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

cOmplete His-Tag Purification Column

Pre-charged, ready-to-use columns

Version: 03

Content Version: June 2021

For small or large-scale purification of His-tagged proteins.

Cat. No. 06 781 535 001	1 column 1 column containing 5 ml of cOmplete His-Tag Purification Resin
Cat. No. 06 781 543 001	5 x 1 ml columns 5 columns containing 1 ml of cOmplete His-Tag Purification Resin

Store the product at +2 to +8°C.

1. General Information

1.1. Contents

Bottle	Label	Function / Description	Catalog Number	Size	Content
1	cOmplete His-Tag Purification Column	▪ Ready-to-use columns for the purification of His-tagged proteins. ▪ Pre-charged with $Ni^{2}+$. ▪ Supplied in a 20% ethanol storage buffer.	06 781 535 001	Inner diameter: 16.2 mm Height: 25 mm	1 Column (5 ml Resin)
			06 781 543 001	Inner diameter: 7.2 mm Height: 25 mm	5 Columns (1 ml Resin each)

1.2. Storage and Stability

Storage Conditions (Product)

When stored at +2 to +8°C, the product is stable through the expiry date printed on the label.

Vial / bottle	Label	Storage
1	cOmplete His-Tag Purification Column	Store at +2 to +8°C.

1.3. Additional Equipment and Reagent required

For purification under native conditions

- NaH_2PO_4
- NaCl
- Imidazole
- Ethanol

For purification under denaturing conditions

- NaH_2PO_4
- Tris-HCl*
- Urea
- Ethanol
- NaOH

For cleaning

- Imidazole
- HCl
- Ethanol
- SDS*
- DTT*
- Guanidinium-HCl

1.4. Application

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

Sample Materials

Lysate preparation

Prior to the lysate preparation, express the target protein recombinantly in a host organism, preferably in *E. coli*. The choice of an *E. coli* strain, expression conditions (temperature, media composition, induction strength, duration of induction), and the lysis buffer can have a significant impact on the yield and purity of the target protein. 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, handle cells and lysates on ice or at +2 to+8°C.

General Considerations

Precautions

- cOmplete His-Tag Purification Columns must always be maintained in buffer and never be allowed to dry.
- 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.

The purification of a single type of protein from a complex mixture includes a series of procedures. Consider the following variables when optimizing the protocols:

Variable	Description and Recommendation
Appropriate host organism	Most proteins are produced in *E. coli* which is often the host of choice as 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. ▪ Specialized mammalian-, insect-, and yeast-based expression systems are also available, and can be used when folding problems or eukaryotic posttranslational modification problems are encountered in *E. coli*.
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, as the vast majority of functional applications rely on a properly folded protein. ▪ Purification under denaturing conditions may be preferred 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 can differ in native and denaturing purification protocols.*
Appropriate length of the His-Tag	The length of the His-tag fused to the target protein determines the binding specificity. ▪ When using hexahistidine tags, histidine amino acids from the tag may not 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.*

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 cOmplete His-Tag Purification Columns, the optimal buffer can be selected for protein purification without having to compromise between protein stability and resin stability. ▪ The 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. Adding 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 cOmplete His-Tag Purification Columns is also pH dependent. *The pK_a value of commonly used buffers changes with the temperature. Adjust the pH value of buffers at the same temperature as the temperature of the purification experiment.* *As E. coli grown with a fermentable carbon source, such as glucose, may produce organic acids that lower the pH value, buffer the media with, for example, K_2HPO_4, and resuspend cells in an alkaline buffer with high buffering capacity. For best results, control the pH value after cell resuspension and readjust if necessary.*
Imidazole concentration for load/wash	Nonspecific binding of proteins without a His-tag to cOmplete His-Tag Purification Resin is low. Use up to 5 mM imidazole in load and/or wash buffers. ⚠ ***Do not use imidazole for new purification assays of His-tag proteins using cOmplete His-Tag Purification Resin.*** To improve the purity of the His-tag protein following this first step, use imidazole in a final concentration of up to 5 mM in a second step.
Imidazole concentration for elution	Up to 500 mM *In contrast to other available resins, bound His-tagged protein typically elutes from cOmplete His-Tag Purification Columns with a lower imidazole concentration, such as 25 to 45 mM.*
Compatibility for long-term storage	20% ethanol, pH 4.0 to pH 9.0.
Compatibility during chromatography	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.
Compatibility during cleaning	4% SDS

