Bio-Works

DATA SHEET

WorkBeads 40 NTA GoBio prepacked columns

WorkBeads™ 40 NTA resins are based on nitrilotriacetic acid (NTA) chelating groups. The resins can easily be charged, before use, with a broad spectrum of divalent or trivalent transition metal ions, including Ni^{2+}, Co^{2+}, Cu^{2+}, Zn^{2+}, Ga^{3+} or Fe^{3+}. They can then be used for Immobilized Metal Ion Affinity Chromatography (IMAC) purification of His-tagged proteins or other proteins with an affinity for metal ions. The selectivity of the metal-charged resin depends on both the choice of ligand and the metal ion used. These resins can also be used for divalent metal ion removal.

WorkBeads™ 40 NTA resins are available in several different ready-to-use prepacked column sizes, from GoBio™ Mini 1 mL to GoBio Prod columns starting from 1 L.

- Resins to be charged with the metal ion of choice
- High binding capacity and flow properties
- Reliable and reproducible results

Resin description

WorkBeads are agarose-based chromatographic resins manufactured using a proprietary method that results in porous beads with a tight size distribution and exceptional 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 40 NTA resins are immobilized with nitrilotriacetic acid (NTA) based chelating ligand, shown in Figure 1.

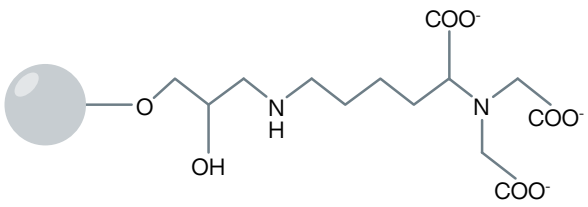


Figure 1. Structure of the chelating ligand used in WorkBeads 40 NTA resins.

These uncharged WorkBeads IMAC resins facilitate charging with a large spectrum of divalent or trivalent transition metal ions to produce IMAC resins. WorkBeads 40 NTA resins are compatible with Ni^{2+}, Co^{2+}, Cu^{2+}, Zn^{2+}, Ga^{3+} and Fe^{3+}.

WorkBeads 40 Ni-NTA is recommended as the starting point of choice for His-tagged protein purification and, in most cases, will give excellent results.

For optimization a screening is recommended with the different WorkBeads IMAC resins to identify the optimal combination of ligand and metal ion. Bio-Works offer prepacked GoBio His-tag Screening kits with all available precharged WorkBeads IMAC resins.

The main characteristics of these resins are shown in Table 1. For more details, please see instruction, IN 40 600 010.

Table 1. Main characteristics of WorkBeads 40 NTA resins.

	WorkBeads 40 NTA
Target substance	His-tagged proteins, proteins containing histidine cysteine and/or tryptophan amino acid side chains
Matrix	Highly cross-linked agarose
Average particle size1 (D_{V50})	45 µm
Chelating ligand	Nitrilotriacetic acid (NTA)
Metal ion capacity[2]	20 – 30 µmol Cu^{2+}/mL resin
Max flow rate (20 cm bed height and 5 bar)[3]	600 cm/h
Chemical stability	Compatible with all standard aqueous buffers used for protein purification, and 8 M urea and 6 M guanidine-HCl, non-ionic detergents, 20% ethanol. Chelating substances (e.g. EDTA) will strip off the metal ions. Stripped column 10 mM HCl (pH 2), 10 mM NaOH (pH 12), 10 mM sodium citrate-HCl (pH 3).
pH stability	2 – 12
Storage	2 to 25 °C

[1] The median particle size of the cumulative volume distribution.
[2] Metal ion capacity is determined by frontal analysis at 50% breakthrough using cupper solution.
[3] Optimal flow rate during binding is depending on the sample.

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" female	1/16" female	1/16" female
	1/16" male (bottom)	(both ends)	(both ends)	(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

WorkBeads 40 NTA resins can successfully be used for metal ion removal or, if charged with a metal ion, for Immobilized Metal Ion Affinity Chromatography (IMAC).

Principle

IMAC utilizes the affinity of histidine, cysteine, and tryptophan amino acid side chains on the protein surface for transition metal ions, such as Ni^{2+}, Co^{2+}, Cu^{2+} and Zn^{2+}, immobilized (via a metal chelating ligand), on the chromatography resin.

IMAC is commonly used for the purification of recombinant His-tagged proteins. The 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 the column. The bound proteins are desorbed utilizing stepwise or gradient elution, using a competing ligand, such as imidazole or lower pH.

Imidazole is routinely recommended for elution, it is the most common used competing ligand, but histidine, ammonium chloride or histamine can also be used. Before applying the sample, the column should be equilibrated with a low concentration of the competing ligand to prevent non-specific binding of endogenous proteins that may bind, for example via histidine clusters for example.

