A newly developed nickel IMAC resin resistant to reducing and chelating agents

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Introduction

Purification of His-tagged proteins from feeds containing reducing agents (DTT) or chelating agents (EDTA) causes stripping of nickel ions from conventional metal ion affinity chromatography (IMAC) resins. Typical sources of this problem are extracts from eukaryotic cells, *e.g.* insect cells, that commonly contain reducing agents added during the extraction. To circumvent this issue, a new precharged IMAC resin resistant to these agents have been developed - WorkBeads™ NiMAC.

WorkBeads NiMAC

- Nickel precharged IMAC resin optimized for purification of His_6-tagged proteins expressed in eukaryotic cells
- Highly resistant to chelating substances and reducing agents

Purification of sample containing DTT and EDTA

His-tagged Green Fluorescent Protein (His_6-GFP) expressed in *E. coli* containing 20 mM DTT and 20 mM Na_2-EDTA was purified with WorkBeads NiMAC and Workbeads 40 Ni-NTA (conventional nickel IMAC), prepacked in GoBio™ Mini columns. UV traces at 490 nm shows higher absorbance in the flowthrough (FT) from WorkBeads 40 Ni-NTA compared to FT from WorkBeads NiMAC (Fig. 1A). This indicates target protein leakage due to stripping of nickel ions from the conventional resin, whereas WorkBeads NiMAC shows no protein leakage and a protein recovery of 95% (Fig. 1B).

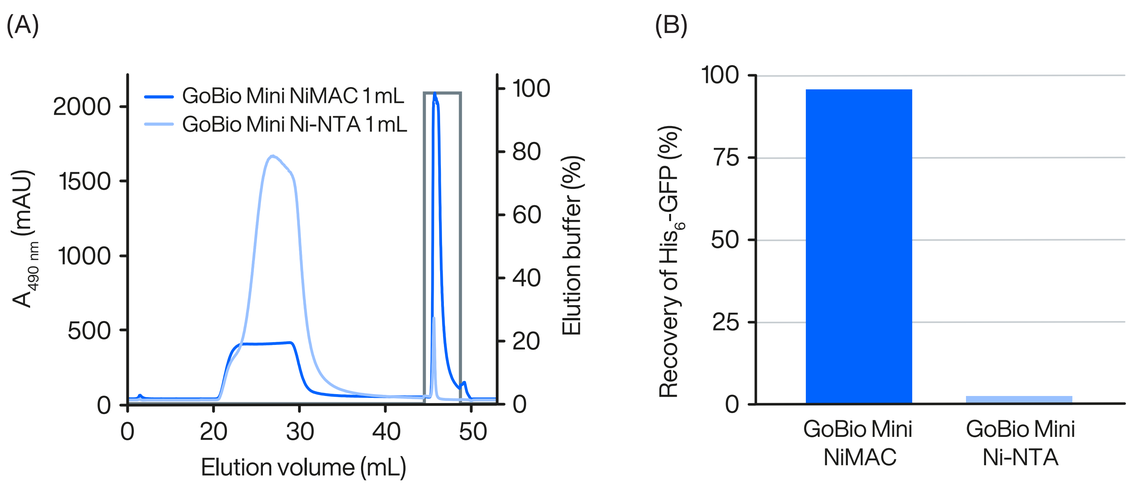

Figure 1. Chromatographic purification profiles for GoBio Mini NiMAC 1 mL (dark blue) and GoBio Mini Ni-NTA 1 mL (light blue), elution gradient (gray) (A). Comparison of target recovery for the two runs (B).

Table 1. Chromatographic running conditions.

Sample	10 mL clarified His_6-GFP incl. 10 mM imidazole, 20 mM DTT, 20 mM Na_2-EDTA
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	Step gradient, 100% elution buffer, 5 CV
Flow rates	0.5 mL/min (78 cm/h; elution) 1 mL/min (156 cm/h; sample loading)

Multiple purification runs on GoBio Mini NiMAC *vs.* HisTrap Excel

Since WorkBeads NiMAC resin cannot be stripped of the Ni^{2+} ions and recharged, the performance after several consecutive runs was investigated. Seven consecutive purifications as described in Figure 1 were run on GoBio Mini NiMAC (Fig. 2A) and a similar resin, HisTrap™ Excel (Cytiva). Purity and recovery were assessed (Fig. 2B).

(A) Elution profile. 7 consecutive runs on GoBio Mini NiMAC.

(B) Recovery and purity assessment after 7 runs.

