Bio-Works

DATA SHEET

WorkBeads NiMAC
GoBio NiMAC prepacked columns

WorkBeads™ NiMAC resin and GoBio™ NiMAC prepacked column family allow quick and easy purification of His-tagged proteins and other proteins with an affinity for nickel ions. WorkBeads NiMAC resin is precharged with extremely strongly bound nickel ions resulting in very high resistance to reducing agents such as DTT and chelating substances such as EDTA. The high stability allows purification of proteins from sources such as eukaryotic cell extracts, that normally would cause significant nickel ion stripping from the resin. This reduces the need for sample pre-treatment. The resin provides high purity and binding capacity and the possibility to use high flow rates for minimized process time.

- 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
- Very high protein binding capacity, higher than 40 mg/mL resin
- High purity and reproducible results
- Prepacked GoBio columns for convenience and reproducibility

Resin description

WorkBeads are agarose-based chromatographic resins manufactured using a proprietary method that results in porous beads with a tight size distribution and high mechanical stability. Agarose based matrices have been successfully used for decades in biotechnology purifications, from research to production scale, due to their exceptional compatibility with biomolecules including proteins, peptides, nucleic acids and carbohydrates. WorkBeads resins are designed for separations requiring optimal capacity and purity.

WorkBeads NiMAC resin is immobilized with chelating groups that bind nickel ions very tightly. The Ni^{2+}-charged resin binds His-tagged proteins but has unusually low affinity for other host proteins that tend to bind other resins made for Immobilized Metal Ion

Affinity Chromatography (IMAC). The very tightly bound nickel ions result in extremely low nickel ion leakage. This property makes WorkBeads NiMAC excellent for purification of His-tagged proteins from large feed volumes containing chelating substances. Typical sources of this problem are extracts from eukaryotic cells, e.g. insect cells, that commonly contain reducing agents added during the extraction. WorkBeads NiMAC resin cannot be stripped of the Ni^{2+} ion and recharged.

The main characteristics of WorkBeads NiMAC resin are shown in Table 1. For more details, see instruction IN 40 653 01.

Table 1. Main characteristics of WorkBeads NiMAC resin.

	WorkBeads NiMAC
Target substance	His-tagged proteins
Matrix	Highly cross-linked agarose
Average particle size (D_{V50})[1]	45 µm
Precharged ions	Nickel (II) ions, Ni^{2+}
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)[4]	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 TCEP, 20 mM ß-mercaptoethanol, 8 M urea, 6 M guanidine-HCl, non-ionic detergents, 500 mM imidazole, 30% isopropanol, 0.5 M NaOH
pH stability	3 – 9 (working range)[5] 2 – 14 (cleaning-in-place)
Storage	2 to 25 °C in 20% ethanol

[1] The median particle size of the cumulative volume distribution.
[2] Binding capacity may vary depending on protein characteristics and on flow rate used. A lower flow rate usually increases the dynamic binding capacity.
[3] Metal ion capacity is determined by frontal analysis at 50% breakthrough using copper solution.
[4] Optimal flow rate during binding is depending on the sample.
[5] This pH range is the most used pH for purification of His-tagged proteins.

GoBio prepacked column family

GoBio prepacked column family is developed for convenient, reproducible and fast results and includes columns with different sizes and formats.

GoBio Mini 1 mL and GoBio Mini 5 mL for small scale purification and screening using a shorter packed bed.

GoBio Screen 7x100 (3.8 mL) for reproducible process development including fast and easy optimization of methods and parameters.

GoBio Prep 16x100 (20 mL) and GoBio Prep 26x100 (53 mL) for lab-scale purifications and scaling up.

GoBio Prep 16x600 (120 mL) and GoBio Prep 26x600 (320 mL) for preparative lab-scale size exclusion chromatography.

GoBio Prod 80x200 (1 L), GoBio Prod 130x200 (2.7 L), GoBio Prod 200x200 (6 L), GoBio Prod 240x200.

