WorkBeads 40 NTA
WorkBeads 40 IDA
BabyBio NTA
BabyBio IDA

WorkBeads™ 40 NTA and WorkBeads 40 IDA resins are based on nitrilotriacetic acid (NTA) and the iminodiacetic acid (IDA) 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 (NTA or IDA) and the metal ion used. These resins can also be used for divalent metal ion removal. These two different resins are available in prepacked ready-to-use BabyBio™ 1 ml and 5 ml columns.

- 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 and WorkBeads 40 IDA resins are immobilized with either nitrilotriacetic acid (NTA) or iminodiacetic acid (IDA) based chelating ligands, shown in Figure 1.

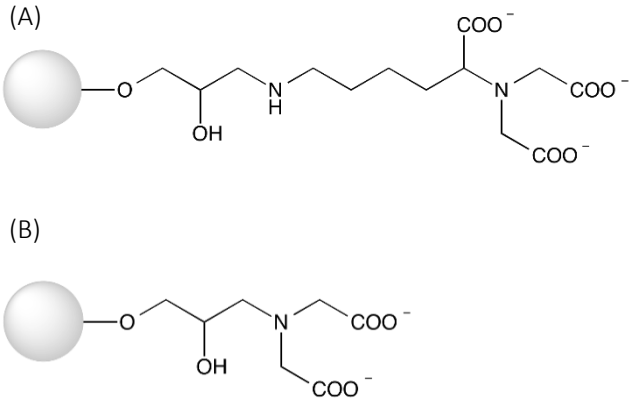

Figure 1. Structure of the chelating ligand used in WorkBeads 40 NTA (A) and WorkBeads 40 IDA (B) resins.

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 and WorkBeads 40 IDA 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 BabyBio His-tag Screening kits with all available WorkBeads IMAC resins.
The main characteristics of these resins are shown in Table 1. For more details, please see instruction, IN 40 600 010.

Column description

The column is made from biocompatible polypropylene which does not significantly interact with biomolecules. The top and bottom filters are made from polyethylene. The ready to use BabyBio columns are delivered with a plug in the inlet, a cut-off outlet and a cap for storage. The columns can be connected to a syringe, pump or chromatography system using finger tight fittings (coned 10–32) for 1/16" o.d. tubing (standard HPLC PEEK tubing).

The main characteristics of BabyBio NTA and BabyBio IDA columns are shown in Table 2. For more details, see instructions IN 45 655 010.

Table 1. Main characteristics of WorkBeads 40 NTA and WorkBeads 40 IDA resins.

	WorkBeads 40 NTA	WorkBeads 40 IDA
Target substance	His-tagged proteins, proteins containing histidine cysteine and/or tryptophan amino acid side chains	His-tagged proteins, proteins containing histidine cysteine and/or tryptophan amino acid side chains
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 - 30 µmol Cu^{2+}/ml resin	50 - 60 µmol Cu^{2+}/ml resin
Max flow rate (20 cm bed height and 5 bar)[3]	600 cm/h	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	2 - 12
Storage	2 to 25 °C	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.

Table 2. Main characteristics of BabyBio NTA and BabyBio IDA.

	BabyBio NTA	BabyBio IDA
Target substance	His-tagged proteins, proteins containing histidine, cysteine and/or tryptophan amino acid side chains	His-tagged proteins, proteins containing histidine, cysteine and/or tryptophan amino acid side chains
Resin	WorkBeads 40 NTA	WorkBeads 40 IDA
Matrix	Rigid, highly cross-linked agarose	Rigid, highly cross-linked agarose
Average particle size[1] (D_{V50})	45 µm	45 µm
Ligand	Nitrilotriacetic acid (NTA)	Iminodiacetic acid (IDA)
Column volume	1 ml 5 ml	1 ml 5 ml
Column dimension	7 x 28 mm (1 ml) 13 x 38 mm (5 ml)	7 x 28 mm (1 ml) 13 x 38 mm (5 ml)
Recommended flow rate[2]		
BabyBio 1 ml	0.25 - 1 ml/min (37 - 150 cm/h)	0.25 - 1 ml/min (37 - 150 cm/h)
BabyBio 5 ml	1.25 - 5 ml/min (56 - 225 cm/h)	1.25 - 5 ml/min (56 - 225 cm/h)
Maximum flow rate[3]		
BabyBio 1 ml	5 ml/min (780 cm/h)	5 ml/min (780 cm/h)
BabyBio 5 ml	20 ml/min (900 cm/h)	20 ml/min (900 cm/h)
Maximum back pressure	0.3 MPa, 3 bar, 43 psi	0.3 MPa, 3 bar, 43 psi
Chemical stability	Chelating substances (e.g. EDTA) will strip off the metal ions. Stripped column: 10 mM HCl (pH 2), 10 mM NaOH (pH 12), 100 mM sodium citrate-HCl (pH 3), 6 M guanidine-HCl	
pH stability	2 - 12 cleaning (stripped column) Do not keep the resin at low pH for prolonged time	2 - 12 cleaning (stripped column) Do not keep the resin at low pH for prolonged time
Storage	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. 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.
3. 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 (use half of the maximum flow rate at 4 °C), or by additives (e.g. use half of the maximum flow rate for 20% ethanol).

Applications

WorkBeads 40 NTA and WorkBeads 40 IDA 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 in order 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 BabyBio NTA 1 ml column charged with Ni-ion.

