

Removal of HCD in complex feeds using a high salt-tolerant anion exchange resin upstream of IMAC

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Introduction

Purification of complex feeds can result in an extensive burden on the capture column in the form of e.g., nucleic acids and different protein impurities when the feed is directly loaded without major pre-treatments. These contaminant species also often bind non-specifically to the target molecules and/or resin, and thus may be co-eluted with the final product.

Introduction of a pre-treatment column upstream of an immobilized metal ion affinity chromatography (IMAC) column will both increase the lifetime of the resin and improve the final purity of the target protein. Here, a high-salt-tolerant anion exchange resin (AIEX) was introduced upstream of IMAC resins to purify a His-tagged protein. Host-cell DNA (HCD), endotoxins, viruses and various host-cell proteins (HCP) adsorb to AIEX resins and thereby reduce the foulant load on the subsequent IMAC column.

WorkBeads 40 TREN

WorkBeads™ 40 TREN is an agarose-based high-salt-tolerant AIEX resin, with Tris(2-aminoethyl)amine (TAEA) ligands that have two positively charged groups below approximately pH 9 (Fig. 1).

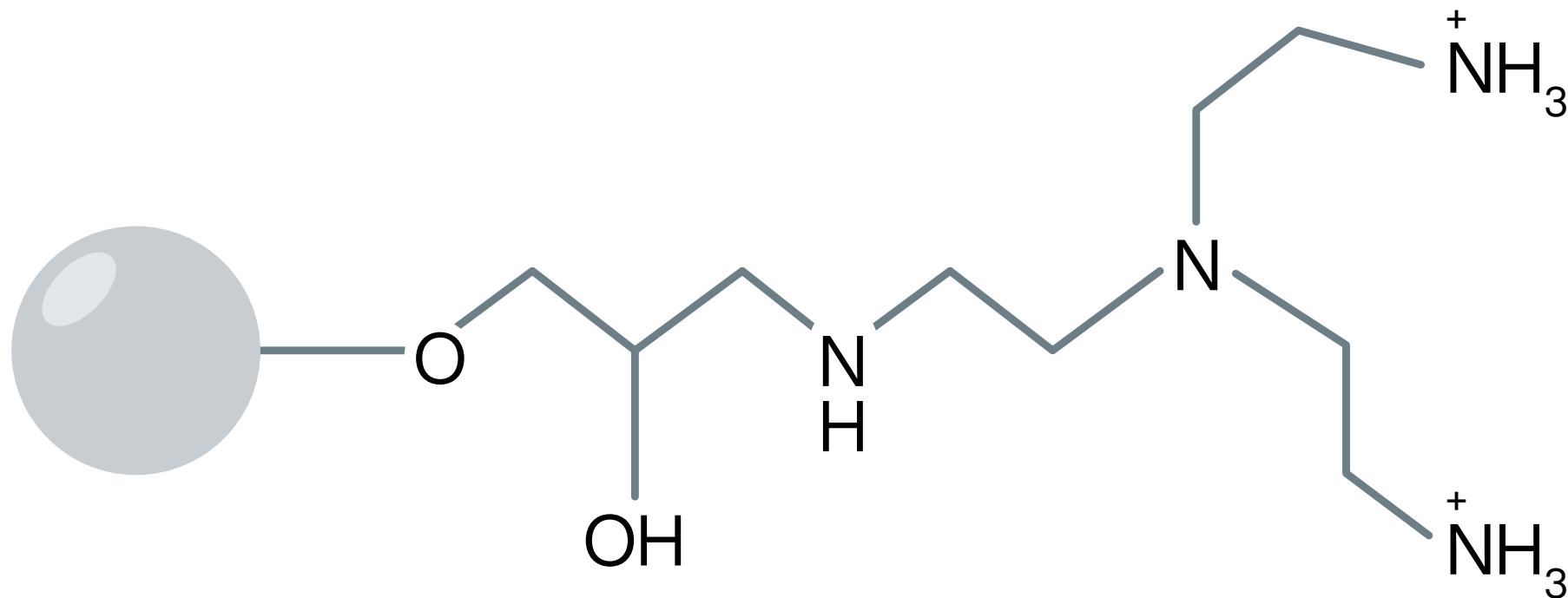


Figure 1. WorkBeads 40 TREN.

Experimental setup

The experiment setup included two separate 1 mL prepacked IMAC columns, GoBio™ Mini Ni-NTA and HisTrap™ FF (Cytiva) with or without a GoBio Mini TREN 5 mL column connected upstream of the two separate IMAC columns (Fig. 2). The experimental running conditions are shown in Table 1.

