Bio-Works

WHITE PAPER

Optimizing your IMAC purification

Results from IMAC purifications depend both on the chelated metal ion and the chelator itself

Immobilized Metal Ion Affinity Chromatography (IMAC) utilizes the affinity of histidine, cysteine and tryptophan residues on the protein surface for metal ions immobilized by chelating ligands on the chromatographic resin. The method can be used for a number of different applications but is most frequently used for the purification of His-tagged proteins.

IMAC purification of His-tagged proteins is an efficient and convenient technique, although very high purity usually requires an additional polishing purification step. IMAC is not only selective for His-tagged proteins but to some extent also for other proteins containing histidine, cysteine or tryptophan residues. Some proteins from the expression host will bind to the IMAC resin, and constitute impurities in the His-tagged protein preparation. Although this is true for protein expressed in *Escherichia coli*, the problem is usually more severe when purifying His-tagged proteins expressed in eukaryotic hosts. Minor differences in surface distribution of the relevant amino acids can greatly change the affinity of a protein for different metal ions. The affinities depend on the metal ion itself, as well as the type of chelating ligand used for their immobilisation.

The typical metal ions used for IMAC often show an increase in affinity in the order Zn^{2+}, Co^{2+}, Ni^{2+} and Cu^{2+}. The type of chelator also tends to affect the affinity of proteins for the immobilised metal ion, likely because of difference in the number of available coordination sites of the metal ion for protein binding. As an example, the nitrilotriacetic (NTA) ligand is believed to chelate Ni^{2+} ions via four coordination sites, leaving two coordination sites of the Ni^{2+} ion left for protein binding, while the iminodiacetic (IDA) ligand is believed to coordinate Ni^{2+} ions via three coordination sites leaving three sites for a stronger protein binding. Such difference in affinity, especially for untagged host cell proteins (the tentative impurities) may partly explain the differences in purification obtained with different resins. It may be added that ligand concentration and structural properties of the resin also affect the purification performance. It can be concluded that testing different IMAC resins for best purification results is highly recommended and a screening kit is a very useful tool.

The GoBio™ Mini His-tag Screening kits are available in two column sizes; 1 mL and 5 mL, pre-packed with WorkBeads™ NTA and WorkBeads IDA, containing the chelating ligands mentioned earlier. The screening kits for each ligand contains ready-to-use, prepacked GoBio Mini columns, charged with four different metal ions, Ni^{2+}, Co^{2+}, Cu^{2+} and Zn^{2+} (Fig. 1). This diversity, combined with the excellent mechanical properties and tight size distribution of the WorkBeads resin, provides the perfect starting point to discover the optimal resin configuration for your protein.

Columns:	GoBio Mini Ni-NTA 1 mL, GoBio Mini Co-NTA 1 mL, GoBio Mini Zn-NTA 1 mL, GoBio Mini Cu-NTA 1 mL, GoBio Mini Ni-IDA 1 mL, GoBio Mini Co-IDA 1 mL, GoBio Mini Zn-IDA 1 mL, GoBio Mini Cu-IDA 1 mL
Binding buffer:	50 mM sodium phosphate, 300 mM NaCl, 10 mM imidazole, pH 8.0
Elution buffer:	50 mM sodium phosphate, 300 mM NaCl, 300 mM imidazole, pH 8.0
Sample:	10 mL clarified extract with His-tagged Green Fluorescent Protein (His_6-GFP) expressed in *E. coli*
Flow rate:	1 mL/min (150 cm/h
Gradient:	Linear, 0–100% elution buffer, 20 column volumes (CV)