Column:	BabyBio 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)

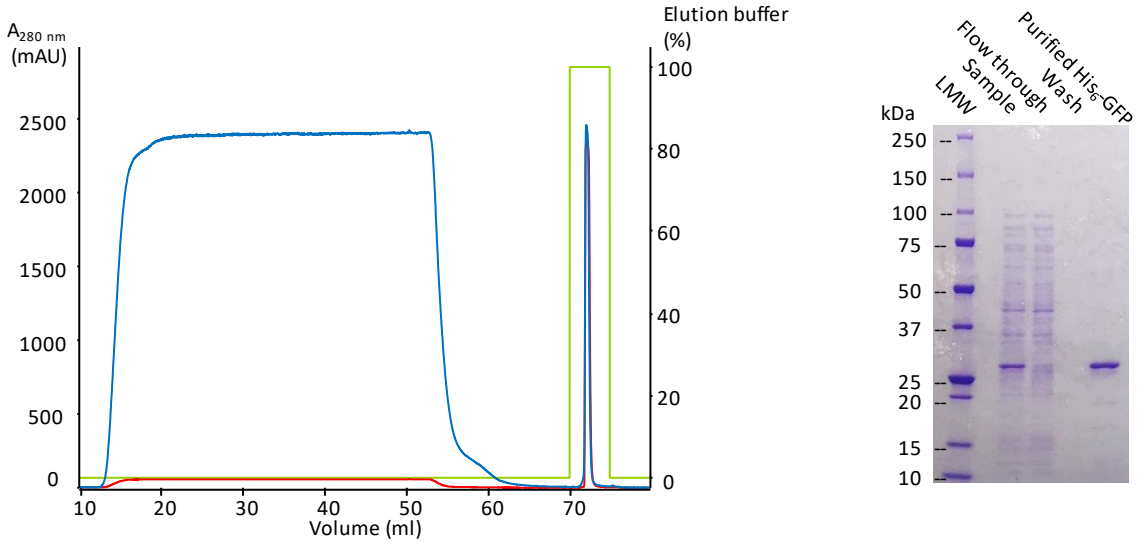

Figure 2. Purification of clarified His_6-GFP on WorkBeads 40 Ni-NTA packed into a BabyBio 1 ml column. (A) Chromatogram of the capture and elution of His_6-GFP. Absorbance at 280 nm (blue), absorbance at 490 nm (red) and percentage of elution buffer (green). (B) SDS-PAGE analysis of sample, flow through,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

Storage

Store at 2 to 25°C in 20 % ethanol.
Equilibrate BabyBio columns with 20% ethanol and close it securely using the included plug and cap.
Store the column at 2 to 25°C.

Related products

Product name	Pack size[1]	Article number
Prepacked columns		
BabyBio NTA His-tag Screening kit 1 ml[2]	1 ml x 4	45 700 101
BabyBio NTA His-tag Screening kit 5 ml[2]	5 ml x 4	45 700 102
BabyBio IDA His-tag Screening kit 1 ml[2]	1 ml x 4	45 700 001
BabyBio IDA His-tag Screening kit 5 ml[2]	5 ml x 4	45 700 002
BabyBio Dsalt 1 ml	1 ml x 5	45 360 103
BabyBio Dsalt 5 ml	5 ml x 5	45 360 107
BabyBio IEX Screening Kit[3]	1 ml x 4	45 900 001
Bulk resins		
WorkBeads 40 Ni-NTA	25 ml	40 651 001
WorkBeads 40 Co-NTA	25 ml	40 651 401
WorkBeads 40 Cu-NTA	25 ml	40 651 301
WorkBeads 40 Zn-NTA	25 ml	40 651 501
WorkBeads 40 Ni-IDA	25 ml	40 650 001
WorkBeads 40 Co-IDA	25 ml	40 650 401
WorkBeads 40 Cu-IDA	25 ml	40 650 301
WorkBeads 40 Zn-IDA	25 ml	40 650 501
WorkBeads Dsalt	300 ml	40 360 003
Accessories		
Column plug male 1/16''	10	70 100 010
Column cap female 1/16''	10	70 100 020

1. Other pack sizes can be found in the complete product list on www.bio-works.com
2. Includes one column each charged with Ni^{2+}, Co^{2+}, Cu^{2+} or Zn^{2+}
3. BabyBio IEX Screening Kit includes one of each: BabyBio S 1 ml, BabyBio Q 1 ml, BabyBio DEAE 1 ml and BabyBio TREN 1 ml.

Ordering information

Product name	Pack size	Article number
BabyBio NTA 1 ml	1 ml x 5	45 655 111
	1 ml x 2	45 655 112
	1 ml x 5	45 655 113
	1 ml x 10	45 655 114
BabyBio NTA 5 ml	5 ml x 1	45 655 115
	5 ml x 2	45 655 116
	5 ml x 5	45 655 117
	5 ml x 10	45 655 118
BabyBio IDA 1 ml	1 ml x 1	45 655 011
	1 ml x 2	45 655 012
	1 ml x 5	45 655 013
	1 ml x 10	45 655 014
BabyBio IDA 5 ml	5 ml x 1	45 655 015
	5 ml x 2	45 655 016
	5 ml x 5	45 655 017
	5 ml x 10	45 655 018
WorkBeads 40 NTA	25 ml	40 602 001
	150 ml	40 602 003
	1 L	40 602 010
WorkBeads 40 IDA	25 ml	40 601 001
	150 ml	40 601 003
	1 L	40 601 010

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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