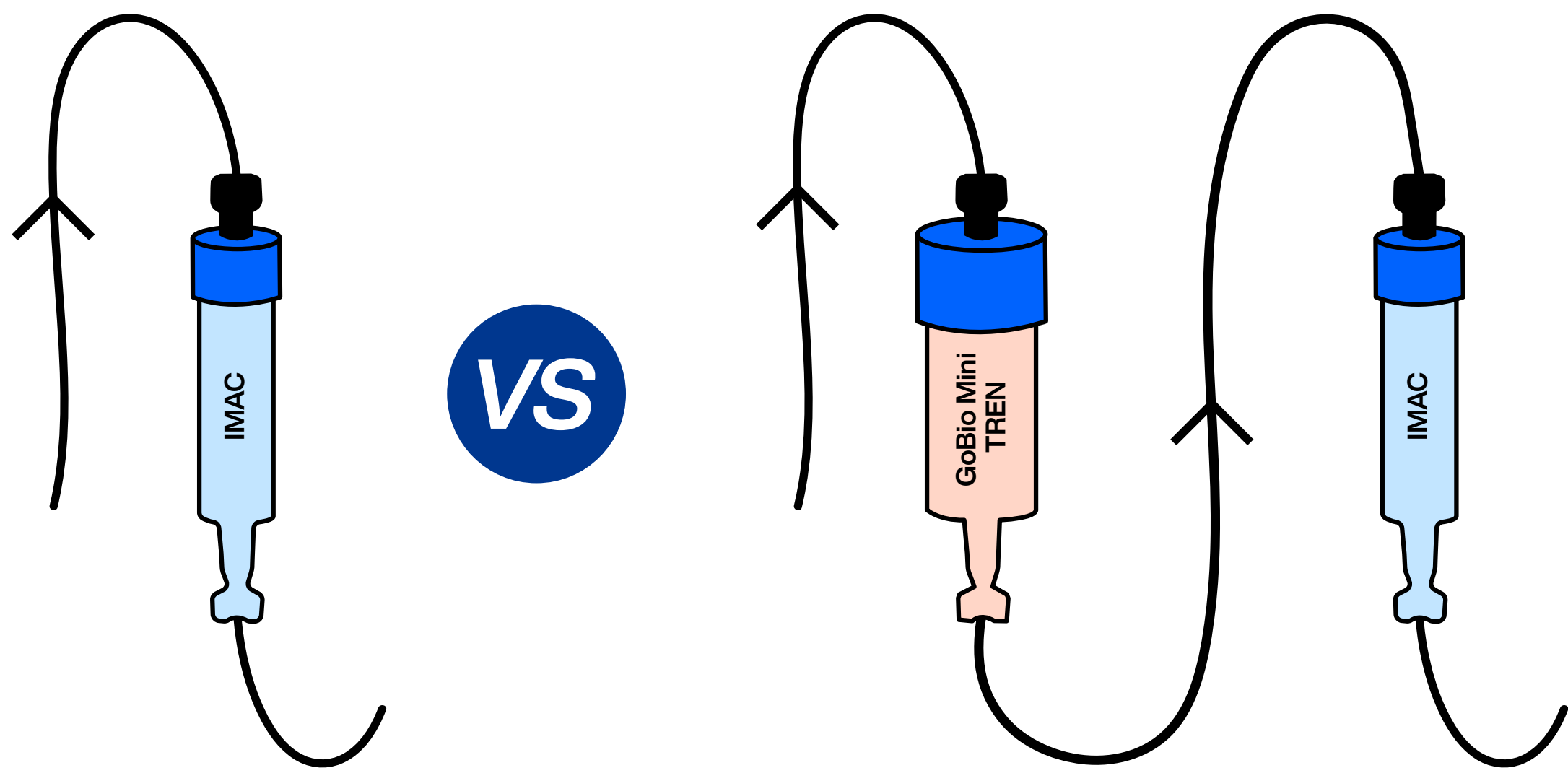


Figure 2. Stand-alone IMAC purification vs. in-line purification setup of GoBio Mini TREN and IMAC columns. Arrows show the flow direction.

Table 1. Chromatographic running conditions.

Binding buffer	50 mM sodium phosphate, 300 mM NaCl, 10 mM imidazole, pH 8.0
IMAC elution buffer	50 mM sodium phosphate, 300 mM NaCl, 300 mM imidazole, pH 8.0
TREN elution buffer	50 mM sodium phosphate, 2.0 M NaCl, pH 8.0
Flow rate	1 mL/min

Target protein

The His-tagged target protein was *E. coli* translation initiation factor 3 (IF-3-His₆). IF-3 binds to the ribosomal subunits 30S and 50S and regulates the formation of the 70S complex. It is used as a model protein for studying the translational mechanism in detail. IF-3, with its nucleic acid binding domain, is expected to co-elute with a significant amount of nucleic acids.

The effects of WorkBeads 40 TREN as a pre-treatment step

- Increased purity in flow-throughs and eluates (Fig. 3A-D)
- HCD removal from feed was >99.9% and 97-98% from eluates (Fig. 3C-D)
- No significant loss of target protein recovery (Fig. 3A)
- ELISA showed that HCP removal from eluates was 49-62%
- Analogous results from GoBio Mini Ni-NTA and HisTrap FF (Fig. 3A, C-D)

