Optimizing your IMAC purification

The purity of your target protein doing IMAC purifications depends both on the immobilized ligand and the metal ion

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 chromatography resin. This purification technique is 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. Apart from the target His-tagged protein some proteins from the expression host may also bind to the IMAC resin. 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 immobilization. Other factors that influence the purity is the length of the His-tag and also where the tag is placed, in the N- or C-terminal.

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 to the immobilized 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 left for protein binding, while the iminodiacetic acid (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) partly explains 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 therefore be concluded that testing different IMAC resins for best purification results is highly recommended and a screening kit is a very useful tool.

The BabyBio™ His-tag Screening kits are available in two column sizes; 1 ml and 5 ml, prepacked with WorkBeads™ 40 NTA and WorkBeads 40 IDA. The screening kits for each ligand contains ready-to-use, BabyBio 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 specific target protein.

Columns:	BabyBio Ni-NTA 1 ml, BabyBio Co-NTA 1 ml, BabyBio Zn-NTA 1 ml, BabyBio Cu-NTA 1 ml, BabyBio Ni-IDA 1 ml, BabyBio Co-IDA 1 ml, BabyBio Zn-IDA 1 ml, BabyBio Cu-IDA 1 ml
Binding buffer:	50 mM Na-phosphate, 300 mM NaCl, 10 mM imidazole, pH 8.0
Elution buffer:	50 mM Na-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:	0 - 100% elution buffer, 20 CV

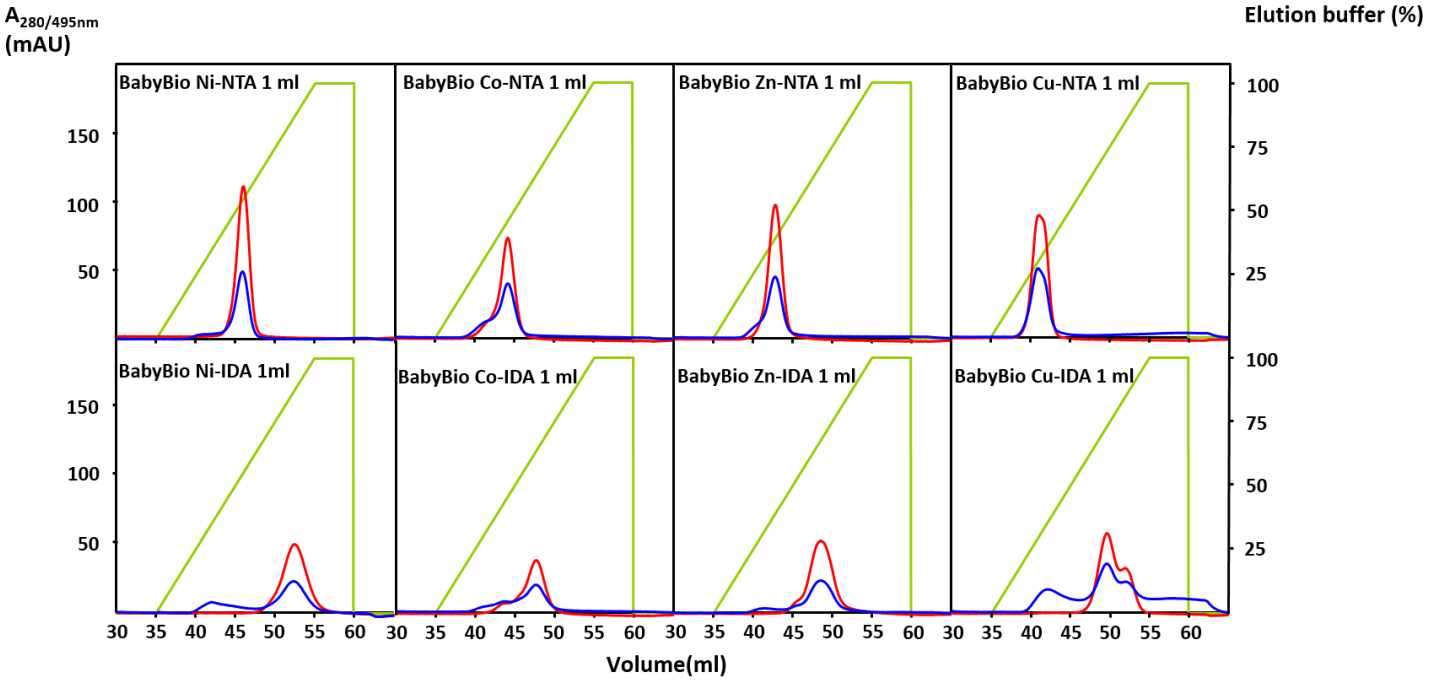

Figure 1: Comparisons of purfications of His_6-GFP on BabyBio NTA 1 ml and BabyBio IDA 1 ml charged with Ni^{2+}, Co^{2+}, Cu^{2+} and Zn^{2+} Blue line: absorbance at 280 nm. Red line: absorbance at 495 nm (specific for His_6-GFP). Green line: elution buffer, %.