(9 L) and GoBio Prod 330x250 (21.4 L) for production-scale purifications.

Table 2. Main characteristics of GoBio Mini, GoBio Screen and GoBio Prep columns.

	GoBio Mini 1 mL & 5 mL	GoBio Screen 7x100	GoBio Prep 16x100	GoBio Prep 26x100
Column hardware	Polypropylene	Acrylic	Acrylic	Acrylic
Top and bottom filters	Polyethylene	Polyamide	Polyamide	Polyamide
Top and bottom plugs	Polypropylene	Polypropylene	Polypropylene	Polypropylene
Connections	1/16" female (top) 1/16" male (bottom)	1/16" female (both ends)	1/16" female (both ends)	1/16" female (both ends)
Column volumes	1 mL 5 mL	3.8 mL	20 mL	53 mL
Column dimensions	7 × 28 mm (1 mL) 13 × 38 mm (5 mL)	7 × 100 mm	16 × 100 mm	26 × 100 mm
Max. column hardware pressure[1]	0.3 MPa, 3 bar, 43 psi	5 bar, 0.5 MPa, 70 psi	5 bar, 0.5 MPa, 70 psi	5 bar, 0.5 MPa, 70 psi
Chemical stability	1 M NaOH, 30% isopropanol, 70% ethanol	1 M NaOH, 20% isopropanol, 20% ethanol	1 M NaOH, 20% isopropanol, 20% ethanol	1 M NaOH, 20% isopropanol, 20% ethanol

[1] 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.

Table 3. Main characteristics of GoBio Prod columns.

	GoBio Prod 80x200, GoBio Prod 130x200, GoBio Prod 200x200, GoBio Prod 280x200, GoBio Prod 330x250
Column hardware	Acrylic
Top and bottom filters	Polyamide
Top and bottom plugs	Polypropylene
Connections	TC-connections
Column volumes	1 L, 2.7 L, 6 L, 9 L, 21.4 L
Column dimensions	80 × 200 mm (1 L), 130 × 200 mm (2.7 L), 200 × 200 mm (6 L), 280 × 200 mm (9 L), 330 × 250 mm (21.4 L)
Max. column hardware pressure[1]	5 bar, 0.5 MPa, 70 psi
Chemical stability	1 M NaOH, 20% isopropanol, 20 % ethanol

[1] 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.

Applications

Principle

WorkBeads NiMAC uses the Immobilized Metal Ion Affinity Chromatography (IMAC) technique. IMAC utilizes the affinity of histidine, cystein and tryptophan amino acid side chains on the protein surface for transition metal ions, such as Ni^{2+}, immobilized (via a metal chelating ligand) on the chromatography resin.

IMAC is most commonly used for the purification of recombinant His-tagged proteins. This His-tag is usually composed of six to ten histidyl groups and is typically placed at the N- or C-terminus of the target protein, although other positions are possible. The His-tagged proteins will bind to the chelating ligand (through the metal ion) and the unbound material will pass through.

Purification of His_6-GFP from *E. coli* extract in the presence of reducing agent and chelator

His_6-GFP was purified from an *E. coli* extract expressing His_6- Green Fluorescent Protein (His_6-GFP) (Fig. 1) using a prepacked GoBio Mini NiMAC 1 mL column. The extract was clarified and supplemented with 20 mM DTT and 20 mM Na_2-EDTA before 10 mL was loaded onto a column equilibrated with 10 column volumes (CV) of binding buffer containing 10 mM imidazole. After sample application, the column was washed with 10 CV of binding buffer to remove unbound impurities. The target protein was then eluted using a 25 CV gradient from 10 to 300 mM imidazole. No significant amount of His_6-GFP was detected in the flow through (or in the wash). The purity of the eluted main peak was 89%, and no impurity peaks are seen in the chromatogram. The emission maximum for His_6-GFP is 490 nm.

