Immobilized metal ion affinity chromatography

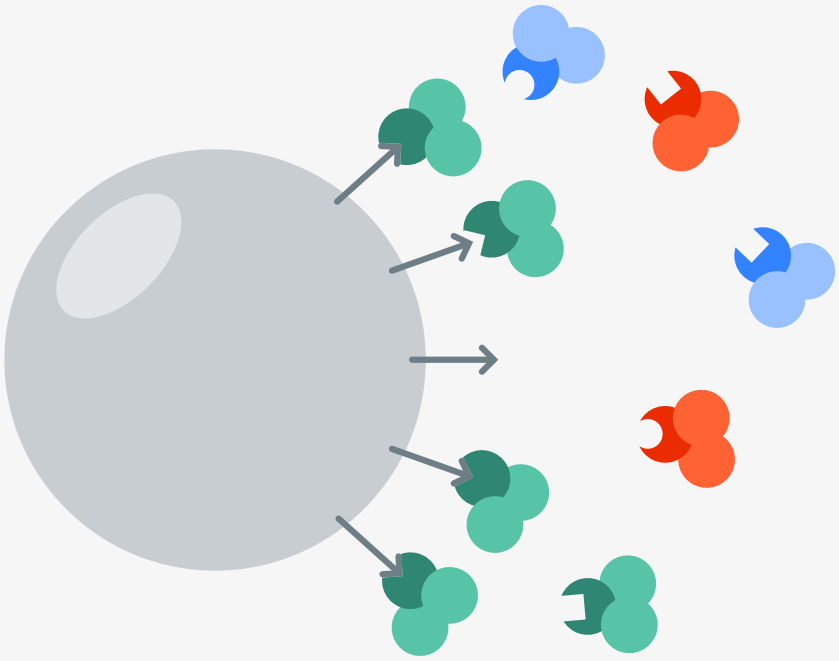
Immobilized metal ion affinity chromatography (IMAC) separates most proteins with exposed histidine, cysteine and tryptophan on their surface. IMAC is an excellent technique for optimization and purification of His-tagged proteins. The technique is ideal for capture directly from clarified cell lysate. The target protein is collected in a highly purified and concentrated form.

Several factors influence the final purity of a His-tagged protein after an IMAC purification, for example, position of the tag (C- or N-terminal), the length of the tag, immobilized metal ion (Ni^{2+}, Co^{2+}, Zn^{2+}, Cu^{2+}) and the ligand immobilized on the matrix (NTA or IDA). To make the optimization of His-tagged protein purifications as efficient as possible, Bio-Works offers products with many combinations of metal ion and immobilized ligand, as well as GoBio Mini His-tag NTA Screening kits and GoBio Mini His-tag IDA Screening kits.

Target molecules

His-tagged proteins and other proteins with exposed histidine, cysteine and tryptophan on their surface.

See schematic depicting immobilized metal ion affinity chromatography.



Precharged IMAC resin resistant to DTT and EDTA

WorkBeads NiMAC

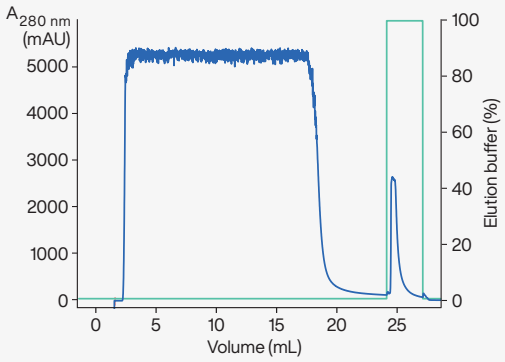
- Resin with extra strongly bound Ni^{2+} resulting in extremely low nickel ion leakage
- Highly resistant to reducing agents up to 20 mM DTT
- Highly resistant to chelating substances present in eukaryotic extracts or up to 20 mM EDTA
- Excellent purity, recovery and reproducible results
- Outstanding alkali stability with 0.5 M NaOH, extends the number of purification cycles
- Available in several different GoBio prepacked columns

