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

These products comprise of WorkBeads™ 40 NTA and WorkBeads 40 IDA resins charged with Ni^{2+}, Co^{2+}, Cu^{2+}, or Zn^{2+} ions to be used for Immobilized Metal Ion Affinity Chromatography (IMAC). The resins are designed for purification of poly-histidine tagged (His-tagged) proteins or other metal ion binding proteins. Metal ions have different affinities for these types of proteins which results in resins with slightly different selectivities.

- Pre-charged resins with different metal ions for optimal purity of the target protein
- Pre-charged resins for ease of use
- High binding capacity and flow properties

Resin description

WorkBeads are agarose-based chromatographic resins manufactured using 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 research from laboratory to production scale, due to their exceptional compatibility with biomolecules including proteins, peptides, nucleic acids and carbohydrates. WorkBeads resins are designed for separations that requiring optimal capacity and purity.

WorkBeads 40 Ni-NTA, WorkBeads 40 Co-NTA, WorkBeads 40 Cu-NTA and WorkBeads 40 Zn-NTA are based on WorkBeads 40 NTA (see *Related products)* with a chelating ligand based on nitrilotriacetic acid (NTA). WorkBeads 40 Ni-IDA, WorkBeads 40 Co-IDA, WorkBeads 40 Cu-IDA and WorkBeads 40 Zn-IDA are based on WorkBeads 40 IDA (see *Related products*) that has a chelating ligand based on iminodiacetic acid (IDA). The pre-charged WorkBeads 40 NTA and WorkBeads 40 IDA resins are available with four metal ions: Ni^{2+}, Co^{2+}, Cu^{2+} or Zn^{2+} as denoted in their names.

The structures of the chelating ligands used in WorkBeads 40 NTA and WorkBeads 40 IDA are shown in Figure 1.

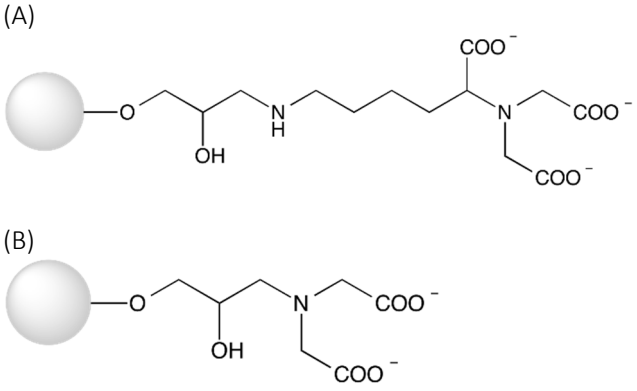

Figure 1. Structure of the chelating ligand used in WorkBeads 40 NTA (A) and WorkBeads 40 IDA (B) resins.

The main characteristics of the resins are shown in Table 1 and Table 2. For more details, please see instruction IN 40 650 010.

Table 1. Main characteristics of WorkBeads 40 Ni-NTA, WorkBeads 40 Co-NTA, WorkBeads 40 Cu-NTA and WorkBeads 40 Zn-NTA resins.

	WorkBeads 40 Ni-NTA	WorkBeads 40 Co-NTA	WorkBeads 40 Cu-NTA	WorkBeads 40 Zn-NTA
Target substance	His-tagged proteins, proteins containing histidine cysteine and/or tryptophan amino acid side chains			
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)	NTA	NTA	NTA
Metal ion	Nickel (II)	Cobalt (II)	Cupper (II)	Zink (II)
Metal ion capacity for the chelating ligand[2]	NA	NA	50 - 60 µmol Cu^{2+}/ml resin	NA
Dynamic binding capacity[3]	> 60 mg His_6-GFP/ml resin	NA	NA	NA
Max flow rate (20 cm bed height and 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 - 9 (working range) 2 - 12 cleaning (stripped resin)	7 - 9 (working range) 2 - 12 cleaning (stripped resin)	7 - 9 (working range) 2 - 12 cleaning (stripped resin)	7 - 9 (working range) 2 - 12 cleaning (stripped resin)
Storage	2 to 25 °C	2 to 25 °C	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. The binding capacity is determined using a BabyBio Ni-NTA 1 ml. The binding capacity is dependent on the size of the target protein, and on the competition of impurities.

Table 2. Main characteristics of WorkBeads 40 Ni-IDA, WorkBeads 40 Co-IDA, WorkBeads 40 Cu-IDA and WorkBeads 40 Zn-IDA resins.