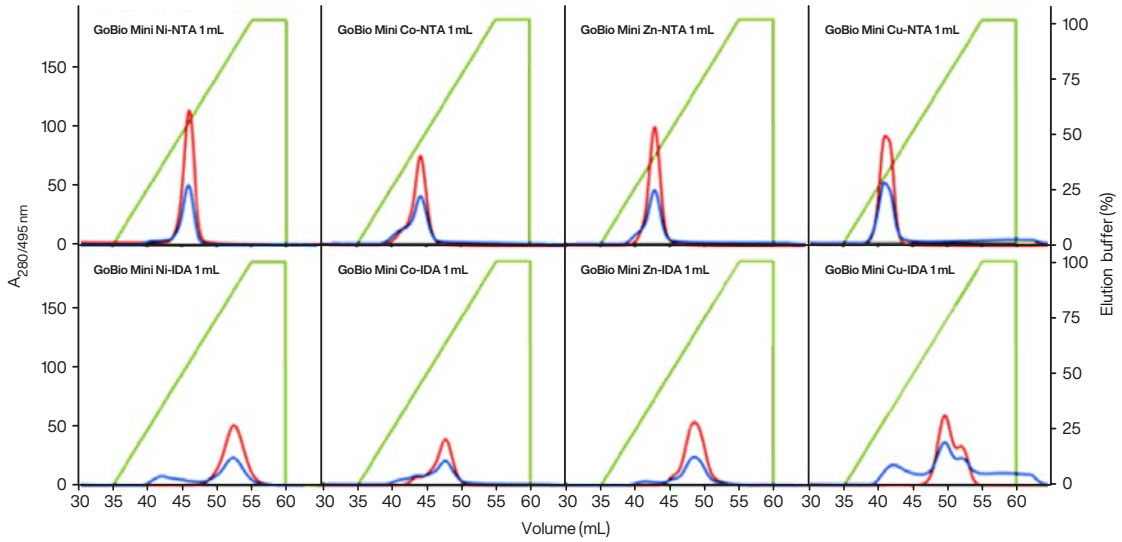

Figure 1. Comparison of purifications of His_6-GFP on GoBio Mini NTA 1 mL and GoBio Mini IDA 1 mL charged with Ni^{2+}, Co^{2+}, Cu^{2+} and Zn^{2+} Blue line: absorbance at 280 nm. Red line: absorbance at 495 nm (specific for His_6-GFP). Green line: elution buffer, %.

Capacity and yield can vary considerably using seemingly very similar resins

Ni-NTA resins are the commonly recommended starting point for His-tagged protein purification since they give excellent results in most purifications. However, Co^{2+}-resins has been increasingly popular since they may further improve purification in some cases. These resins often give higher purity, but usually to the cost of product yield. Therefore, testing other metal ion and chelator combinations as well as resin matrices may further improve your results.

Purification results can also vary considerably, also when working with highly concentrated target proteins at small sample volumes (Figure 2 and Table 1). As shown, the product yield can improve considerably changing to the Bio-Works product brand, without having to exchange with metal ion you use.

Column:	GoBio Mini Co-NTA 1 mL, HiTrap™ TALON® Crude 1 mL, HisPur™ Cobalt 1 mL, GoBio Mini Ni-NTA, Superflow™ Ni-NTA, HisPur Ni-NTA, HisTrap™ FF
Binding buffer:	50 mM sodium phosphate, 300 mM NaCl, 10 mM imidazole, pH 8.0
Elution buffer:	50 mM sodium phosphate, 300 mM NaCl, 300 mM imidazole, pH 8.0
Sample:	10 mL clarified extract with His-tagged Green Fluorescent Protein (His_6-GFP) expressed in *E. coli*
Flow rate:	1 mL/min (150 cm/h)
Gradient:	Linear, 0–100% elution buffer, 20 CV

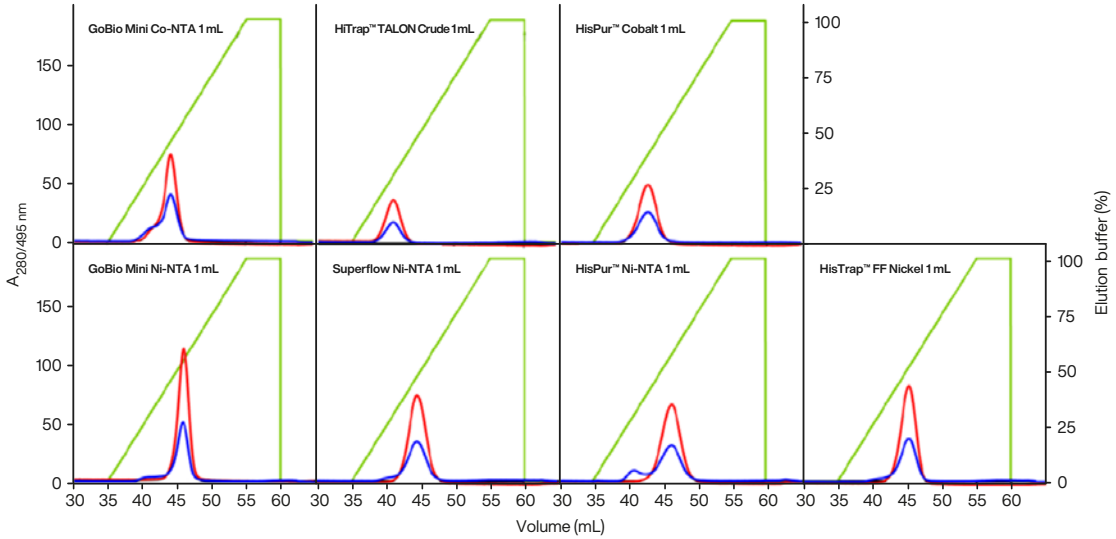

Figure 2. Comparison of purifications of His_6-GFP on GoBio Mini NTA 1 mL charged with Ni^{2+}, Co^{2+} and other supplier's products. Blue line: absorbance, 280 nm. Red line: absorbance, 495 nm (specific for His_6-GFP). Green line: elution buffer, %.