Applications

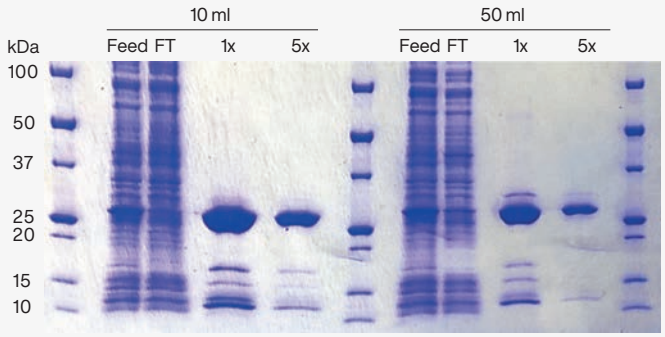
Larger sample load incl. 20 mM DTT and 20 mM Na_2-EDTA

Column:	GoBio Mini NiMAC 1 mL
Sample:	50 mL His_6-GFP in binding buffer with 20 mM DTT and 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
Elution:	Step gradient, 100% elution buffer, 10 column volumes (CV)
Flow rates:	0.5 mL/min (78 cm/h; elution); 1 mL/min (loading)

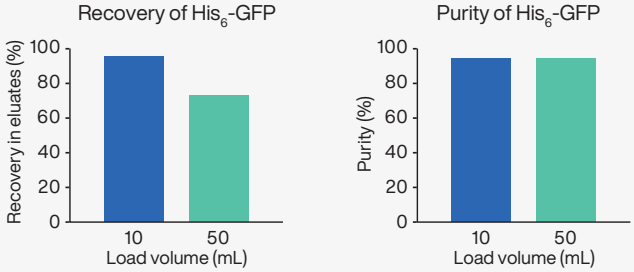
(A)



(B)



(C)



(A) Chromatogram with 50 mL load of His_6-GFP, (B) SDS-PAGE under reducing conditions of the feed, flow-through (FT) and eluted pool (1x: concentrated eluate, 5x: 1:5 diluted eluate) from 10 mL sample load and 50 mL sample load. (C) Comparison of target recovery and purity for the two different sample load purifications.

IMAC resins precharged with different metal ions

WorkBeads 40 Ni-NTA
WorkBeads 40 Co-NTA
WorkBeads 40 Zn-NTA
WorkBeads 40 Cu-NTA
WorkBeads 40 Ni-IDA
WorkBeads 40 Co-IDA
WorkBeads 40 Zn-IDA
WorkBeads 40 Cu-IDA

- Resins immobilized with either NTA (Nitrilotriacetic acid) or IDA (Iminodiacetic acid) and four different choices of metal ions Ni^{2+}, Co^{2+}, Zn^{2+} or Cu^{2+}
- Precharged with different metal ions for ease of use
- Low leakage of immobilized ligand and metal ions
- Resistant to harsh cleaning agents (NaOH). Note! The metal ions have to be stripped off before cleaning
- High binding capacity and flow rate
- Available in several different GoBio prepacked columns

Applications

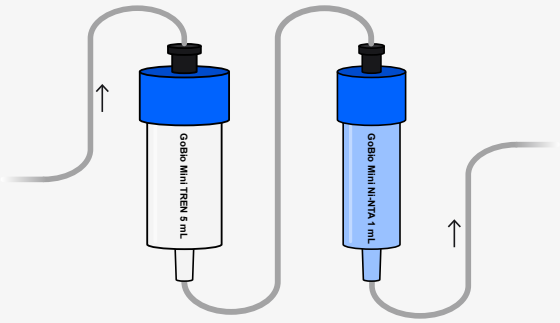
Purification of His-tagged protein on GoBio Mini Ni-NTA *vs.* HisTrap™ FF +/- upstream GoBio Mini TREN

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

One example of such a complex feed is His-tagged proteins expressed in bacteria. Therefore, WorkBeads 40 TREN prepacked in a GoBio Mini TREN column was introduced as a pre-treatment step upstream the IMAC column when purifying the *E. coli* translation initiation factor 3 (IF-3-His_6). Since this protein has a nucleic acid binding domain, host cell nucleic acids can potentially be a major co-eluting impurity in the eluates.

The GoBio Mini TREN column was operated in flow-through mode to capture impurities. This resin binds host cell nucleic acid (HCD), viruses and various host cell proteins (HCP), thereby reducing the foulant load on the subsequent IMAC column. The effect of the TREN column was evaluated by examining the removal of HCD and HCPs, see analysis.