Column:	GoBio Mini NiMAC 1 mL
Sample:	10 mL clarified His_6-GFP including 10 mM imidazole, 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:	Linear gradient, 0-100% elution buffer, 25 CV
Flow rates:	0.5 mL/min (78 cm/h; elution); 1 mL/min (loading)

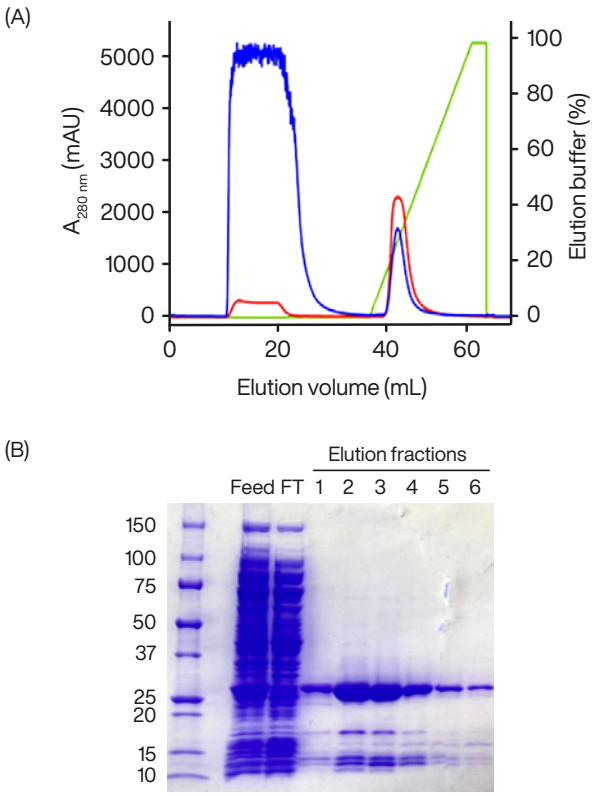


Figure 1. Purification of His_6-GFP from clarified *E. coli* extract using GoBio Mini NiMAC 1 mL column. (A) Chromatogram showing UV absorbance at 280 nm (blue) and 490 nm (red), and percentage of elution buffer (green). (B) SDS-PAGE analysis. Marker, feed, flowthrough (FT) and eluted peak fractions of 1.5 mL (lanes 1-6).

WorkBeads NiMAC *vs.* WorkBeads Ni-NTA (conventional IMAC resin)

Purification of His-tagged proteins from feeds containing reducing agents, such as DTT or chelating agents, such as EDTA, causes stripping of nickel ions from conventional IMAC resins. To analyse the difference between WorkBeads NiMAC resin and a conventional resin, comparative experiments were performed using GoBio Mini 1 mL columns (Fig. 2).

Aliquots of 10 mL of clarified *E. coli* extract containing His_6-GFP with 20 mM EDTA and 20 mM DTT were applied to GoBio Mini NiMAC 1 mL and GoBio Mini Ni-NTA 1 mL, the latter being a conventional column for His-tagged protein purification. The target His-tagged protein was eluted stepwise using 300 mM imidazole. Imidazole at a concentration of 10 mM was included in both sample and binding buffer to reduce unspecific binding. Higher imidazole concentrations increase purity but may reduce the yield.

The difference in performance between the two resins is seen in Figure 2, where the nickel ions are stripped off from the conventional resin that turns white, whereas WorkBeads NiMAC has an outstanding recovery of protein (95%) and maintains its blue color. Even after 300 mL sample load containing 20 mM EDTA and 20 mM DTT the blue color was maintained.

