



APPLICATION NOTE

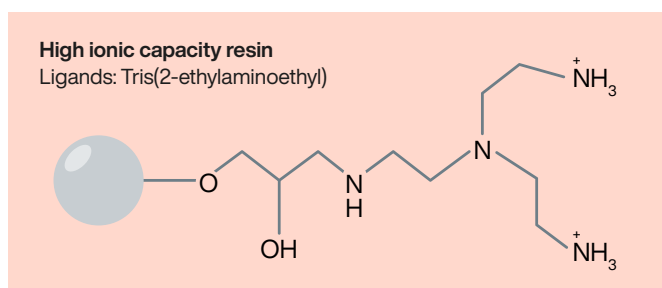
Bulk impurity removal prior to IMAC for increased purity of nucleic acid binding proteins

Proteins containing DNA-binding domains, such as translation factors, are often difficult to purify due to high levels of co-eluting DNA impurities after the capture step. High levels of nucleic acids also have the tendency of increasing viscosity of the sample, giving rise to down-stream purification issues, such as column clogging. To circumvent these problems, a high salt tolerant resin, WorkBeads™ 40 TREN, was placed upstream the capture step to act as a bulk impurity filter, removing the nucleic acids, thereby protecting the capture column, and increasing the final purity of the eluted target. The target studied was a recombinant expressed translation initiator factor, IF-3, which contained a fusion tag to facilitate purification. The IMAC resin WorkBeads Ni-NTA was used as the capture step.

This study shows that running WorkBeads 40 TREN prior to IMAC captures impurities and removes ~98% more dsDNA compared to direct IMAC, leading to a much purer product. WorkBeads 40 TREN is therefore highly advantageous to include early in a purification process to remove co-eluting nucleic acid fragments when purifying a DNA or RNA binding proteins. Depending on the application, the downstream polishing step may be omitted.

TREN – IMAC study

IF-3 is a bacterial translation initiation factor that interacts with the ribosomal subunits to regulate the formation of the 70S complex. The proteins involved in the translation process are of great interest when studying antibiotics-induced translational-inhibiting mechanisms and they are often His-tagged to simplify purification by using IMAC. In this study IF-3 was recombinantly expressed in *Escherichia coli* and had a His-tag of six histidine residues fused to the C-terminus (Figure 1A). To purify IF-3-His₆, a high salt tolerant anion exchange resin (WorkBeads™ 40 TREN) was placed upstream an IMAC resin (WorkBeads 40 Ni-NTA). Since IF-3 has a high isoelectric point (pI) it will elute in the AEX flow-through (FT) at the standard operating pH condition of 8 to subsequently be adsorbed to the nickel attached to the IMAC resin (Figure 1B).



Commonly the feed is directly loaded onto an IMAC resin without any thorough pre-treatments. Applying this new resin setup should generate a purer product, especially regarding host cell DNA (HCD) levels, and furthermore protect the IMAC resin from fouling host cell derived impurities. See Figure 2 for a standard IMAC purification of a His-tagged protein with the following analysis of eluate vs. feed and flow-through. This illustrates the need to reduce the impurity levels of the feed to protect the IMAC resin from fouling and to increase the purity for more “sticky” proteins.