Schematic view of GoBio Mini TREN upsteam GoBio Mini Ni-NTA



IF-3-His_6 purification on GoBio Mini Ni-NTA and HisTrap FF +/- upstream GoBio Mini TREN

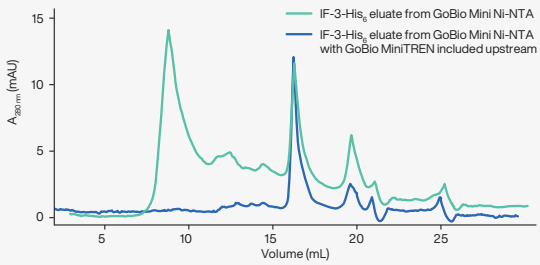
The effects of WorkBeads 40 TREN as a pre-treatment step:

- Increased purity in flow-throughs and eluates (Figures A-D)
- > 99.9% HCD removal in feed and 96-98% HCD removal in eluates
- No significant loss of target protein recovery (Figure B)
- ELISA showed that GoBio Mini TREN removes 49-62% more HCPs in eluates
- GoBio Mini Ni-NTA and HisTrap FF show analogous results (Figures B-D)

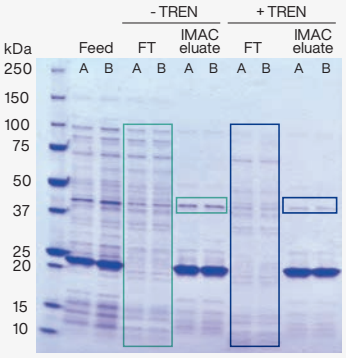
Analytical SEC of eluted IF-3-His_6 from only GoBio Mini Ni-NTA compared to GoBio Mini Ni-NTA in combination with GoBio Mini TREN

Sample volumes: 200 µL
Column: Superdex™ 200 10/300 GL (Cytiva)
Buffer: 20 mM PBS, pH 7.4
Flow rate: 30 cm/h (0.3 mL/min)

(A)

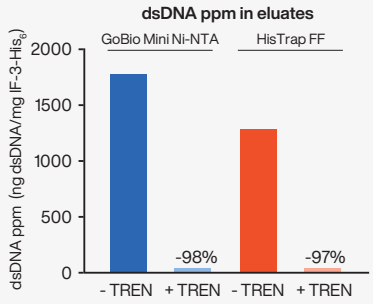


(B)

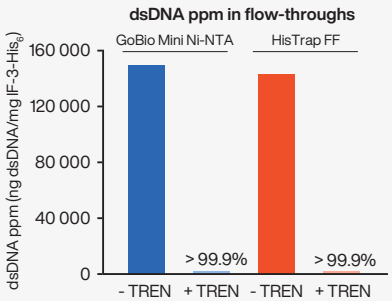


A – Samples from GoBio Mini Ni-NTA run
B – Samples from HisTrap FF (Cytiva) run

(C)



(D)



Conclusion

The final purity of the eluted His-tagged proteins, regarding HCD presence, was increased 97–98% when GoBio Mini TREN was placed upstream the IMAC column compared to a stand-alone step with only IMAC. Moreover, the purity was increased in terms of less co-eluted HCPs. In conclusion, the addition of an upstream pre-treatment step is highly advantageous to include early in a purification process to remove especially host-cell DNA that tend to interfere with downstream chromatographic processes.

Uncharged IMAC resins

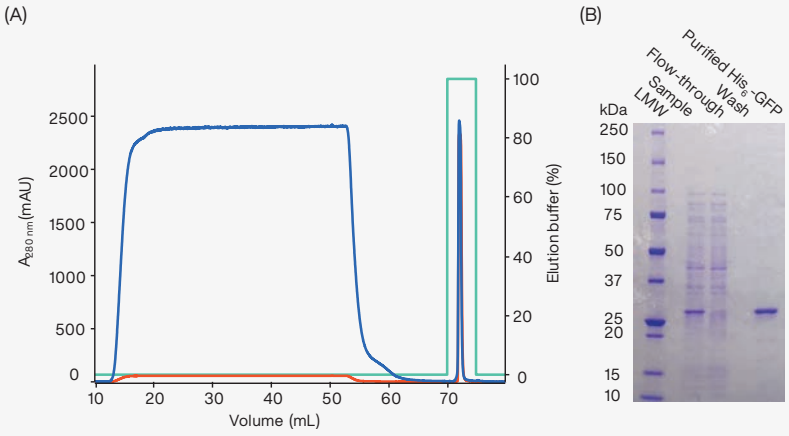
WorkBeads 40 NTA
WorkBeads 40 IDA

- Resins immobilized with either NTA (nitrilotriacetic acid) or IDA (iminodiacetic acid) for immobilization of your choice of metal ion
- Low leakage of immobilized ligand and metal ions of choice
- Resistant to harsh cleaning agents (NaOH). Note! The metal ions have to be stripped off before cleaning
- High binding capacity and flow rate
- Available in several different GoBio prepacked columns