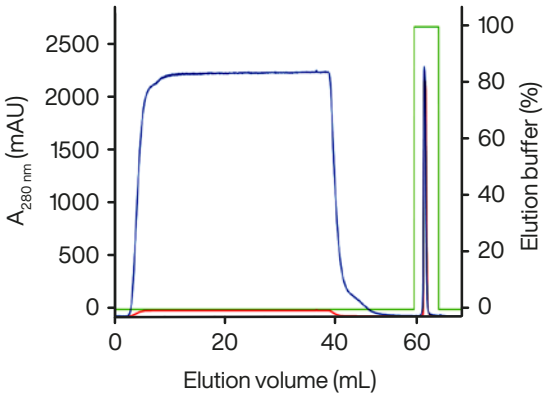
For more detailed instruction about the IMAC principle please see instructions IN 40 600 010 and IN 45 655 010.

Purification of His-tagged proteins

Figure 2 shows an example of the purification of clarified Histidine-tagged Green Fluorescent Protein (His_6-GFP) using GoBio Mini NTA 1 mL column charged with Ni^{2+}-ion.

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 (78 cm/h)

(A)



(B)

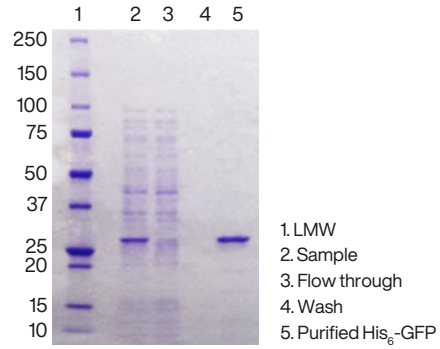


Figure 2. Purification of clarified His_6-GFP on WorkBeads 40 Ni-NTA packed into a GoBio Mini 1 mL column. (A) Chromatogram of the capture and elution of His_6-GFP. Absorbance at 280 nm (blue), absorbance at 490 nm (red) and percentage of elution buffer (green). (B) SDS-PAGE analysis of sample, flow through, wash and eluted peak

Cleaning-in-place

During purification impurities such as cell debris, lipids, nucleic acids, and protein precipitates from the samples may gradually build up in the resin. The severity of this process depends on the type of sample applied to the column, and the pre-treatment of the sample. The impurities may reduce the performance of the column over time. Regular cleaning (Cleaning-in-Place, CIP) keeps the resin clean, reduces the rate of further contamination, and prolongs the capacity, resolution, and flow properties of the column. Cleaning of a column using 1 M NaOH applied by a low reversed flow for 2 hours or overnight is often sufficient. Note! NaOH should only be used on metal stripped resin.

Sanitization (reduction of microorganism) can be done using combinations of NaOH and ethanol (e.g., incubation with a mixture of 0.5 M NaOH and 40% ethanol for 3 hours). The sanitization procedure and its effectiveness will depend on the microorganism to be sanitized and needs to be evaluated for each case. Before the cleaning of IMAC resins the metal ions must be removed from the resin using, for example, 50 mM Na_2EDTA, pH 8.5. After the cleaning the resin can be re-charged with fresh metal ions.

Scale-up

Scale-up can conveniently be carried out from a 1 mL GoBio Mini column to GoBio Prod columns starting from 1 L. Bulk packages of WorkBeads resins can also be packed into other column formats of choice.

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.

Check stored columns every 6 months and replenish the syringe buffer (20% Ethanol) with up to 5 mL if needed.

Related products

Product name	Pack size[1]	Article number
Prepacked columns		
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[2]	20 mL × 1	55 700 021
GoBio Prep 26x100 Dsalt[2]	53 mL × 1	55 700 031
Bulk resins		
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] Packed on request.

Ordering information

Product name	Pack size	Article number
Prepacked column		
GoBio Mini NTA 1 mL	1 mL × 1 1 mL × 5 1 mL × 10	45 655 111 45 655 113 45 655 114
GoBio Mini NTA 5 mL	5 mL × 1 5 mL × 5 5 mL × 10	45 655 115 45 655 117 45 655 118
GoBio Screen 7x100 NTA[1]	3.8 mL × 1	55 602 001
GoBio Prep 16x100 NTA[1]	20 mL × 1	55 602 021
GoBio Prep 26x100 NTA[1]	53 mL × 1	55 602 031
GoBio Prod 80x200 NTA[1]	1 L	55 602 042
GoBio Prod 130x200 NTA[1]	2.7 L	55 602 062
GoBio Prod 200x200 NTA[1]	6 L	55 602 072
GoBio Prod 240x200 NTA[1]	9 L	55 602 082
GoBio Prod 330x250 NTA[1]	21.4 L	55 602 093
Bulk resin		
WorkBeads NTA	25 mL 150 mL 1 L	40 602 001 40 602 003 40 602 010

[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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