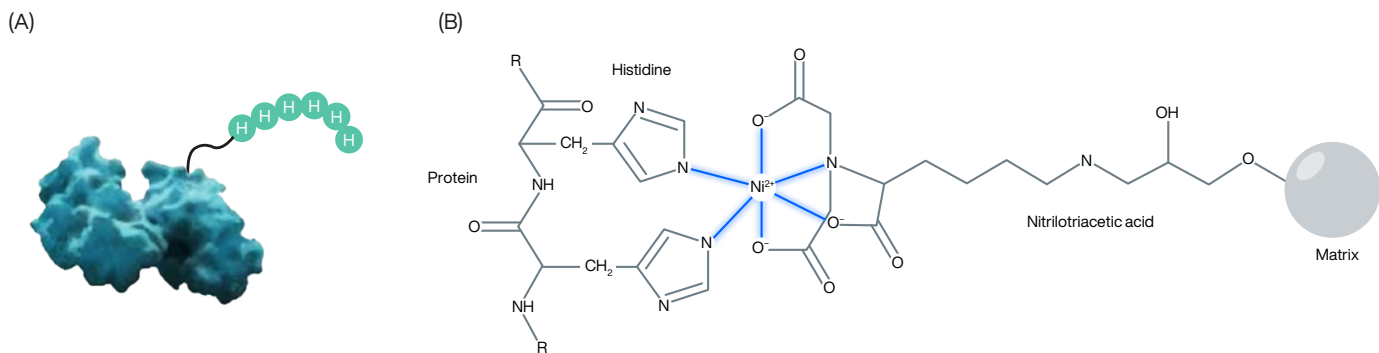


Figure 1. (A) An illustration of the C-terminus His-tagged translation initiation factor 3 (IF-3). (B) Interactions between the histidines and the nickel metal charged on the NTA ligands on the resin are indicated by blue lines.

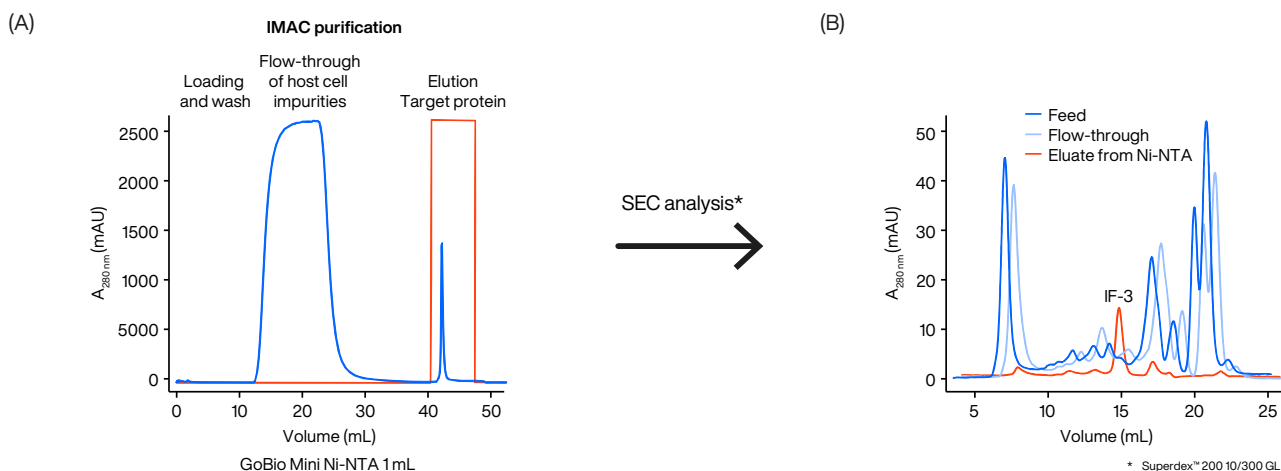


Figure 2. (A) Purification of 10 mL clarified His-tagged lysate on a GoBio™ Mini Ni-NTA 1 mL column at a flow rate of 1 mL/min using common IMAC buffers (see Table 1). (B) SEC analysis of eluate vs. feed and flow-through.

In Figure 3A, the standard versus the new resin setup is shown. In both cases, 10 mL of clarified lysate was loaded onto either a GoBio™ Mini Ni-NTA or a HisTrap™ FF (Cytiva) column, operated separately. For the new setup, a GoBio™ Mini TREN 5 mL column was connected upstream of the IMAC column to improve purity. Analyses of the two setups are shown in Figures 3B–C.