Columns:	GoBio Mini NiMAC 1 mL and GoBio Mini Ni-NTA 1 mL
Sample:	10 mL clarified His_6-GFP including 10 mM imidazole, 20 mM DTT and 20 mM Na2-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, 5 CV
Flow rates:	0.5 mL/min (78 cm/h; elution); 1 mL/min (loading)

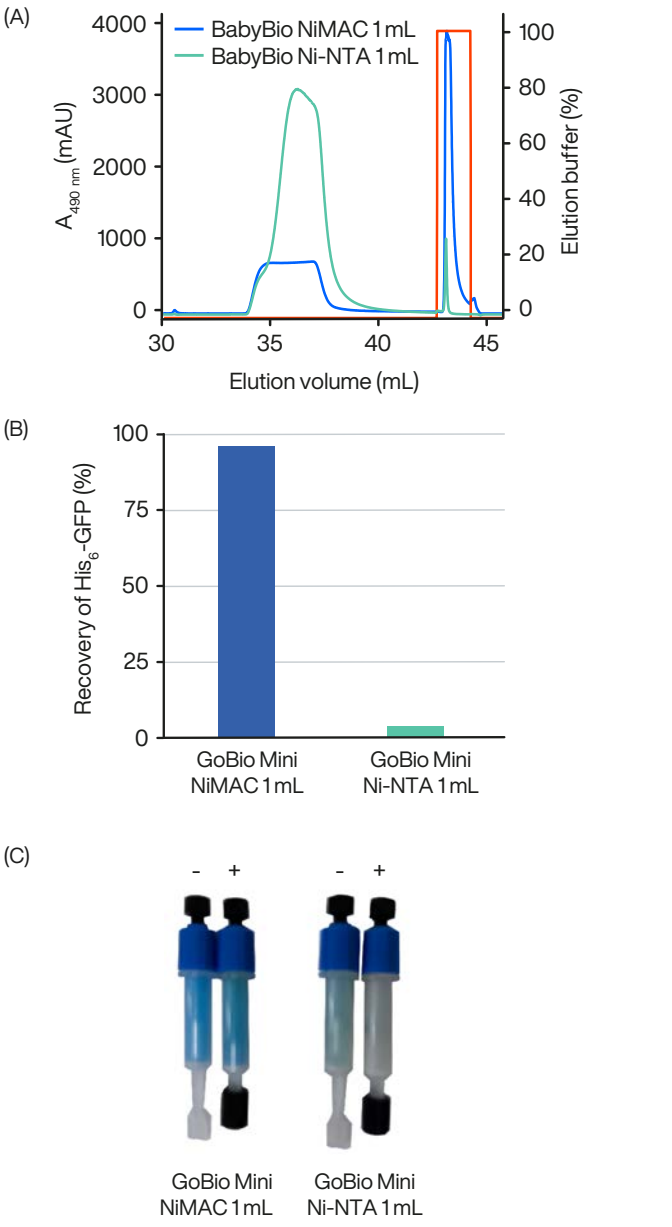


Figure 2. Purification of clarified His_6-GFP using GoBio Mini NiMAC 1 mL column compared to a conventional GoBio Mini Ni-NTA 1 mL. (A) Chromatogram of the capture, wash and elution of His_6-GFP expressed in *E. coli.* Absorbance at 490 nm for GoBio Mini NiMAC (blue) and GoBio Mini Ni-NTA (red). (B) Comparison of target recovery for the two columns. (C) The columns before (-) and after (+) the purification runs.

Multiple runs

The advantage of using WorkBeads NiMAC when purifying His-tagged proteins from a cell culture medium containing e.g. reducing agents that often cause metal stripping is visualized in Figure 2. But it is also of high importance that resins can be used for multiple runs without losing performance, for example binding capacity, even when large amounts of reducing agents and chelators are included in the sample and/or buffers.