Applications

Purification of clarified His_6-GFP on GoBio Mini Ni-NTA

Column:	GoBio Mini Ni-NTA 1 mL
Sample:	40 mL His_6-GFP in binding buffer
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
Elution:	100% elution buffer in 5 CV
Elution flow rate:	0.5 mL/min (75 cm/h)



(A) Chromatogram of the capture and elution of His_6-GFP. Absorbance at 280 nm (blue), absorbance at 490 nm (red) and percentage of elution buffer (green). (B) SDS-PAGE analysis of sample, flow-through, wash and eluted peak.

Technical specifications

	WorkBeads NiMAC
Target substance	His-tagged proteins
Matrix	Highly cross-linked agarose
Average particle size[1] (D_{V50})	45 µm
Precharged ions	Nickel (II)
Static binding capacity	> 80 mg/mL resin
Dynamic binding capacity[2]	> 40 mg/mL resin
Metal ion capacity[3]	> 60 µmol Cu^{2+}/mL resin
Max flow rate (20 cm bed height and 5 bar)	600 cm/h
Chemical stability	Compatible with all standard aqueous buffers used for protein purification, and additives such as 20 mM Na_2-EDTA, 20 mM dithiothreitol (DTT), 20 mM TECP, 20 mM β-mercaptoethanol, 8 M urea, 6 M guanidine-HCl, non-ionic detergents, 500 mM imidazole, 30% isopropanol, 0.5 M NaOH
pH stability	3 to 9 (working range) 2 to 14 (cleaning-in-place)
Storage	2 to 25°C in 20% ethanol

1 Optimal flow rate during binding is depending on the sample. During column wash and elution, a flow rate of 1 mL/min and 5 mL/min can be used for 1 mL and 5 mL columns, respectively. Note: The maximum pressure the packed bed can withstand depends on the sample/liquid viscosity and chromatography resin characteristics. The pressure also depends on the tubing used to connect the column and the system restrictions after the column outlet.
2 Maximum flow rate for aqueous buffers at 20°C. Decrease the maximum flow rate if the liquid has a higher viscosity. Higher viscosities can be caused by low temperature or presence of additives. Use half of the maximum flow rate for 20% ethanol for example.

	WorkBeads 40 Ni-NTA WorkBeads 40 Ni-IDA	WorkBeads 40 Co-NTA WorkBeads 40 Co-IDA	WorkBeads 40 Cu-NTA WorkBeads 40 Cu-IDA	WorkBeads 40 Zn-NTA WorkBeads 40 Zn-IDA
Matrix	Highly cross-linked agarose	Highly cross-linked agarose	Highly cross-linked agarose	Highly cross-linked agarose
Average particle size[1] (D_{V50})	45 µm	45 µm	45 µm	45 µm
Chelating ligand	Nitrilotriacetic acid (NTA) or Iminodiacetic acid (IDA)	NTA or IDA	NTA or IDA	NTA or IDA
Metal ion	Nickel (II)	Cobalt (II)	Copper (II)	Zink (II)
Metal ion capacity for the chelating ligand[2]	N/A	N/A	50 to 60 µmol Cu^{2+}/mL (WorkBeads 40 Cu-IDA)	N/A
Dynamic binding capacity[3] (DBC)	> 60 mg His_6-GFP/mL resin	N/A	N/A	N/A
Maximum flow rate (20 cm bed height, 5 bar)	600 cm/h	600 cm/h	600 cm/h	600 cm/h
Chemical stability	Compatible with all standard aqueous buffers used for protein purification, 8 M urea, 6 M guanidine-HCl, non-ionic detergents, 20% ethanol. Chelating substances (e.g. EDTA) will strip off the metal ions. Stripped resin: 10 mM HCl (pH 2), 10 mM NaOH (pH 12), 10 mM sodium citrate-HCl (pH 3).			
pH stability	7 to 9 (working) 2 to 12 (cleaning, stripped resin)	7 to 9 (working) 2 to 12 (cleaning, stripped resin)	7 to 9 (working) 2 to 12 (cleaning, stripped resin)	7 to 9 (working) 2 to 12 (cleaning, stripped resin)
Storage	2 to 25°C in 20% ethanol	2 to 25°C in 20% ethanol	2 to 25°C in 20% ethanol	2 to 25°C in 20% ethanol