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 have been increasingly popular since 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 when working with highly concentrated target proteins and small sample volumes (Fig. 2 & Table 1). As shown, the product yield can improve considerably changing to the Bio-Works product brand, without changing the metal ion.

Columns:	BabyBio Co-NTA 1 ml, HiTrap TALON Crude 1 ml, HisPur Cobalt 1 ml, BabyBio Ni-NTA 1 ml, Ni-NTA Superflow cartridge 1 ml, HisPur Ni-NTA cartridge 1 ml, HisTrap FF 1 ml
Binding buffer:	50 mM Na-phosphate, 300 mM NaCl, 10 mM imidazole, pH 8.0
Elution buffer:	50 mM Na-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:	0 - 100% elution buffer, 20 CV

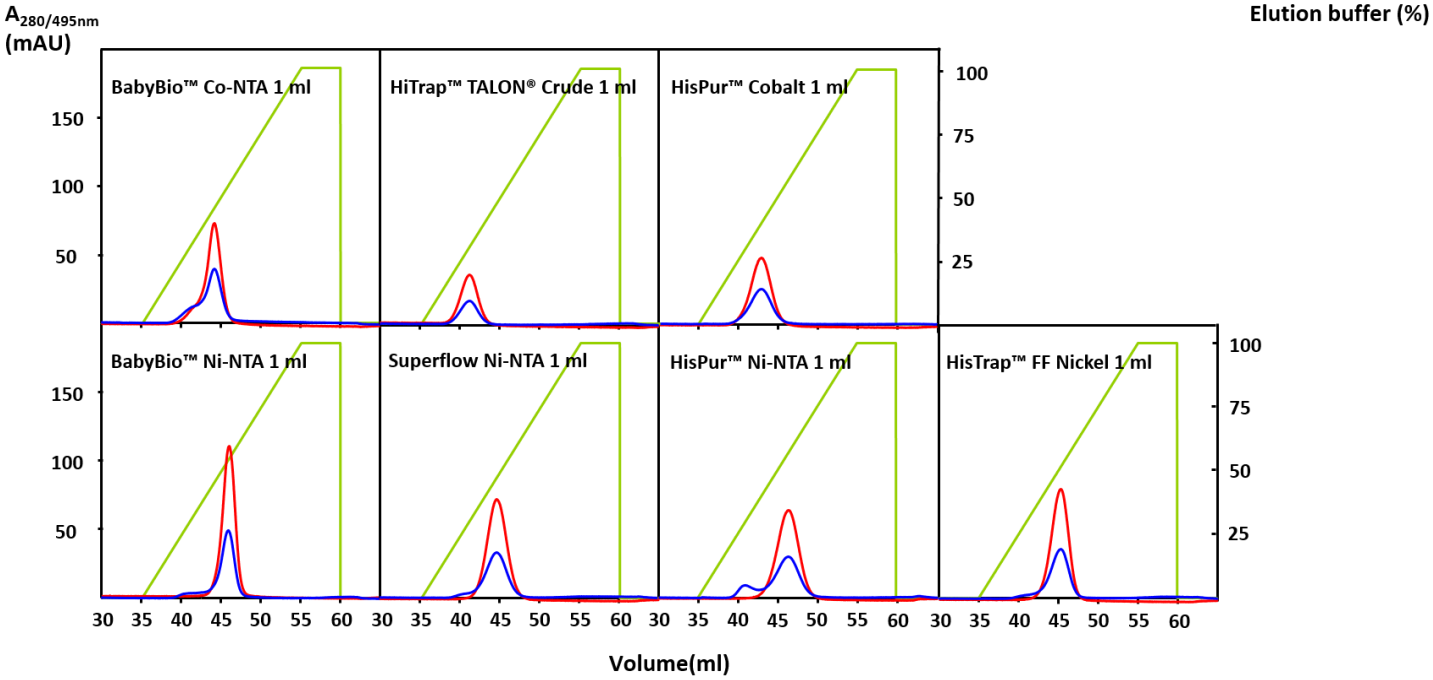

Figure 2: Comparisons of purifications of His_6-GFP on BabyBio Co-NTA 1 ml and BabyBio Ni-NTA 1 ml with similar products from other suppliers. Blue line: absorbance, 280 nm. Red line: absorbance, 495 nm (specific for His_6-GFP). Green line: elution buffer.

