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# Evaluation of immobilized metal affinity chromatography kits for the purification of histidine-tagged recombinant CagA protein

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ABSTRACT

Immobilized metal affinity chromatography (IMAC) technique is used for fast and reliable purification of histidine(His)-tagged recombinant proteins. The technique provides purification under native and denaturing conditions. The aim of this study is to evaluate three commercially available IMAC kits (Thermo Scientific, GE Healthcare and Qiagen) for the purification of a 6xHis-tagged recombinant CagA (cytotoxin-associated gene A) protein from IPTG-induced *Escherichia coli* BL21(DE3) culture. The kits were tested according to the manufacturer instructions and the protein was purified with only GE Healthcare and Qiagen kits under denaturing conditions. 1% (w/v) SDS was used as denaturing agent in PBS instead of extraction reagent of Thermo Scientific kit to lyse bacterial cells from 100 ml culture. The 6xHis-tagged recombinant protein was purified by the three kits equally.



## 1. Introduction

Immobilized metal affinity chromatography (IMAC) was first developed by Porath et al. in 1975 [1]. The technique is fast, reliable and commonly used for the purification of histidine-tagged recombinant proteins [2–6]. The IMAC resin is composed of a support (e.g., agarose), linker, a chelating compound (iminodiacetic acid, nitrilotriacetic acid or tris'carboxymethyl' ethylenediamine) and a divalent transition metal ion (Zn(II), Cu(II), Ni(II) or Co(II)). The chelating compounds that are bound to agarose by a linker immobilize and coordinate the metal ions [7]. The metal ions interact with nitrogen on imidazole rings of histidine (His) amino acids placed in peptides and on protein surfaces [2,3].

The performance of the IMAC is influenced by the type of the chelating compound. Tridentate (iminodiacetic acid) chelating compound occupies three of the six coordination sites of the Ni(II) leaving the remaining sites for reversible protein binding. However, the tetradentate (nitrilotriacetic acid) chelating compund reacts with four coordination sites of the Ni(II), immobilize them and prevents the leaching of metal ions during purification [3,8]. The remaining two coordination sites on the metal ion interact with His residues and provide similar quality purifications. In addition, the nickel-nitrilotriacetic acid (Ni-NTA) based IMAC is stable under native and denaturing conditions and compatible with a lot of chemicals up to specified limits [2]. On the other hand, pentadentate (tris'carboxymethyl' ethylenediamine) chelating compund binds to five of the six coordination sites of Ni(II) leaving one free site for protein binding which results in lower protein purification [2,3].

The six consecutive histidine residues (6xHis) at either the N- or C-terminal or both terminal ends of the protein are most commonly used to provide six binding sites for metal ions [9]. The small size of the His-tag does not affect the structure and function of the purified protein, thus there will be no need to remove it following purification [6,10]. The effect of the presence of the 6xHis-tag at either the N- or C-terminal position on protein expression in bacterial and eukaryotic cell was investigated and it was found that N-terminal 6xHis-tagged proteins were highly expressed than the C-terminal ones [2,11]. The bound 6xHis-tagged protein can be eluted by lowering pH of the buffer which leads to the dissociation

*Abbreviations:* IMAC, immobilized metal affinity chromatography; His, histidine; CagA, cytotoxin-associated gene A; rCagA, recombinant CagA; IPTG, isopropyl β-D-1-thiogalactopyranoside; SDS, sodium dodecyl sulfate; PBS, phosphate buffered saline; IDA, iminodiacetic acid; NTA, nitrilotriacetic acid; TED, tris(carboxymethyl)ethylenediamine; Zn, zinc; Cu, copper; Ni, nickel; Co, cobalt.

