# cOmplete His-Tag Purification Resin

*Truly compatible with DTT and EDTA*

# cOmplete His-Tag Purification Resin

*Purify protein without compromises*

## *Choose buffer conditions that suit your target protein*

cOmplete His-Tag Purification Resin is an innovative high-capacity IMAC matrix for convenient single-step purification of His-tagged proteins from lysates. Roche's new proprietary nickel-chelate chemistry is compatible with commonly used reducing agents (such as DTT), chelating metalloprotease inhibitors (such as EDTA), and a wide range of buffer substances and salt conditions. The wide choice of compatible ingredients makes it easy to optimize buffers for high protein stability and solubility and to protect your proteins from proteases and oxidation.

- **Stabilize your protein with DTT and EDTA.** Use any buffer with EDTA or DTT for the highest protein stability without loss of capacity.
- **Produce large amounts of highly purified proteins.** High binding capacity and specificity are ideal for one-step purifications.
- **Generate less toxic waste.** Negligible nickel leakage reduces toxic waste and avoids nickel recharging.

## *Reduce nickel leakage*

In contrast to nitrilotriacetic acid (NTA) resins or resins using iminodiacetic acid (IDA) chelator, nickel ions are immobilized on the cOmplete His-Tag Purification Resin using a proprietary chemistry using one of the strongest chelators known.
Even with EDTA and DTT, nickel ion leakage from the resin is negligible, eliminating the need for recharging (see Figure 1).

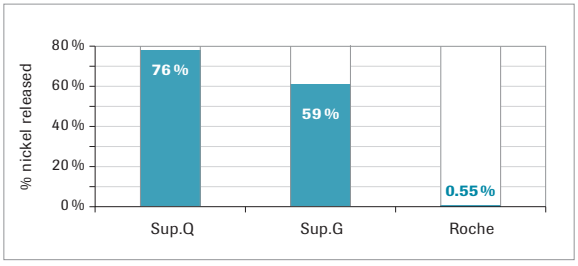


**Figure 1: Loss of resin Ni ions under stringent conditions by different supplier resins.** cOmplete His-Tag Purification Resin (Roche) and two commercially available resins using Ni-NTA (Sup. Q) and another chelator (Sup. G) were incubated for 1 hour at room temperature in 9 volumes of a buffer containing 10 mM EDTA, 10 mM DTT, 500 mM imidazole, 300 mM NaCl, and 50 mM $NaH_2PO_4$, at pH 8.0. The amount of nickel ions released into the buffer was measured by inductively-coupled plasma mass spectrometry (ICP-MS).

**Result:** During the incubation, cOmplete His-Tag Purification Resin lost less than 1 % of nickel ions. In comparison, resins from Sup. Q and Sup. G released 76 % and 59 % of their nickel ions into the buffer.

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Not for use in diagnostic procedures.

## *Eliminate frequent regeneration cycles*

Choose optimal buffer conditions for your target protein without compromising resin stability. Using cOmplete His-Tag Purification Resin with buffers containing reducing agents (such as DTT) and/or metalloprotease inhibitors (such as EDTA) does not alter the capacity of the resin (see Figure 2). This is due to the new chelator which binds nickel ions tightly to the resin (see Figure 2).

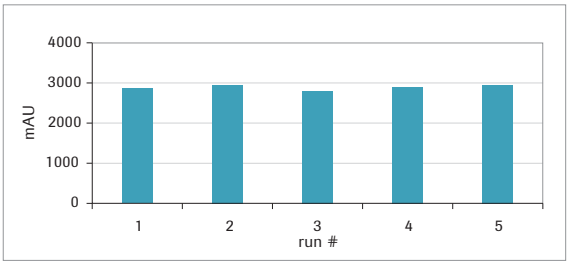


**Figure 2: Capacity of resin after multiple use without Ni-recharging.** cOmplete His-Tag Purification Resin (0.5 ml) was loaded with 7 ml lysate containing $His_6$-CFP (cyan fluorescent protein). The lysate buffer contained 10 mM EDTA and 10 mM DTT. The resin was washed with 5 ml buffer A (50 mM $NaH_2PO_4$ pH 8.0/RT, 300 mM NaCl, 10 mM EDTA, 10 mM DTT). After washing, bound protein was eluted with 1.5 ml buffer B (buffer A + 250 mM imidazole) and quantified by integration of the protein peaks. To prepare for the next run, the resin was washed with 15 ml buffer A. Calculated mAU after five consecutive runs (corresponding to the total protein released from the column) was plotted.

**Result:** Even under such harsh buffer conditions, the capacity of the cOmplete His-Tag Purification Resin remained stable within 5 protein purification runs. Regeneration with reloading of nickel ions was not needed.

## *Economize with a high capacity resin*

Binding capacity is greatly dependent on the characteristics of the target protein, its molecular weight, its Stokes radii, the binding conditions, and additives. The capacity of cOmplete His-Tag Purifcation Resin was carefully selected to balance an economical alternative with high performance. Exceeding a resin's capacity can destabilize proteins. The cOmplete His-Tag Purification Resin shows highest capacity for small proteins. When increasing protein size, capacity decreases (see Table 1).

| Target Protein | Size | Binding Capacity per ml resin |
|---|---|---|
| $His_{14}$-GFP | 30 kD | 75 mg |
| $His_{10}$-T4-DNA-Ligase | 58 kD | 40 mg |
| $His_6$-MBP | 42 kD | 40 mg |

**Table 1: Binding capacities of various proteins.**

The capacity of the cOmplete His-Tag Purification Resin shows – as with other resins – a time dependency. Extending the incubation time allows reaching maximum capacity (see Figure 3).