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

Column (1 ml)	Supplier	Area 280nm (AU)	Area 495nm (AU)	A495nm Peak width (ml)
BabyBio Co-NTA	Bio-Works	66.3	172.8	8.07
HiTrap TALON Crude	GE Healthcare	29.6	75.04	5.11
HisPur Cobalt	Thermo Fisher	65.5	115.7	6.04
BabyBio Ni-NTA	Bio-Works	103.1	195.7	4.88
Ni-NTA Superflow	Qiagen	82.4	179.9	5.93
HisPur Ni-NTA	Thermo Fisher	64.3	170.3	6.25
HisTrap FF	GE Healthcare	74.7	189.8	5.91

Considerations of using IMAC in bioprocessing

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

Fortunately Zn^{2+} has been found to be a good non-toxic substitute for Ni^{2+}, providing comparable purity, yield and elution pattern, making it an excellent alternative to resins immobilized with Ni^{2+} (or Co^{2+}). The similarities in functionality of the two resins (Zn^{2+} and Ni^{2+}) are consistent also between elution modes, i.e., stepwise or gradient elution, as well as protein load (Fig. 3).

Columns:	BabyBio Ni-NTA 1 ml, BabyBio Zn-NTA 1 ml
Binding buffer:	50 mM Na-phosphate, 300 mM NaCl, 10 mM imidazole, pH 8.0
Elution buffer:	50 mM Na-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:	0 - 100% elution buffer, 20 CV or 100% elution buffer, 5 CV

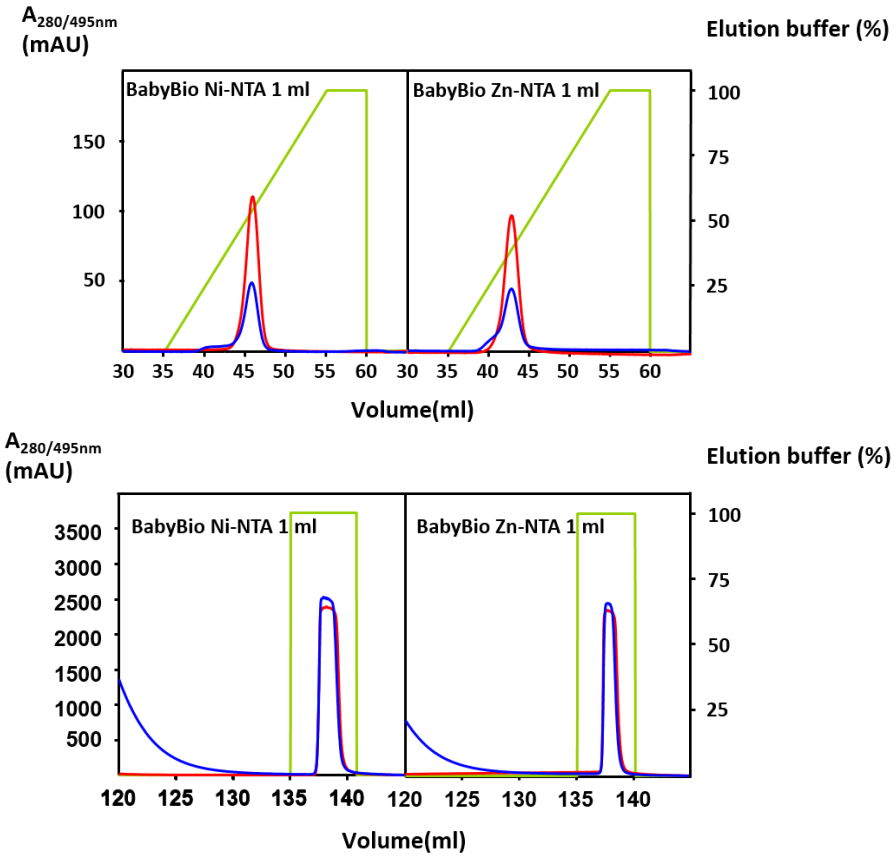

Figure 3: Comparisons of purifications of His_6-GFP on BabyBio Ni-NTA 1 ml and BabyBio Zn-NTA using different protein loads and elution modes. Blue line: absorbance, 280 nm. Red line: absorbance, 495 nm (specific for His_6-GFP). Green line: elution buffer, %.

Conclusions

- The choice of metal ion and chelator ligand of IMAC resins affect purification results.
- BabyBio His-tag Screening kits are excellent tools for effective screening of metal ion and chelating ligand combinations to get the optimal result when purifying His-tagged proteins.
- Due to the health and safety issues related to using toxic metal ions in purification of pharmaceuticals, Zn^{2+} is an attractive non-toxic alternative to Ni^{2+} and Co^{2+}.

Bio-Works
Virdings allé 18
754 50 Uppsala
Sweden