1 The median particle size of the cumulative volume distribution.
2 Metal ion capacity is determined by frontal analysis at 50% breakthrough using copper solution.
3 The binding capacity is determined using a GoBio Mini Ni-NTA 1 mL, equal value is expected for IDA resins. The binding capacity is dependent on the size of the target protein, and on the competition from impurities.

	WorkBeads 40 NTA	WorkBeads 40 IDA
Matrix	Highly cross-linked agarose	Highly cross-linked agarose
Average particle size[1] (D_{V50})	45 µm	45 µm
Chelating ligand	Nitrilotriacetic acid (NTA)	Iminodiacetic acid (IDA)
Metal ion capacity[2]	20 to 30 µmol Cu^{2+}/mL resin	50 to 60 µmol Cu^{2+}/mL resin
Maximum flow rate	600 cm/h (20 cm bed height, 5 bar)	600 cm/h (20 cm bed height, 5 bar)
Chemical stability	Compatible with all standard aqueous buffers used for protein purification, 8 M urea, 6 M guanidine-HCl, non-ionic detergents, 20% ethanol. Chelating substances (e.g. EDTA) will strip off the metal ions. Stripped resin: 10 mM HCl (pH 2), 10 mM NaOH (pH 12), 10 mM sodium citrate-HCl (pH 3).	
pH stability	7 to 9 (working range) 2 to 12 (cleaning, stripped resin)	7 to 9 (working range) 2 to 12 (cleaning, stripped resin)
Storage	2 to 25°C in ethanol	2 to 25°C in ethanol

1 The median particle size of the cumulative volume distribution.
2 Metal ion capacity is determined by frontal analysis at 50% breakthrough using copper solution.

Ordering information

Product name	Pack size	Article number
WorkBeads NiMAC	25 mL 150 mL 1 L 5 L	40 653 001 40 653 003 40 653 010 40 653 050
WorkBeads 40 Ni-NTA	25 mL 150 mL 1 L 5 L 10 L	40 651 001 40 651 003 40 651 010 40 651 050 40 651 060
WorkBeads 40 Co-NTA	25 mL 150 mL 1 L	40 651 401 40 651 403 40 651 410
WorkBeads 40 Cu-NTA	25 mL 150 mL 1 L	40 651 301 40 651 303 40 651 310
WorkBeads 40 Zn-NTA	25 mL 150 mL 1 L	40 651 501 40 651 503 40 651 510
WorkBeads 40 Ni-IDA	25 mL 150 mL 1 L 5 L 10 L	40 650 001 40 650 003 40 650 010 40 650 050 40 650 060
WorkBeads 40 Co-IDA	25 mL 150 mL 1 L	40 650 401 40 650 403 40 650 410
WorkBeads 40 Cu-IDA	25 mL 150 mL 1 L	40 650 301 40 650 303 40 650 310
WorkBeads 40 Zn-IDA	25 mL 150 mL 1 L	40 650 501 40 650 503 40 650 510
WorkBeads 40 NTA	25 mL 150 mL 1 L 5 L 10 L	40 602 001 40 602 003 40 602 010 40 602 050 40 602 060
WorkBeads 40 IDA	25 mL 150 mL 1 L 5 L 10 L	40 601 001 40 601 003 40 601 010 40 601 050 40 601 060

More information

Data Sheet, DS 40 653 010
WorkBeads NiMAC, GoBio prepacked columns

Data Sheet, DS 40 600 010
WorkBeads 40 NTA, WorkBeads 40 IDA, GoBio prepacked columns

Data Sheet, DS 40 650 010
WorkBeads 40 Ni-NTA, WorkBeads 40 Co-NTA, WorkBeads 40 Zn-NTA, WorkBeads 40 Cu-NTA, GoBio prepacked columns

WorkBeads 40 Ni-IDA, WorkBeads 40 Co-IDA, WorkBeads 40 Zn-IDA, WorkBeads 40 Cu-IDA, GoBio prepacked columns

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