Technical specifications

Specification	Value
Matrix	Sepharose-CL 6B
Bead size	45 – 165 µM
Maximal linear flow rate	1,420 cm/hour
Recommended volumetric flow rate	5 ml column: 2.5 to 10 ml/min. 1 ml column: 0.5 to 2.0 ml/min. The volumetric flow rate is a function of the cross-section of the column. *Using the following formula, a linear flow rate can be converted to a volumetric flow (ml/min): Linear flow rate (cm/hour) × column cross-sectional area (cm^2)/60* *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.*

Working Solution

Buffer	Composition	For use in...
Buffer A	▪ 50 mM NaH_2PO_4, pH 8.0 ▪ 300 mM NaCl	▪ Purification under native conditions. ▪ Purification under denaturing conditions (optional).
Buffer B	▪ 50 mM NaH_2PO_4, pH 8.0 ▪ 300 mM NaCl ▪ 250 mM imidazole	Purification under native conditions.
Buffer C	▪ 100 mM NaH_2PO_4 ▪ 10 mM Tris-HCl* ▪ 8 M urea ▪ pH 8.0	Purification under denaturing conditions.
Buffer D	▪ 100 mM NaH_2PO_4 ▪ 10 mM Tris-HCl* ▪ 8 M urea ▪ pH 6.3	Purification under denaturing conditions.
Buffer E	▪ 100 mM NaH_2PO_4 ▪ 10 mM Tris-HCl* ▪ 8 M urea ▪ pH 5.9	Purification under denaturing conditions.
Buffer F	▪ 100 mM NaH_2PO_4 ▪ 10 mM Tris-HCl* ▪ 8 M urea ▪ pH 4.5	Purification under denaturing conditions.

2.2. Protocols

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

Purification under native conditions

See section, **Working Solution** *for additional information on preparing solutions.*

The purification of native proteins requires optimal buffer conditions for the target protein. This document describes well established examples and can be adapted to achieve optimal conditions for a specific target protein. cOmplete His-Tag Purification Columns offer full flexibility in selecting the optimal buffer conditions without compromises.

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.

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

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

1. Wash the pump with 10 to 20 ml Buffer A using the System Wash function of the ÄKTAexplorer System at a starting flow rate of 10 ml/minute.
   Ensure that all air is displaced from the pumps and tubing of the system.

2. Remove the plug at the column outlet and attach it to the outlet tubing of the ÄKTAexplorer System.
   Save the plug of the column outlet if the column needs to be stored or is to be reused.

3. As soon as Buffer A is running out of the inlet tubing, remove the upper plug from the column and immediately attach it to the inlet tubing.
   – Continuously measure OD_{280} values.
   If a fluorescent protein is purified, continuously measure the OD values at the absorption maximum of the fluorescence dye, for example, OD_{485} for GFP (Green Fluorescent Protein) or OD_{435} for CFP (Cyan Fluorescent Protein).
   Save the plug of the column inlet if the column needs to be stored or is to be reused.

4. Define the flow rate as 10 ml/minute for the 5 ml column or 2 ml/minute for the 1 ml column, and equilibrate the column with 10 column volumes of Buffer A.

5. Pause the run.
   – Load the cleared sample, for example, after an ultracentrifugation or filtration step onto the column with a volumetric flow rate of 2.5 ml/minute for the 5 ml column or 0.5 to 1 ml/minute for the 1 ml column.
   Remove insoluble material prior to loading the column to prevent blockage.
   Due to the high binding specificity of the resin, the kinetics of adhesion of the protein to the resin is slower than other available resins. If high volumetric flow rates for loading are used, protein yield can decrease.