To show that multiple runs can be done using WorkBeads NiMAC without losing performance, seven purification cycles were run on GoBio Mini NiMAC 1 mL (Fig. 3). In each cycle 10 mL clarified *E. coli* extract expressing His_6-GFP was applied. The runs were repeated without any intermediate treatment of the column. The protein was eluted by a step gradient with 300 mM imidazole. Figure 3A shows the elution profiles for each run in order, with the first run shown in dark blue. The reproducibility was excellent with a recovery reduction of less than 5% over the seven consecutive purifications. The relative purity of the eluted His_6-GFP was measured by photometry as the ratio of $A_{490\,nm}/A_{280\,nm}$. The purity in percentage was calculated based on the fact that 100% pure His_6-GFP has a ratio of 2.13, as determined after polishing by size-exclusion chromatography, and the assumption that no tentative impurity from the extract shows any absorbance at A_{490nm}. Protein purity was found to be 94.2% across the purifications (Fig. 3B). Complete capture of the target protein to the resin from the feed in the loading step was confirmed by performing SEC analysis of the feed, the flowthrough and the eluate fractions (Fig. 3C).

Comparison of reproducibility

A comparative study was performed with HisTrap™ Excel (Cytiva), another product that is tolerant against reducing agents and chelating substances. The purifications described in Fig. 3 were repeated in seven cycles also with HisTrap Excel (not shown). The recovery of His_6-GFP in run 1 was estimated to be 84% for HisTrap Excel and 95% for GoBio Mini NiMAC. The reduction in recovery in run 7 compared to run 1 was 5% for GoBio Mini NiMAC and 10% for HisTrap Excel, showing a significantly better stability of GoBio Mini NiMAC (Fig. 4A).

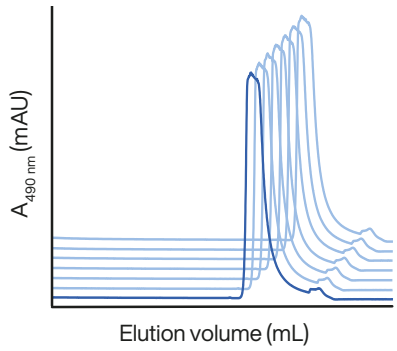
The purity was found to be 92.4% for HisTrap Excel compared with 94.2% for GoBio Mini NiMAC. The purity and yield are also visualized by SDS-PAGE (Fig. 4B). All these data demonstrate excellent reusability properties of WorkBeads NiMAC (Table 1).

Table 1. Data for the consecutive runs on GoBio Mini NiMAC and HisTrap Excel.

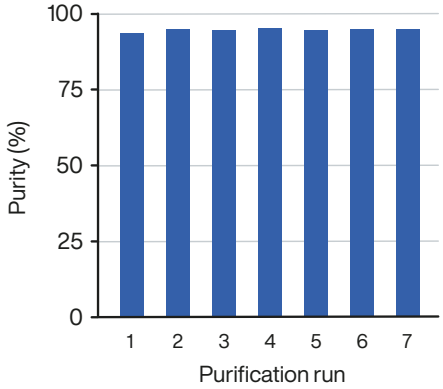
	GoBio Mini NiMAC	HisTrap Excel
Purity (average %)	94.2	92.4
Recovery (%)	95	84
Loss of recovery after seven runs (%)	4.6	9.8

Column: GoBio Mini NiMAC 1 mL
Sample: 7 x 10 mL clarified His6-GFP including 10 mM imidazole, 20 mM DTT and 20 mM Na2-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, 5 column volumes (CV)
Flow rates: 0.5 mL/min (78 cm/h; elution); 1 mL/min (loading)

(A)



(B)



(C)

SEC analysis
Column: Superdex™ 200 Increase 10/300 GL (Cytiva)
Sample: 60 µl flow through fraction or eluted sample
Elution buffer: 20 mM PBS, pH 7.4
Flow rate: 0.3 mL/min

