH₂PO₄ • 10 mM Tris-HCl • 8 M urea • pH 5.9
Buffer F	<ul style="list-style-type: none"> • 100 mM NaH₂PO₄ • 10 mM Tris-HCl • 8 M urea • pH 4.5

④ The following protocol describes the experimental procedures when using an ÄKTAexplorer 100 System (GE Healthcare Life Sciences) for FPLC purification.

Step Protocol

- ① Wash the pump with 10 to 20 ml **Buffer C** using the *System Wash* function of the ÄKTAexplorer System at a flow rate of, e.g., 10 ml/min. Ensure that all air is displaced from the pumps and tubings of the system.
- ② Remove the plug at the column outlet and attach it to the outlet tubing of the ÄKTAexplorer System.
 - ④ Save the plug of the column outlet in case the column needs to be stored or is to be reused.
- ③ As soon as **Buffer C** is running out of the inlet tubing of the ÄKTAexplorer System, remove the upper plug from the column and immediately attach it to the inlet tubing of the ÄKTAexplorer System. Continuously measure OD₂₈₀ values on the ÄKTAexplorer System.
 - ④ If a fluorescent protein is purified, continuously measure the OD values at the absorption maximum of the fluorescence dye; e.g., OD₄₈₅ for GFP (Green Fluorescent Protein) or OD₄₃₅ for CFP (Cyan Fluorescent Protein).
 - ④ Save the plug of the column inlet in case the column needs to be stored or is to be reused.

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- 4 Define the flow rate as **10 ml/min for the 5 ml column** or **2 ml/min for the 1 ml column** and equilibrate the column with 10 column volumes of **Buffer C**.

 - 5 Pause the run. Load the cleared sample (e.g., after an ultracentrifugation or filtration step) onto the column with a volumetric flow rate of **2.5 ml/min for the 5 ml column** or **0.5 to 1 ml/min for the 1 ml column**.
 - ⚠ **To prevent blockage of the column, remove insoluble material prior to loading the column.**
 - ⚠ Since the binding specificity of the resin is high, the kinetics of adhesion of the protein to the resin is slower than other available resins. If using high volumetric flow rates for loading, protein yield can decrease.

 - 6 Wash the column with **Buffer C** until the OD₂₈₀ value reaches the baseline level (approximately 10 column volumes).

 - 7 Wash with 10 to 20 column volumes of **Buffer D**.

 - 8 Wash with 10 to 20 column volumes of **Buffer E**.

 - 9 Elute the His-tagged protein with 10 to 20 column volumes of **Buffer F**.
 - 🕒 Alternatively, the elution can also be performed with a gradient up to 250 mM imidazole solution using **Buffer A** and **Buffer B** instead of the pH shift option (refer to the elution step within section *Purification Under Native Conditions*).

 - 10 Wash and equilibrate for the next run under denaturing conditions with **Buffer C** or wash with **Buffer A** to remove the denaturing agents if the column will next be used under native conditions. For details, refer to section *Cleaning Procedures*.
 - ⚠ If the column is not immediately reused, clean the column with 2 column volumes of 2 M imidazole to remove nonspecific binding of proteins. Equilibrate the column in a 20% ethanol solution and tightly close the column at both threads with plugs. Store at +2 to +8°C to prevent cell growth.

 - 🕒 Refer to sections *Purification Process Optimization* and *Troubleshooting* for technical advice in optimizing the purification results.

2.4 Cleaning Procedures

cOmplete His-Tag Purification Columns can be used multiple times without loss of binding capacity. Over time, however, some protein aggregates might accumulate, leading to a decrease of efficiency of the resin within the columns. This can be identified by a slower flow rate or a higher back pressure.

The cleaning procedures remove aggregates for further efficient use of the columns. Different cleaning procedures can be carried out, based on the different applications. Once the cleaning procedure is completed, the resin should be transferred to 20% ethanol.

Stringent Native Cleaning

This method is appropriate when non-aggregating proteins have been purified, and if the column is used again for purifying the same protein.

- Wash with 10 column volumes of 1 M imidazole/HCl, pH 7.5,
- Wash with 10 column volumes of 4 M imidazole/HCl, pH 7.5,
- Equilibrate the column with binding buffer and proceed to the next round of purification or transfer the material to 20% ethanol.

Denaturing Cleaning with SDS

This method is appropriate to remove aggregated proteins and lipids.

