Easy optimization of His-tagged protein purification

Kajsa Eriksson, Anna Heijbel and Lars Haneskog
Bio-Works, Uppsala, Sweden

Introduction

Immobilized Metal Ion Affinity Chromatography (IMAC) utilizes the affinity of histidine, cysteine and tryptophan on the protein surface for binding to metal ions immobilized on the resin. IMAC is commonly used for purification of His-tagged proteins.

BabyBio™ Ni-NTA prepacked column is recommended as the starting point for His-tagged protein purification and, in most cases, will give excellent results. However, the complexity of working with recombinant His-tagged proteins, i.e., the variety of expression host cells, different host cell proteins and expression levels may require optimized purifications to get the required purity. Therefore, we have developed screening kits for a quick and convenient screening of the optimal combination of ligand and metal ion, getting the highest purity of the His-tagged protein.

BabyBio His-tag Screening kits are available in two column sizes; 1 ml and 5 ml, and are based on two different chelating ligands, nitrilotriacetic acid (NTA) or iminodiacetic acid (IDA) (Fig 1). The screening kits for each ligand contains ready-to-use, prepacked BabyBio columns, charged with four different metal ions, Ni^{2+}, Co^{2+}, Cu^{2+} and Zn^{2+}, immobilized via the chelating ligand.

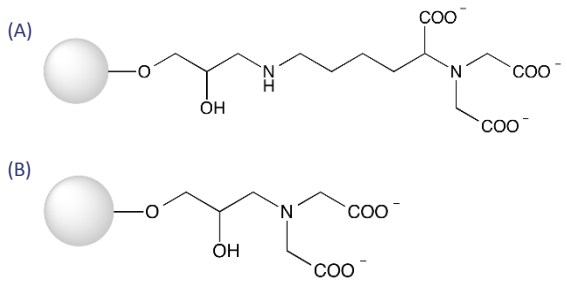

Figure 1. Structure of the chelating ligands used in WorkBeads™ NTA (A) and WorkBeads IDA (B).

Example

Purifications of His_6-tagged Green Fluorescent Protein (His_6-GFP) expressed in *E. coli* using BabyBio NTA His-tag Screening kit 1 ml and BabyBio IDA His-tag Screening kit 1 ml are shown in Fig 2. The result show that depending on the target protein, each specific chelating ligand together with the different metal ion attached give significant differences in the purification result.

Sample:	2 ml clarified extract from *E. coli* expressing His_6-GFP in binding buffer
Columns:	BabyBio NTA His-tag Screening kit 1 ml BabyBio IDA His-tag Screening kit 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
Gradient:	0 to 100% elution buffer in 15 column volumes
Flow rate:	0.5 ml/min (75 cm/h)

Conclusion

The two different BabyBio His-tag Screening kits offer a quick and convenient way of screening for optimization of His-tag protein purification. After ligand and metal optimization, the purification can easily be scaled up using precharged WorkBeads IMAC resins, available in the same range of ligands and metal ions as the screening kits.

For very high purity requirements, it is common to add a second purification step to remove final impurities and for buffer exchange and salt removal. This can be carried out using size exclusion chromatography (gel filtration), with WorkBeads SEC resins.

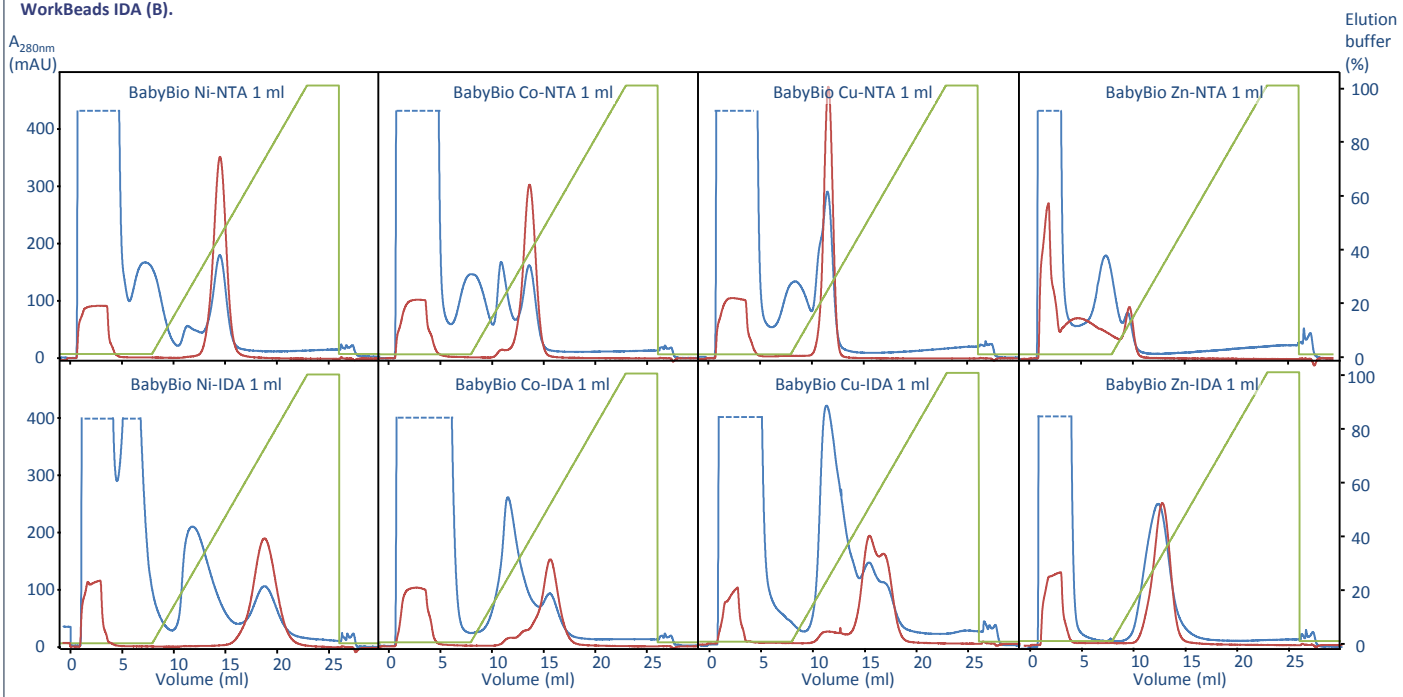

Figure 2. Comparison of purifications of His_6-GFP on BabyBio NTA 1 ml and BabyBio IDA 1 ml charged with Ni^{2+}, Co^{2+}, Cu^{2+} and Zn^{2+}. Blue line: absorbance, 280 nm. Red line: absorbance, 490 nm (specific for His_6-GFP). Green line: % elution buffer.

Bio-Works

Purification made simple

PS45655101 AA