Table 1. Description of the yields and peaks width of each individual chromatogram presented in Figure 2.

Column ID	Area280nm (AU)	Area495nm (AU)	A495nm Peak width (mL)
GoBio Mini Co-NTA (Bio-Works)	66.3	172.8	8.07
HiTrap TALON Crude (Cytiva)	29.6	75.04	5.11
HisPur Cobalt (Thermo Fisher Scientific)	65.5	115.7	6.04
GoBio Mini Ni-NTA (Bio-Works)	103.1	195.7	4.88
Superflow Ni-NTA (Qiagen)	82.4	179.9	5.93
HisPur Ni-NTA (Thermo Fisher Scientific)	64.3	170.3	6.25
HisTrap FF (Cytiva)	74.7	189.8	5.91

Calls to exchange toxic metal compounds in bioprocessing

Recent times and development of IMAC for process-scale purification of biopharmaceuticals have raised concerns regarding the health and environmental issues associated with the use of toxic metal ions, such as Ni^{2+} and Co^{2+}. Both are known to cause allergic reactions upon contact with the human skin as well as being carcinogenic when ingested. The inevitable leaching of ions from the resin during purification therefore makes such products questionable when processing pharmaceutical compounds.

Zn^{2+} has been found to be a possible non-toxic substitute for Ni^{2+}, and can provide comparable purity, yield and elution pattern, making it a good alternative to resins based on Ni^{2+} (or Co^{2+}). The similarities in functionality of the two resins is consistent between elution modes, i.e., stepwise or gradient elution, as well as protein load (Fig. 3)

Column:	GoBio Mini Ni-NTA 1 mL, GoBio Mini Zn-NTA 1 mL
Binding buffer:	50 mM sodium phosphate, 300 mM NaCl, 10 mM imidazole, pH 8.0
Elution buffer:	50 mM sodium phosphate, 300 mM NaCl, 300 mM imidazole, pH 8.0
Sample:	10 mL or 100 mL clarified extract with His_6-GFP expressed in *E. coli*
Flow rate:	1 mL/min (150 cm/h)
Gradient:	Linear, 0–100% elution buffer, 20 CV or one-step 100% elution buffer, 5 CV

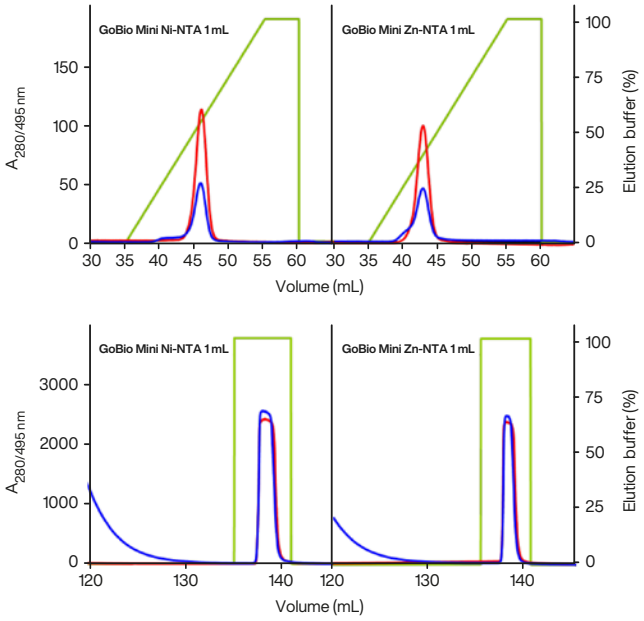

Figure 3. Comparison of purifications of His_6-GFP on GoBio Mini NTA 1 mL charged with Ni^{2+} and Zn^{2+} using different protein loads and elution modes. Blue line: absorbance, 280 nm. Red line: absorbance, 495 nm (specific for His_6-GFP). Green line: elution buffer, %.

Conclusions

- The choice of metal ion and chelator ligand of IMAC resins affect purification results
- GoBio Mini His-tag Screening kits can be used for effective screening of metal ion and chelating ligands combinations to get the best results when purifying His-tagged proteins
- Due to the health and safety issues related to using toxic metal ions in purification of pharmaceuticals as well as other process scale compounds, Zn^{2+} is an attractive non-toxic alternative to Ni^{2+}-ion- and Co^{2+}-ion-based IMAC for the purification of future His-tagged biopharmaceuticals

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