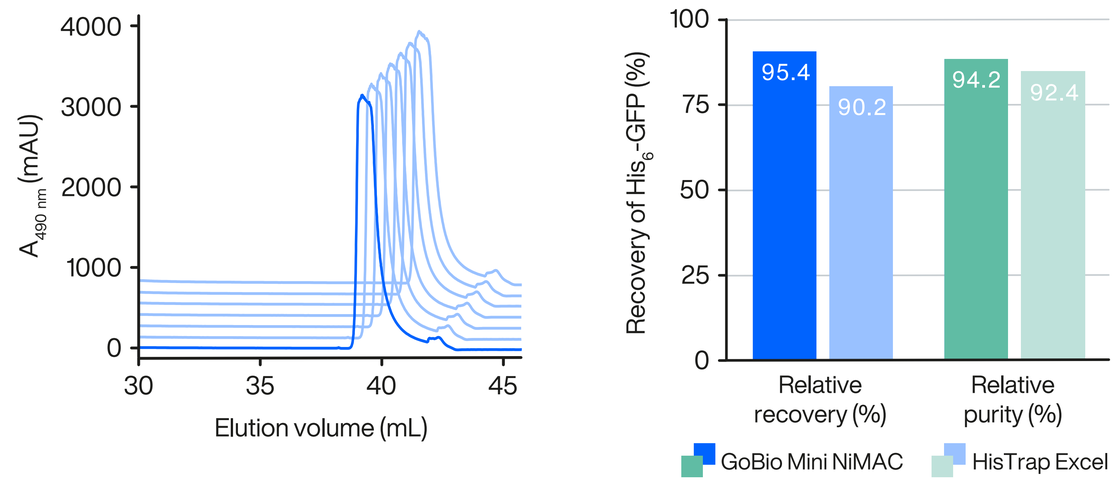

Figure 2. Seven consecutive purifications of clarified feed expressing His_6-GFP using GoBio Mini NiMAC 1 mL. Chromatograms of the elution profiles of His_6-GFP (chromatograms shifted). Absorbance at 490 nm for the first run (dark blue) and consecutive runs (light blue in run order) (A). Remaining recovery and purity for GoBio Mini NiMAC (dark blue and dark green) *vs.* HisTrap Excel (light blue and light green) after seven consecutive purifications (B).

Scale-up purification

The scalability of WorkBeads NiMAC was investigated by a 5-fold sample loading (Fig. 3A).

Purification was done by applying 50 mL feed/mL resin of His_6-GFP, containing ≈ 50 mg target protein. The comparison of purity and recovery between small and large sample loading is shown in Fig. 3B.

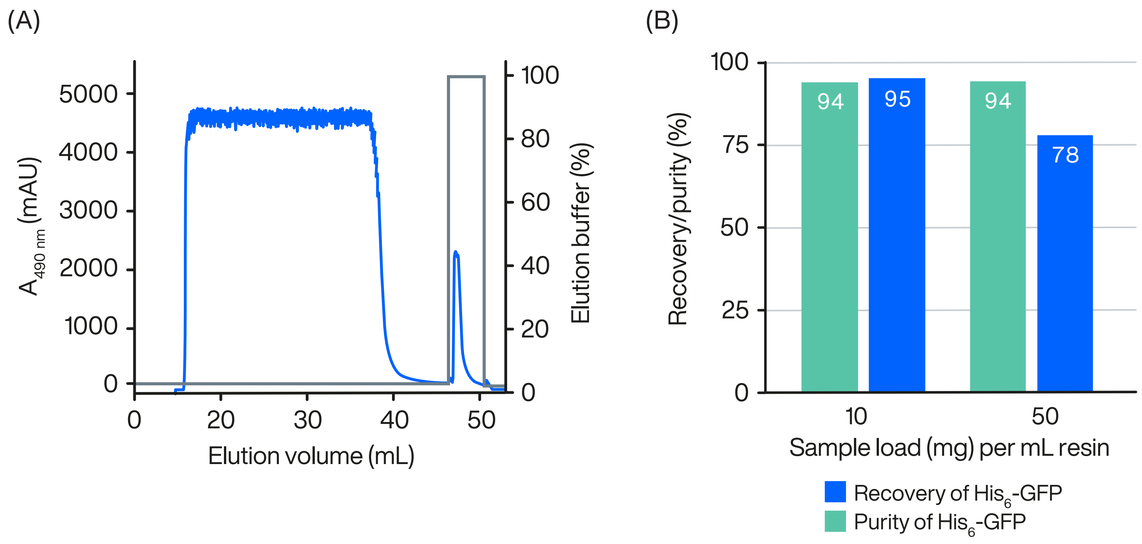

Figure 3. 50 mL load of His_6-GFP eluted in 10 CV step gradient with 100% elution buffer (A). Comparison of target recovery and purity for the two different sample load purifications (B).

Conclusions using WorkBeads NiMAC

- Higher stability and recovery for sample containing up to 20 mM DTT and 20 mM EDTA compared to conventional nickel pre-charged IMAC
- High stability towards up to 20 mM TECP and β-mercaptoethanol (data not shown)
- Excellent recoveries and purities after consecutive runs and no significant nickel stripping
- Excellent choice when purifying His-tagged proteins in cell cultures containing reducing and chelating substances

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