⚠ This cleaning procedure has to be performed at +15 to +25°C because the solubility of SDS is more effective at this temperature than at +2 to +8°C.

Ⓢ The SDS buffer may also contain 50 mM DTT.

⚠ Avoid using K⁺ in this buffer to prevent precipitation with SDS.

- Wash with 10 column volumes of 1 M imidazole/HCl, pH 7.5,
- Wash 2 times with 10 column volumes of 1 M imidazole/HCl, pH 7.5, 20% ethanol, 2 to 4% SDS,
- Remove SDS with 3 times 10 column volumes of 20% ethanol.

Denaturing Cleaning with Guanidinium-HCl

This method is appropriate to remove aggregated proteins.

Ⓢ The guanidinium-HCl buffer may also contain 50 mM DTT.

- Wash with 10 column volumes of 1 M imidazole/HCl, pH 7.5,
- Wash 2 times with 10 column volumes of 6 M guanidinium-HCl, 1 M imidazole, pH 7.5,
- Wash 2 times with 10 column volumes of 20% ethanol.

Ⓢ In general, the choice of cleaning method depends on the protein type.

Ⓢ The denaturing cleaning procedure with guanidinium-HCl presents fewer constraints than the denaturing cleaning method with SDS.

3. Purification Process Optimization

The parameters allowing for the maximal protein yield and purity might vary significantly depending on the characteristics of a given target protein.

To optimize the protein purification procedure for highest protein purity, determine the optimal operating conditions for the specific target protein.

Both purity and yield of a protein preparation depends on the sample amount. If the amount of sample is too high, the resin's binding capacity may not be sufficient to bind all target protein, and this will result in a suboptimal protein yield. If the amount of sample is too low, the remaining binding sites on the resin may enable background binding of lysate components.

Optimal results are obtained when the amount of target protein matches the amount of resin within the columns.

The capacity for a given target protein depends on several factors such as target protein size, conformation, multimerization status, length and accessibility of the His-tag, expression level and solubility of the His-tagged protein, lysate concentration, as well as the buffer pH and composition.

For best results, determine the optimal ratio of the volume of lysate and resin within the columns required for the purification of a specific protein of interest, which is dependent on the expression rate of the protein:

- Incubate the columns with varying volumes of lysates, in parallel test experiments,
 - Wash the columns and elute the bound proteins,
 - Determine the amount of target protein in the unbound fractions and in the eluate by SDS-PAGE,
 - The volume of lysate is optimal when only a small amount of target protein remains in the flow through and the maximal amount of protein is detected in the eluate fractions.
- ③ The yield of the target protein can be optimized by allowing more time for the protein to bind to the resin. This can be performed by reducing the flow rate during the loading step of the chromatography purification.
 - ③ The optimal concentration of imidazole during binding, washing, and elution steps can also be determined during pretrial experiments.
 - ③ Optimal results can typically be achieved with buffers containing a high salt concentration (300 mM) at pH 8.0 for target proteins compatible with those conditions.

4. Troubleshooting

Problem	Possible Cause	Recommendation
Bubbles form in the bed resin.	<ul style="list-style-type: none"> Mixing of the storage buffer (20% ethanol) with aqueous buffer. 	<ul style="list-style-type: none"> After storage at +2 to +8°C, bring the columns to +15 to +25°C. Degas the buffer prior to equilibration of the column.
The sample does not flow easily through the columns (low flow rate or high back pressure).	<ul style="list-style-type: none"> Particulates from the lysates may have clogged the columns. 	<ul style="list-style-type: none"> Centrifuge or ultracentrifuge the sample prior to loading on the column. Reduce the flow rate. Clean the columns using a denaturing cleaning procedure.
Inefficient binding of the target protein to the resin within the columns.	<ul style="list-style-type: none"> Suboptimal buffer conditions during the binding step. Incubation time is too short. The His-tag is not accessible. 	<ul style="list-style-type: none"> Lower the imidazole concentration and/or increase the pH during the binding step. Extend the incubation time. Lower the flow rate during binding. Change the position of the His-tag. Use a longer His-tag.
Inefficient or no elution of the target protein.	<ul style="list-style-type: none"> The target protein multimerizes and binds more avidly to the resin. The protein precipitates on the resin prior to elution. The target protein precipitates during a pH shift elution. 	<ul style="list-style-type: none"> Increase the imidazole concentration during elution. Increase ionic strength to minimize isoelectric precipitations. Elute under denaturing conditions. Elute with imidazole instead.