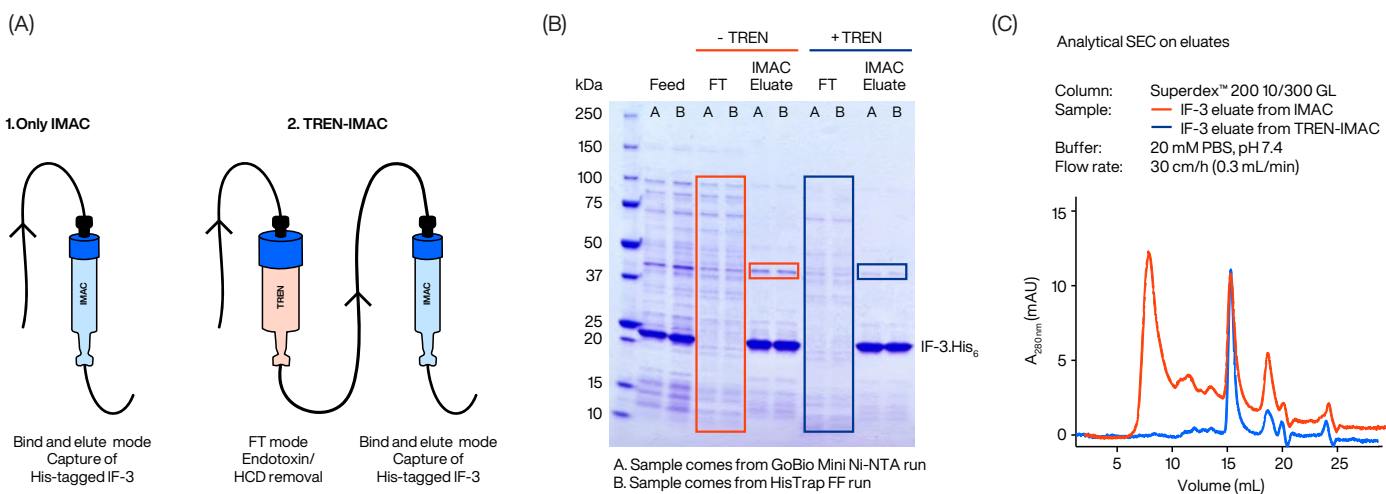


Figure 3. (A) IMAC only (1), and TREN followed by IMAC (2). vs. TREN – IMAC. IMAC: GoBio Mini Ni-NTA 1 mL or HisTrap™ FF 5 mL, TREN: GoBio Mini TREN 5 mL. (B) SDS-PAGE analysis of fractions from the IMAC and TREN-IMAC purifications. The boxes highlight the impurity levels without (red) or with (blue) TREN in the setup. (C) Analytical SEC on IF-3-His₆ eluates from IMAC with (blue) or without (red) pre-treatment using WorkBeads™ 40 TREN.

Table 1. Running conditions for the IMAC purifications

Running conditions	
Binding buffer	50 mM sodium phosphate, 300 mM NaCl, 10 mM imidazole, pH 8
Elution buffer	50 mM sodium phosphate, 300 mM NaCl, 300 mM imidazole, pH 8
Flow rate	1 mL/min (1 minute residence time)

The SDS-PAGE and the analytical SEC of the eluates demonstrated a purer product regarding host cell proteins (HCP) when WorkBeads 40 TREN had been connected upstream of the IMAC resin. In Table 2 both HCP and even more HCD levels are shown to be significantly reduced by the presence of WorkBeads 40 TREN.

Table 2. HCD and HCP levels detected in eluates and flow-throughs for the two setups

Sample	Resins	HCD ppm	HCP ppm
IF-3-His ₆ eluate	WorkBeads 40 Ni-NTA	1780	18
IF-3-His ₆ eluate	WorkBeads 40 TREN + WorkBeads 40 Ni-NTA	41	bdl ^a
IF-3-His ₆ FT	WorkBeads 40 Ni-NTA	15100	–
IF-3-His ₆ FT	WorkBeads 40 TREN + WorkBeads 40 Ni-NTA	bdl ^a	–

^a Value is below the stable range of detection. HCD levels were determined by Quant-IT PicoGreen dsDNA Assay Kit and HCP levels were determined by ELISA.

Relatively high levels of HCD (1780 ppm) was detected after only IMAC due to co-elution of nucleic acid fragments, but the upstream inclusion of WorkBeads 40 TREN in the setup, reduced these levels by ~98% (<50 ppm). The HCD levels in the flow-through was below the detection limit, reducing the risk of fouling the capture column, which is an important factor when later scaling up the processes.

Conclusion

Even though IMAC is a very good capture step for His-tagged proteins in general, it may be advantageous to clean up your feed using WorkBeads 40 TREN in FT mode when working with nucleic acid binding proteins. For IF-3-His₆, ~98% reduction of HCD levels was obtained.

The removal of impurities in an upstream step to circumvent the need for a potential down-stream polishing step – and simultaneously protect the IMAC column – should enable loading of a broader range of samples and result in a more efficient purification process.

- Take control of your feed and get better performances
- Improve efficiency and lifetime of your affinity resin

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