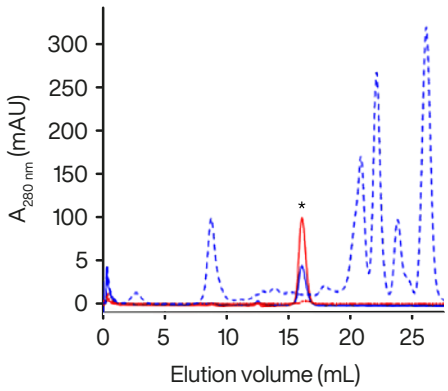


Figure 3. Seven consecutive purifications of clarified *E. coli* extract expressing His_6-GFP using a GoBio Mini NiMAC 1 mL column. (A) Chromatograms of the elution profile of His_6-GFP for 7 repeated runs (chromatograms shifted). Absorbance at 490 nm for the first run (blue) and consecutive runs (light blue in run order). (B) Purity measured off-line for elution pools for each purification. (C) SEC analysis of flow through fraction (dashed lines) and eluted His_6-GFP (solid lines). Absorbances in chromatogram: $A_{280\,nm}$ (blue) and $A_{490\,nm}$ (red). The asterisk highlights the His_6-GFP peak.

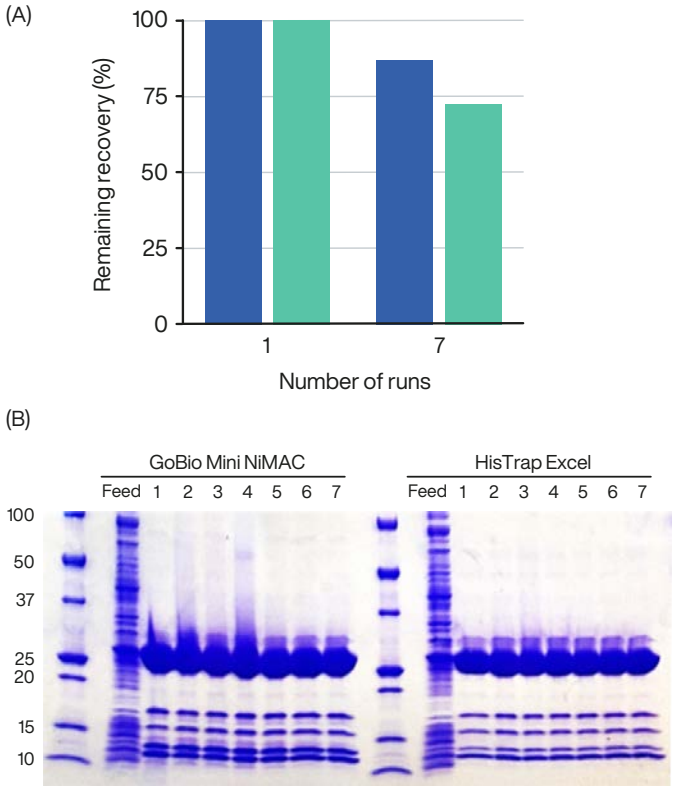


Figure 4. Seven consecutive purifications of clarified *E. coli* extract expressing His_6-GFP using a GoBio Mini NiMAC 1 mL column. (A) Loss of recovery for GoBio Mini NiMAC (blue) *vs.* HisTrap Excel (green) (not shown) over the 7 consecutive runs. (B) SDS-PAGE under reducing conditions of the feed and eluates from the multiple runs for both GoBio Mini NiMAC and HisTrap Excel.

Larger sample loads

The scalability of WorkBeads NiMAC was investigated by a 5-fold purification scale-up of His_6-GFP (Fig. 5).

Purification was done by applying 50 mL *E. coli* extract expressing His_6-GFP on a GoBio Mini NiMAC 1 mL column (Fig. 5A). Purification was done by applying 50 mL *E. coli* extract expressing His_6-GFP on a GoBio Mini NiMAC 1 mL column (Fig. 5A). The purity was as good as purification using just 10 mL of feed, whereas the recovery was 73.5% for the 50 mL purification compared to 95% in the 10 mL purification (Fig. 5C).