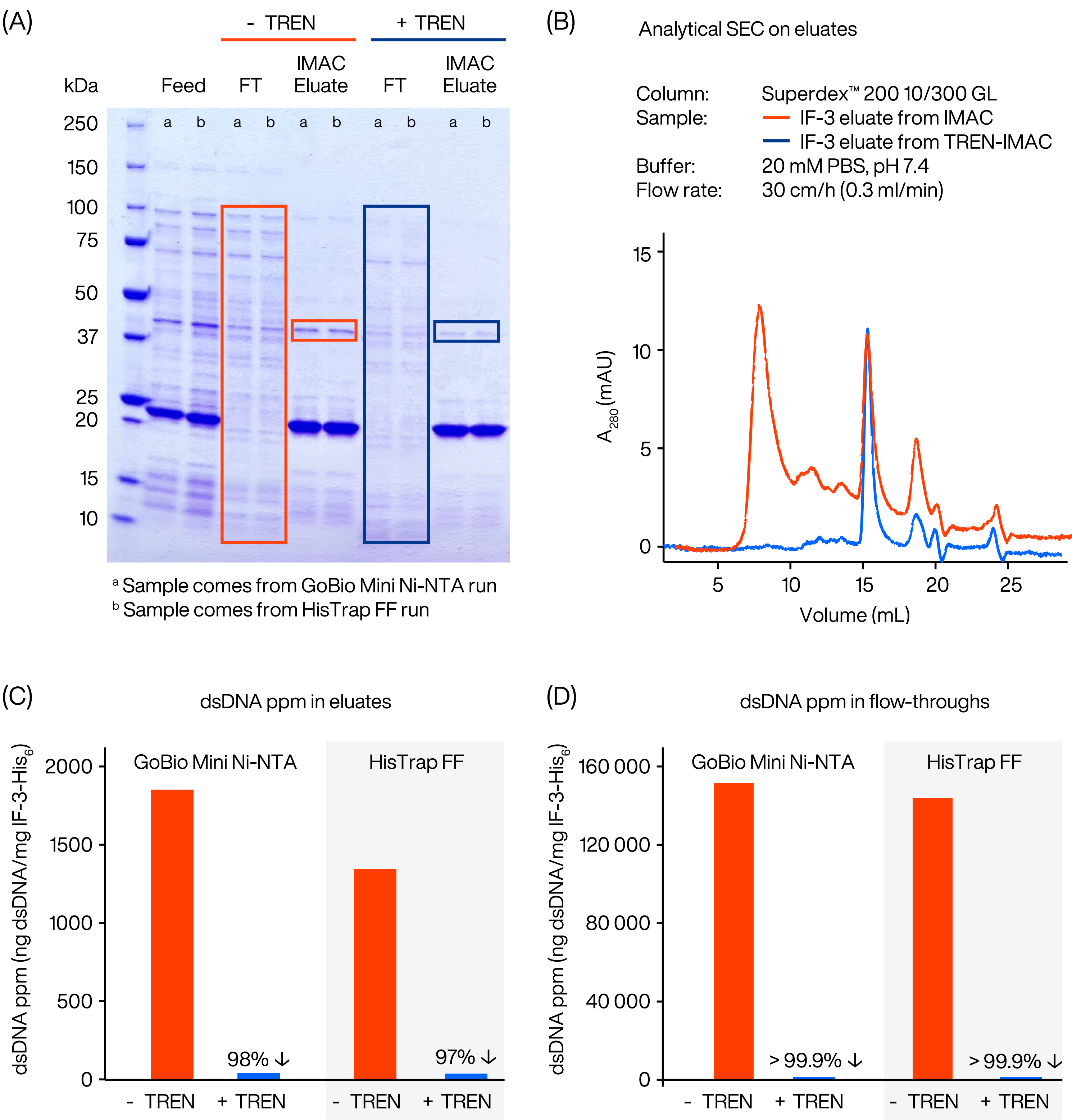


Figure 3. (A) SDS-PAGE analysis of fractions from runs with combinations of IMAC resins +/- pre-treatment using WorkBeads 40 TREN. The boxes highlight the impurity levels without (red) or with (blue) TREN in the setup. (B) Purity profiles of eluates +/- TREN run on analytical SEC. Eluates (C) and flow-throughs (D) +/- TREN prior IMAC purification of 10 mL IF-His₆ feed.

The HCD ppm (ng dsDNA/ mg IF-3-His₆) values are found in Table 2.

Table 2. IF-3-His₆ yields and HCD ppm in eluates and flow-throughs +/-TREN prior GoBio Mini Ni-NTA/HisTrap FF.

Sample	Resin	IF-3-His ₆ (mg)		HCD ppm	
		GoBio Mini Ni-NTA	HisTrap FF	GoBio Mini Ni-NTA	HisTrap FF
Eluate	IMAC	1.9	2.3	1780	1300
Eluate	TREN + IMAC	1.6	1.8	41	37
FT	IMAC	–	–	15100	14000
FT	TREN + IMAC	–	–	bdl ^a	bdl ^a

^a Below detection limit

Conclusions

Including WorkBeads 40 TREN as a pre-treatment step early in the purification process is highly advantageous, particularly for removing host cell DNA, which can interfere with downstream chromatographic processes. Additionally, WorkBeads 40 TREN reduces the amount of co-eluted HCPs in the final product while protecting the IMAC resin. Introducing this pre-step before IMAC also allows for the omission of a subsequent SEC polishing step.