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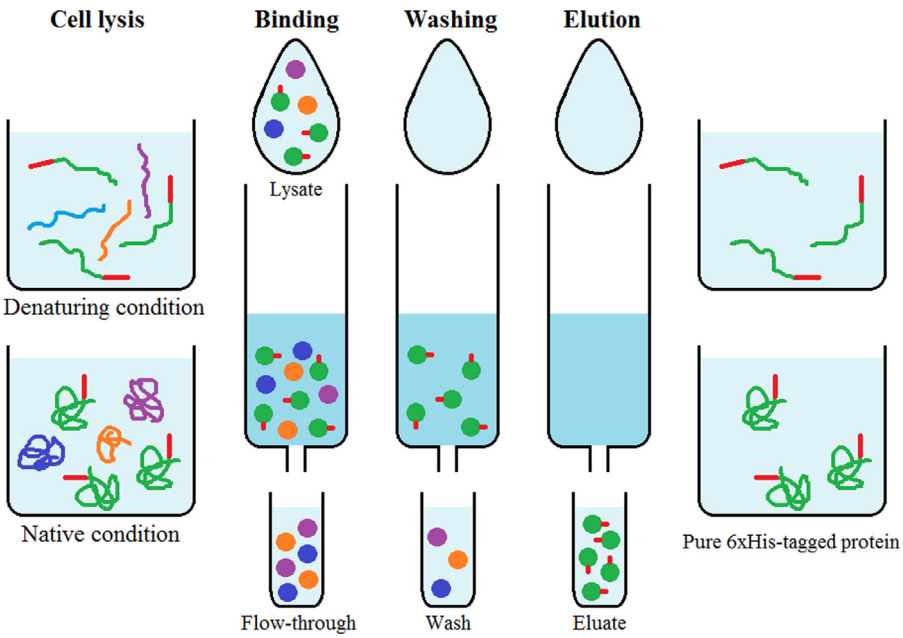


**Fig. 1.** An illustration of IMAC for protein purification under native or denaturing conditions. Insoluble proteins are solubilized by denaturing agent in lysis buffer. The red colored line connected to the protein indicates 6xHis-tag as shown in the eluate. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

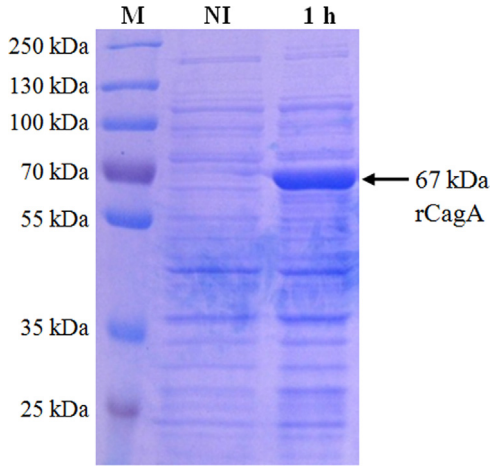


**Fig. 2.** An SDS-PAGE image showing the 67 kDa rCagA protein band expressed after 1 h induction with IPTG. Non-induced *E. coli* BL21(DE3) cells showed no band. M: molecular marker, NI: non-induced control, 1 h: induction hour.

of the imidazole ring of histidine from the metal ion or by adding a competitive imidazole to the buffer for displacement [12,13].

The aim of this study is to evaluate three commercially available IMAC kits for the purification of a 6xHis-tagged recombinant CagA protein from IPTG-induced *Escherichia coli* BL21(DE3) culture. For this purpose, His GraviTrap (GE Healthcare, USA), QIAexpress Ni-NTA Fast Start Kit (Qiagen, Germany) and B-PER 6xHis Fusion Protein Purification Kit (Thermo Scientific, USA) were investigated. Fig. 1 shows the basic principles of the IMAC for the purification of protein under a native or denaturing conditions. A comparative analysis of the properties of these kits were made and given in Table 1. The resin of each comprises Ni(II) ions that are chelated by either IDA or NTA. 6xHis-tagged protein is generally eluted competitively by adding imidazole in elution buffer. The Thermo Scientific kit contains reagents that purify soluble 6xHis-tagged proteins under native conditions only. The manufacturer recommends another reagent to solubilize inclusion body (protein aggregates) for the purification of insoluble proteins. While GE Healthcare and Qiagen kits provide reagents to purify soluble and insoluble proteins both under native and denaturing conditions.

## 2. Material and methods

### 2.1. Materials

The following IMAC kits His GraviTrap (GE Healthcare, Piscataway, NJ, USA), QIAexpress Ni-NTA Fast Start Kit (Qiagen, Hilden, Germany) and B-PER 6xHis Fusion Protein Purification Kit (Thermo Scientific, Rockford, IL, USA) were used in this study. The buffer constituents of each kit for both native and denaturing purification conditions are shown in Tables 2 and 3respectively. In Table 2, the buffers constituents under native conditions according to manufacturer data sheets are shown. Although Thermo Scientific and Qiagen kits contain lysis, wash and elution buffers, the GE Healthcare kit contains only a lysis buffer (that can also be used as washing buffer) and an elution buffer. Table 3 shows the