Column:	GoBio Mini NiMAC 1 mL
Sample:	50 mL clarified His_6-GFP including 10 mM imidazole, 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)

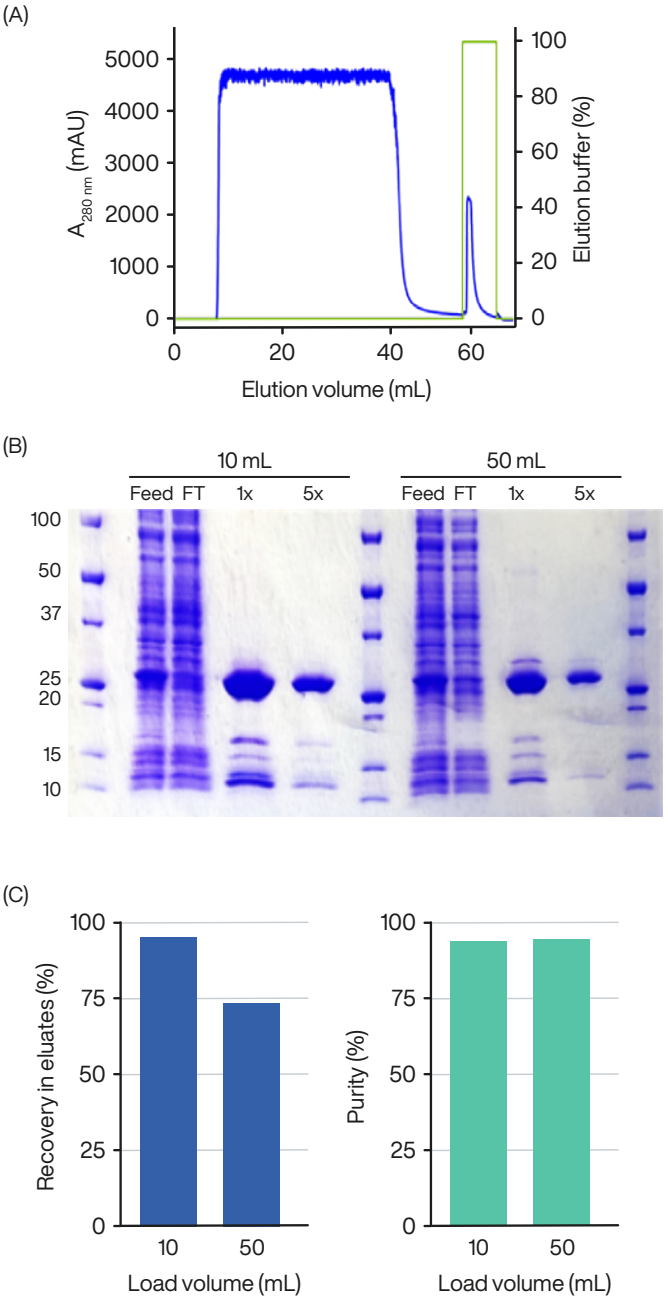


Figure 5. Large sample load on WorkBeads NiMAC (A) Chromatogram with 50 mL load of His_6-GFP eluted in 10 CV step gradient with 100% elution buffer. (B) SDS-PAGE under reducing conditions of the feed, flowthrough (FT) and eluted pool (1x: concentrated eluate, 5x: 1:5 diluted eluate) from 10 mL sample load and 50 mL sample load. (C) Comparison of target recovery and purity for the two different sample load purifications.

Conclusion

WorkBeads NiMAC is an excellent choice when purifying His-tagged proteins in cell cultures containing reducing agents, such as DTT or chelating substances, such as EDTA. This particularly applies to eukaryotic cell cultures, e.g. insect cells. The EDTA concentrations used in the purification examples shown here, 20 mM, exceed the normal concentrations found in most enzyme inhibitor cocktails.

WorkBeads NiMAC resists nickel stripping from the resin and can easily be reused for multiple purification cycles with retained purity and recovery performances.

