

WorkBeads 40 NTA

WorkBeads 40 IDA

The 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 Ni²⁺, Co²⁺, Cu²⁺, Zn²⁺, Ga³⁺ or Fe³⁺. They can then be used for the 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.

- Resin charged with the metal ion of choice
- High binding capacity and purity. Outstanding 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 available with nitrilotriacetic acid (NTA) or iminodiacetic acid (IDA) based chelating ligands, shown in Figure 1.

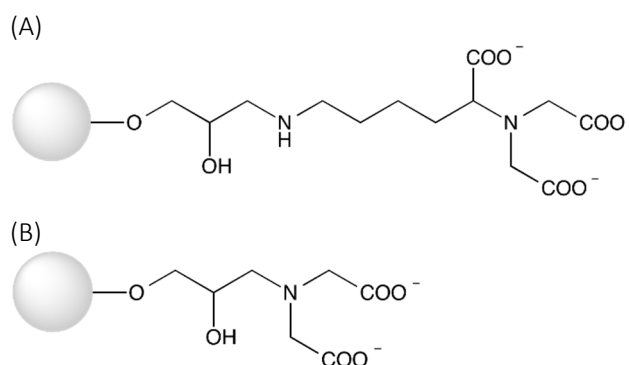


Figure 1. Structure of the chelating ligand used in WorkBeads 40 NTA (A) and WorkBeads 40 IDA (B) resins.

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 Ni²⁺, Co²⁺, Cu²⁺, Zn²⁺, Ga³⁺ and Fe³⁺.

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 more difficult

purifications, a screening is recommended with the different WorkBeads IMAC resins to identify the optimal combination of ligand and metal ion. Bio-Works offer Screening kits with our range of WorkBeads IMAC resins pre-packed in BabyBio columns.

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

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	50 - 60 µmol Cu ²⁺ /ml resin	50 - 60 µmol Cu ²⁺ /ml resin
Max flow rate (20 cm bed height and 5 bar)	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 copper solution.

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 Ni²⁺, Co²⁺, Cu²⁺ and Zn²⁺, immobilized (via a metal chelating ligand), on the chromatography resin.

IMAC is most 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 or lower pH.

Imidazole is routinely recommended for elution, it is the most commonly 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 instruction IN 40 600 010.

Purification of His-tagged proteins

Figure 2 shows an example of the purification of clarified Histidine-tagged Green Fluorescent Protein (His₆-GFP) using WorkBeads 40 Ni-NTA packed in a BabyBio 1 ml column.

Column: BabyBio Ni-NTA 1 ml
 Sample: 40 ml His₆-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)

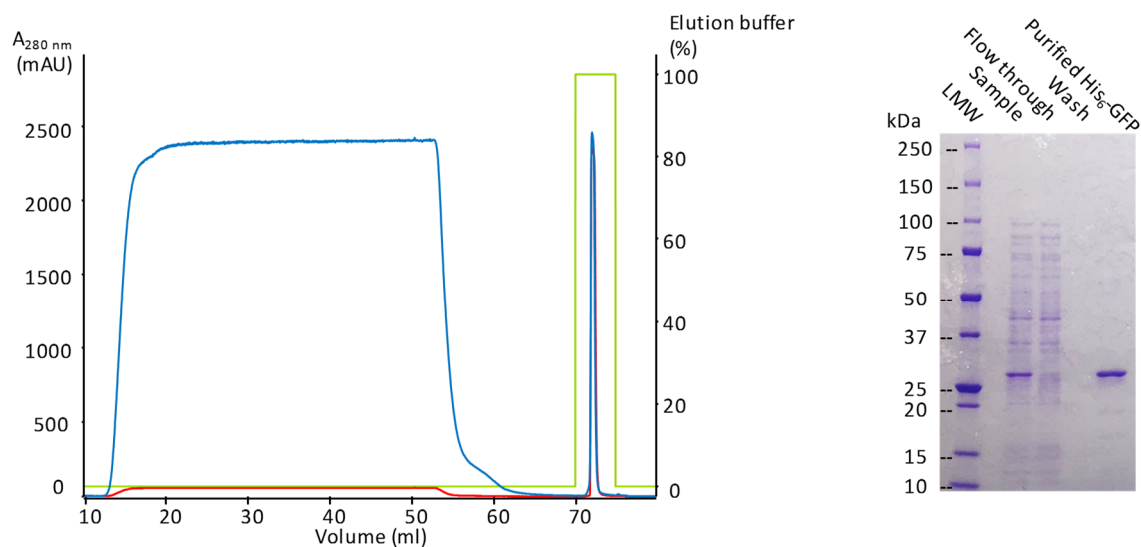


Figure 2. Purification of clarified His₆-GFP on WorkBeads 40 Ni-NTA packed into a BabyBio ml column. (A) Chromatogram of the capture and elution of His₆-GFP. Absorbance at 280 nm (blue), absorbance at 490 nm (red) and percentage of elution buffer (green). (B) SDS-PAGE analysis of sample, flow through, 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.

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 Na₂EDTA, 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.

Related products

Product name	Pack size ¹	Article number
Prepacked columns		
BabyBio IDA His-tag Screening kit 1 ml ²	1 ml x 4	45 700 001
BabyBio NTA His-tag Screening kit 1 ml ²	1 ml x 4	45 700 101
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

1. Other pack sizes can be found in the complete product list on www.bio-works.com

2. Includes one column each charged with Ni²⁺, Co²⁺, Cu²⁺ or Zn²⁺

Ordering information

Product name	Pack size	Article number
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 please visit www.bio-works.com or contact us at info@bio-works.com



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