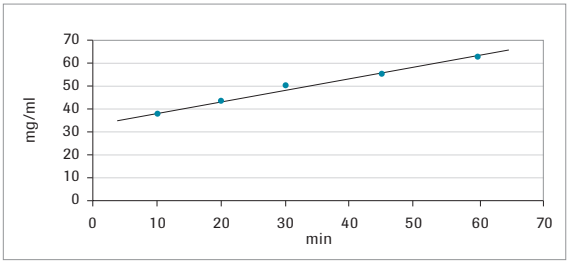


**Figure 3: Correlation between time allowed for binding and bound protein.** Each 250 µl of cOmplete His-Tag Purification Resin was equilibrated with buffer A and incubated on a roller with equal amounts of $His_6$-tagged T4 Gene 32 Protein solution for 10, 20, 30, 45, and 60 minutes, respectively. After centrifugation, protein concentration in the supernatant was determined. The adsorbed protein was calculated as the difference between protein added and protein found in the supernatant.

## *Obtain highly purified protein*

The cOmplete His-Tag Purification Resin is a Sepharose-based, pre-charged and ready-to-use $Ni^{2+}$-chelate matrix for analytical- and large-scale purification of His-tagged proteins. It allows for the production of highly purified proteins from crude lysates using a simple one-step purification process. Highest protein purity after only a single run (see Figure 4) can be achieved.

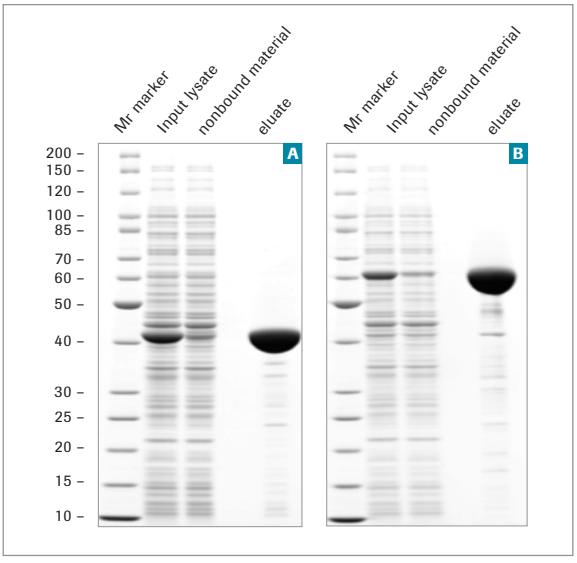


**Figure 4: Efficient purification of $His_6$- and $His_{10}$-tagged proteins.** Two milliliters of native lysate containing moderate amounts of $His_6$-MBP (A) or $His_{10}$-T4 DNA Ligase (B), were incubated for 2 hours with 50 or 40 µl of cOmplete His-Tag Purification Resin in buffer A (150 mM NaCl, 50 mM Tris-HCl pH 7.5, 2 mM DTT) containing 5 or 10 mM imidazole, respectively. Unbound material was washed off with the same buffers and eluted in buffer A supplemented with 400 mM imidazole.

**Result:** The new cOmplete His-Tag Purification Resin produces highly pure target proteins after just one run.

## *Avoid handling of toxic nickel solution*

$Ni^{2+}$ is toxic to living organisms and is a known carcinogen. The toxicity of nickel is essentially exerted on enzymes and DNA since nickel has a high affinity for ligands containing oxygen, nitrogen, and sulfur donors. Nickel is chemically similar to zinc, a known cofactor of a variety of enzymes. There is enormous potential for harm from Ni when it occupies Zn-binding sites. Excess nickel may cause cancer of the lungs, nose, and bone. Due to the chemical characteristics of the chelator used for the binding of $Ni^{2+}$ to the resin, cOmplete His-Tag Purification Resin shields the metal ion against reducing agents and complexing agents. Nickel leakage from the column is therefore minimal. As a consequence, frequent regeneration cycles (including recharging the resin using nickel solutions) are not needed, significantly reducing the risk of handling toxic nickel solutions and lowering both waste disposal costs and environmental impact.

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

## *Order your free sample today*

Interested in trying out cOmplete His-Tag Purification Resin?
A free sample is waiting for you.
Just enter your details into the sample ordering tool at **www.proteomics.roche.com**


After evaluating, please provide feedback via our website: **www.proteomics.roche.com/feedback**
On the same website you can also find additional information such as technical tips and application notes.

More details are provided in the downloadable Instructions for Use by entering Catalog Number 05 893 682 001 at **www.instructions.roche.com**

### Ordering Information

| Product | Catalog Number | Pack Size |
|---|---|---|
| **cOmplete His-Tag Purification Resin** | 05 893 682 001 | 25 ml settled resin volume |
| **cOmplete His-Tag Purification Resin** | 05 893 801 001 | 200 ml settled resin volume |

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