Initial wash and recharging of metal ions

When using affinity chromatography it is recommended to do an initial wash before the first usage to wash out any loosely bound ligands and/or Ni^{2+}-ions to stabilize the binding capacity over time. We recommend an initial wash of WorkBeads NiMAC with 0.5 M NaOH for 15 minutes.

It is not possible to strip and recharge WorkBeads NiMAC due to the very tight binding of the nickel ions to the chelating group immobilized on the matrix.

Cleaning-in-place (CIP)

When running complex feeds, small amounts of impurities tend to adsorb to the resin by unspecific interactions. Cleaning of resin with up to 0.5 M NaOH for 15 minutes followed by 15 minutes distilled H_2O in repeated cycles are recommended if resin gets fouled.

Storage

Store at 2 to 25 °C in 20% ethanol.

For prolonged storage of the prepacked GoBio Screen and GoBio Prep columns connect the included transport syringe filled with storage solution to the bottom end of the column.

Related products

Product name	Pack size[1]	Article number
Prepacked columns		
GoBio Mini NTA His-tag Screening kit 1 mL[2]	1 mL × 4	45 700 101
GoBio Mini NTA His-tag Screening kit 5 mL[2]	5 mL × 4	45 700 102
GoBio Mini IDA His-tag Screening kit 1 mL[2]	1 mL × 4	45 700 001
GoBio Mini IDA His-tag Screening kit 5 mL[2]	5 mL × 4	45 700 002
Go Bio Mini Ni-NTA 1 mL	1 mL × 5	45 655 103
Go Bio Mini Ni-NTA 5 mL	5 mL × 5	45 655 107
Go Bio Mini Ni-IDA 1 mL	1 mL × 5	45 655 003
Go Bio Mini Ni-IDA 5 mL	5 mL × 5	45 655 007
GoBio Mini Dsalt 1 mL	1 mL × 5	45 360 103
GoBio Mini Dsalt 5 mL	5 mL × 5	45 360 107
GoBio Prep 16x100 Dsalt[3]	20 mL × 1	55 700 021
GoBio Prep 26x100 Dsalt	53 mL × 1	55 700 031
Bulk resins		
WorkBeads 40 Ni-NTA	25 mL 150 mL	40 651 001 40 651 003
WorkBeads 40 Ni-IDA	25 mL 150 mL	40 650 001 40 650 003
WorkBeads Dsalt	300 mL 1 L	40 360 003 40 360 010

[1] Other pack sizes can be found in the complete product list on www.bio-works.com
[2] Includes one column each precharged with Ni^{2+}, Co^{2+}, Cu^{2+} or Zn^{2+}
[3] Packed on request.

Ordering information

Product name	Pack size	Article number
Prepacked columns		
GoBio Mini NiMAC 1 mL	1 mL × 1 1 mL × 5 1 mL × 10	45 655 311 45 655 313 45 655 314
GoBio Mini NiMAC 5 mL	5 mL × 1 5 mL × 5 5 mL × 10	45 655 315 45 655 317 45 655 318
GoBio Screen 7x100 NiMAC[1]	3.8 mL × 1	55 653 001
GoBio Prep 16x100 NiMAC[1]	20 mL × 1	55 653 021
GoBio Prep 26x100 NiMAC[1]	53 mL × 1	55 653 031
GoBio Prod 80x200 NiMAC[1]	1 L	55 653 042
GoBio Prod 130x200 NiMAC[1]	2.7 L	55 653 062
GoBio Prod 200x200 NiMAC[1]	6 L	55 653 072
GoBio Prod 280x200 NiMAC[1]	9 L	55 653 082
GoBio Prod 330x250 NiMAC[1]	21.4 L	55 653 093
Bulk resins		
WorkBeads NiMAC	25 mL 150 mL 1 L	40 653 001 40 653 003 40 653 010

[1] Packed on request.

Orders: sales@bio-works.com or contact your local distributor.

For more information about local distributor and products visit **www.bio-works.com** or contact us at **info@bio-works.com**

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