Problem	Possible Cause	Recommendation
Recovery of the target protein is too low.	<ul style="list-style-type: none"> • The target protein may be degraded. • The His-tag might not be accessible. • The His-tag might have been digested by proteases. • The target protein might not be soluble. • The resin is limiting. 	<ul style="list-style-type: none"> • Add protease inhibitors to the sample if degradation occurs during cell lysis. • Protein degradation can also be prevented by working at +2 to +8°C. • Use a longer His-tag. • Check if the target protein contains the His-tag. • Optimize expression conditions and buffers. • Change the localization of the His-tag. • Change to another expression host. • Use protease inhibitors. • Lower the expression temperature, strength, and duration of induction. • Purification under denaturing conditions. • Include solubility-enhancing fusion partners. • Verify that the expressed His-tagged protein is proportionate to the resin within the columns.
Target protein elutes with contaminants.	<ul style="list-style-type: none"> • The host proteins interact with the resin. • DNA and/or RNA contaminants. 	<ul style="list-style-type: none"> • Increase the stringency during the loading and washing step by increasing the imidazole concentration/lowering the pH. • Increase the amount of the sample. • Wash the column with a stringent buffer. • Purify under denaturing conditions. • Include a DNase I digestion step and/or a Polymin P-mediated precipitation step prior to adding the lysate to the columns.
Target protein is degraded during or following the cell lysis.	<ul style="list-style-type: none"> • Insufficient protection from proteases. 	<ul style="list-style-type: none"> • Add protease inhibitors to the buffers and/or culture. • Optimize the experimental workflow. • Strictly work on ice.
Target protein is degraded in the host cell.		<ul style="list-style-type: none"> • Use a protease-deficient host strain. • Reduce the induction time.

5. Additional Information on this Product

5.1 Quality Control

cOmplete His-Tag Purification Columns are function tested using the following procedures:

Binding capacity is determined using the His-tagged T4 gene 32 protein under standard conditions.

6. Supplementary Information



6.1 Conventions

Text Conventions To make information consistent and easy to understand, the following text conventions are used in this document:

Text Conventions	Usage
Numbered stages labeled 1 , 2 , etc.	Steps in a procedure that must be performed in the order listed.

Symbols

In this document, the following symbols are used to highlight important information:

Symbol	Description
	Information Note: Additional information about the current topic or procedure.
	Important Note: Information critical to the success of the procedure or use of the product.

Abbreviations

In this document the following abbreviations are used:

Abbreviation	Meaning
DTT	Dithiothreitol
EDTA	Ethylendiaminetetraacetic Acid
FPLC	Fast Protein Liquid Chromatography
IDA	Iminodiacetic Acid
IMAC	Immobilized Metal Ion Affinity Chromatography
NTA	Nitrilotriacetic Acid
pK _a	Acid Dissociation Constant
SDS	Sodium Dodecyl Sulfate
PAGE	Polyacrylamide Gel Electrophoresis

6.2 Changes to Previous Version

Editorial changes

6.3 Ordering Information

Product	Pack Size	Cat. No.
cOmplete His-Tag Purification Resin	25 ml 200 ml	05 893 682 001 05 893 801 001
cOmplete Protease Inhibitor Cocktail Tablets		
cOmplete ULTRA Tablets, EDTA-free, glass vials	2 × 10 tablets in glass vial 6 × 10 tablets in glass vial	05 892 953 001 06 538 282 001
cOmplete ULTRA Tablets, glass vials	2 × 10 tablets in glass vial 6 × 10 tablets in glass vial	05 892 988 001 06 538 304 001
cOmplete ULTRA Tablets, Mini, EDTA-free, <i>EASYPack</i>	30 tablets, individually packed in foil blister pack	05 892 791 001
cOmplete ULTRA Tablets, Mini, <i>EASYPack</i>	30 tablets, individually packed in foil blister pack	05 892 970 001
PhosSTOP Phosphatase Inhibitor Cocktail Tablets		
PhosSTOP	20 tablets in <i>EASYPacks</i> (foil blister)	04 906 837 001
	10 tablets in <i>EASYPacks</i> (foil blister)	04 906 845 001

6.4 License Limitations

For patent license limitations for individual products please refer to:
[List of biochemical reagent products](#)

6.5 Trademarks

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6.6 Regulatory Disclaimer

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