6. Wash the column with Buffer A until the OD_{280} value reaches the baseline level, approximately 10 column volumes.

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 cOmplete His-Tag Purification Columns, a protein can already be eluted with approximately 25 mM imidazole.***
   ⚠ ***The amount of imidazole required for efficient release of the target protein from the resin depends on various parameters, such as the length and accessibility of the His-tag.***

8. Wash and equilibrate for the next run. For details, see section, **Cleaning**.
   ⚠ ***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 information in optimizing the purification results.*

Purification under denaturing conditions

ⓘ *See section,* **Working Solution** *for additional information on preparing solutions.*
The purification of denatured proteins requires optimal buffer conditions for the target protein. This document describes well established examples and can be adapted to achieve optimal conditions for a specific target protein. cOmplete His-Tag Purification Columns offer full flexibility in selecting the optimal buffer conditions without compromises.cOmplete 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.*

⚠ ***cOmplete His-Tag Purification Columns have been optimized using Buffer C, Buffer D, Buffer E, and Buffer F specified in the table. Other buffers might function as well, but need to be tested prior to use.***

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

1. Wash the pump with 10 to 20 ml Buffer C using the System Wash function of the ÄKTAexplorer System at a starting flow rate of 10 ml/minute.
   ⚠ ***Ensure that all air is displaced from the pumps and tubing of the system.***

2. Remove the plug at the column outlet and attach it to the outlet tubing of the ÄKTAexplorer System.
   ⓘ *Save the plug of the column outlet if 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.
   – Continuously measure the OD_{280} values.
   ⚠ ***If a fluorescent protein is purified, continuously measure the OD values at the absorption maximum of the fluorescence dye, for example, OD_{485} for GFP (Green Fluorescent Protein) or OD_{435} for CFP (Cyan Fluorescent Protein).***
   ⓘ *Save the plug of the column inlet if the column needs to be stored or is to be reused.*

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

5. Pause the run.
   – Load the cleared sample, for example, after an ultracentrifugation or filtration step onto the column with a volumetric flow rate of 2.5 ml/minute for the 5 ml column or 0.5 to 1 ml/minute for the 1 ml column.
   ⚠ ***Remove insoluble material prior to loading the column to prevent blockage.***
   ⚠ ***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 OD_{280} 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.
   i *Alternatively, perform the elution with a gradient up to 250 mM imidazole solution using Buffer A and Buffer B instead of the pH shift option, see the elution step in 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, see section, **Cleaning**.
    ⚠ ***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.***

i *Refer to sections,* **Purification process optimization** *and* **Troubleshooting** *for technical information in optimizing the purification results.*

Cleaning

cOmplete His-Tag Purification Columns can be used multiple times without loss of binding capacity. Over time, some protein aggregates might accumulate leading to a decrease in efficiency of the resin showing a slower flow rate or a higher back pressure. The cleaning procedures remove aggregates for further use of the resin. Different cleaning procedures can be carried out, based on the different applications. Once the cleaning procedure is completed, transfer the resin to 20% ethanol.

Stringent native cleaning

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

1. Wash with 10 column volumes of 1 M imidazole/HCl, pH 7.5.
2. Wash with 10 column volumes of 4 M imidazole/HCl, pH 7.5.
3. 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 used to remove aggregated proteins and lipids.

⚠ ***This cleaning procedure must be performed at +15 to +25°C to achieve the optimal solubility of SDS.***
ⓘ *The SDS buffer may contain 50 mM DTT.*
⚠ ***Avoid using K+ in this buffer to prevent precipitation with SDS.***

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

Denaturing cleaning with guanidinium-HCl

This method is used to remove aggregated proteins.

ⓘ *The guanidinium-HCl buffer may contain 50 mM DTT.*

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

ⓘ *The choice of the cleaning method depends on the protein type.*
ⓘ *The denaturing cleaning procedure with guanidinium-HCl presents fewer constraints than the one with SDS.*

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, resulting 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:

1. Incubate the columns with varying volumes of lysates in parallel test experiments.

2. Wash the columns and elute the bound proteins.

3. Determine the amount of target protein in the unbound fractions and in the eluate by SDS-PAGE.

4. 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.
   - *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.*
   - *The optimal concentration of imidazole during binding, washing, and elution steps can be determined during pretrial experiments.*
   - *Optimal results can be achieved with buffers containing a high salt concentration (300 mM) at pH 8.0 for target proteins compatible with those conditions.*

2.3. Parameters

Affinity/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 or by increasing the incubation time during the batch purification procedure.

3. Troubleshooting

Observation	Possible cause	Recommendation
Bubbles form in the bed resin.	Mixing of the storage buffer (20% ethanol) with aqueous buffer.	After storage at +2 to +8°C, equilibrate the resin to +15 to +25°C prior to packing the column.
		Degas the buffer prior to equilibration of the column.
The sample does not flow easily through the resin (low flow rate or high back pressure).	Particulates from the lysates may have clogged the columns.	(Ultra)centrifuge the sample prior to loading on the column.
		Reduce the flow rate.
		Clean the resin using a denaturing cleaning procedure.
Inefficient binding of the target protein to the resin within the columns.	Suboptimal buffer conditions during the binding step.	Lower the imidazole concentration and/or increase the pH during the binding step.
	Incubation time is too short.	Extend the incubation time.
		Lower the flow rate during binding.
	The His-tag is not accessible.	Change the position of the His-tag.
		Use a longer His-tag.
Inefficient or no elution of the target protein.	The target protein multimerizes and binds more avidly to the resin.	Increase the imidazole concentration during elution.
	The protein precipitates on the resin prior to elution.	Increase ionic strength to minimize isoelectric precipitations.
		Elute under denaturing conditions.
	The target protein precipitates during a pH shift elution.	Elute with imidazole instead.
Recovery of the target protein is too low.	The target protein may be degraded.	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.
	The His-tag might not be accessible.	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.
	The His-tag might have been digested by proteases.	Change to another expression host.
		Use protease inhibitors.
	The target protein might not be soluble.	Lower the expression temperature, strength, and duration of induction.
		Purification under denaturing conditions.
		Include solubility-enhancing fusion partners.
	The resin is limiting.	Verify that the expressed His-tagged protein is proportionate to the resin within the columns.

Target protein elutes with contaminants.	The host proteins interact with the resin.	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.
	DNA and/or RNA contaminants.	
		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 resin.
Target protein is degraded during or following the cell lysis.	Insufficient protection from proteases.	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.	Wrong host strain.	Use a protease-deficient host strain.
	Induction time too long.	Reduce the induction time.

4. Additional Information on this Product

4.1. Test Principle

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 $Ni^{2}+$

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 poly-histidine tag (His-tag). This tag has 6 to14 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 $Ni^{2}+$, $Co^{2}+$, or $Zn^{2}+$. This property can be used to separate polyhistidine-tagged target proteins from other proteins. $Ni^{2}+$ show the highest affinity and selectivity for His-tags, and are therefore the preferred ions. Using a specific chelator covalently linked to a matrix, $Ni^{2}+$ are immobilized to still permit interactions with histidine side chains. When His-tagged proteins are applied to such a $Ni^{2}+$ resin, they specifically bind to the resin via $Ni^{2}+$, while most untagged proteins do not. Bound proteins are released from the resin using mild conditions. Imidazole competes for coordination sites on $Ni^{2}+$ 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 $Ni^{2}+$ 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 $Ni^{2}+$ 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.

Properties

cOmplete His-Tag Purification Columns include a sepharose-based, pre-charged, ready-to-use $Ni^{2}+$ 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 $Ni^{2}+$ 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 $Ni^{2}+$ 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 allows 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, Protease inhibitor cocktails containing EDTA, and with PhosSTOP Tablets for inhibiting phosphatases, as well as with common reducing agents, such as DTT.

4.2. Quality Control

For lot-specific certificates of analysis, see section **Contact and Support**.

5. Supplementary Information

5.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

5.2. Changes to previous version

Layout changes.
Editorial changes.

5.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Tris hydrochloride	500 g	10 812 846 001
1,4-Dithiothreitol	2 g	10 197 777 001
	10 g	10 708 984 001
	25 g	11 583 786 001
Sodium Dodecyl Sulfate (SDS)	1 kg	11 667 289 001

5.4. Trademarks

PHOSSTOP is a trademark of Roche.
All other product names and trademarks are the property of their respective owners.

5.5. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products.

5.6. Regulatory Disclaimer

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

5.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

5.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

0621.07012543001 ③

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