	WorkBeads 40 Ni-IDA	WorkBeads 40 Co-IDA	WorkBeads 40 Cu-IDA	WorkBeads 40 Zn-IDA
Target substance	His-tagged proteins, proteins containing histidine cysteine and/or tryptophan amino acid side chains.			
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	Iminodiacetic acid (IDA)	IDA	IDA	IDA
Metal ion	Nickel (II)	Cobalt (II)	Cupper (II)	Zink (II)
Metal ion capacity for the chelating ligand[2]	NA	NA	50 - 60 µmol Cu^{2+}/ml resin	NA
Dynamic binding capacity[3]	> 60 mg His_6-GFP/ml resin	NA	NA	NA
Max flow rate (20 cm Bed height and 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 - 9 (working range) 2 - 12 cleaning (stripped resin)	7 - 9 (working range) 2 - 12 cleaning (stripped resin)	7 - 9 (working range) 2 - 12 cleaning (stripped resin)	7 - 9 (working range) 2 - 12 cleaning (stripped resin)
Storage	2 to 25 °C	2 to 25 °C	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. The binding capacity is determined using a BabyBio Ni-NTA 1 ml, equal value is expected for IDA resins.

Applications

Metal ion charged WorkBeads 40 NTA and WorkBeads 40 IDA resins are designed to be used in 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 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. 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 by stepwise or gradient elution, using a competing agent or lower pH.

Imidazole is recommended for elution. This is the most commonly used competing agent but histidine, ammonium chloride or histamine can also be used. Before sample application the resin should be equilibrated with a low concentration of the competing agent in order to prevent non-specific binding of endogenous proteins that may bind via histidine clusters for example. This is done easily by using the recommended binding buffer.

Elution with a decrease of pH is also an option. At pH 3 - 5, the histidine residues (pK_a approx. 6) are protonated which leads to the loss of affinity for the metal ion and thus to the release of the protein. However, it is important to consider the target protein stability at low pH.

For more detailed description of the IMAC principle, see instruction IN 40 650 010.

Purification of His-tagged proteins

Figure 2 shows an example of purification of clarified histidine-tagged Green Fluorescent Protein (His_6-GFP) expressed in *E. coli* using WorkBeads 40 Ni-NTA packed into a BabyBio 1 ml column.

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 columns volumes (CV)
Flow rate:	0.5 ml/min (78 cm/h)

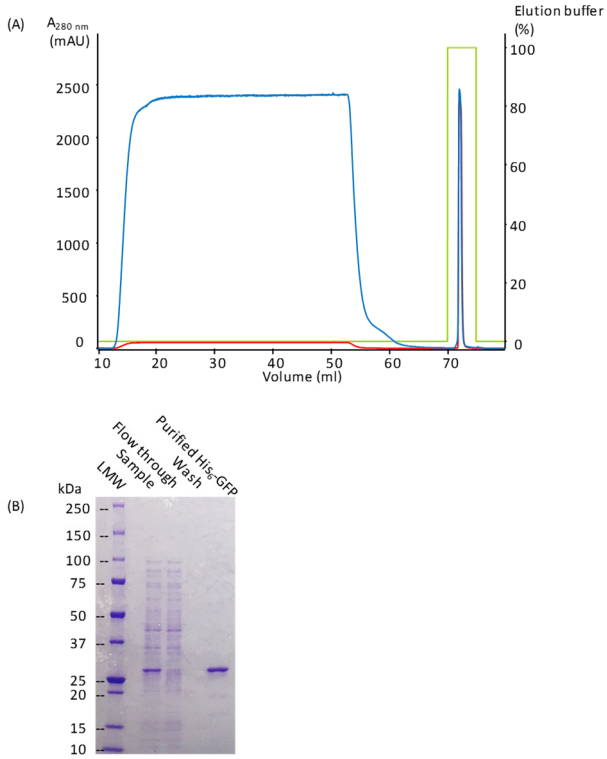

Figure 2. Purification of clarified His_6-GFP using BabyBio Ni-NTA 1 ml. (A) Chromatogram showing the purification of His_6-GFP. Absorbance at 280 nm (blue), absorbance at 490 nm (red) and percentage of elution buffer (green). (B) SDS-PAGE analysis of sample, flow through, wash and eluted peak.

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 with 1 M NaOH applied by a low reversed flow for 2 hours or overnight is often sufficient. Before 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.

Sanitization (reduction of microorganisms) is done by 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 microorganisms to be removed, and needs to be evaluated for each case.

Storage

Store at 2 to 25°C in 20 % ethanol.

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 IDA His-tag Screening kit 1 ml[2]	1 ml x 4	45 700 001
Bulk resins		
WorkBeads 40 NTA	25 ml	40 602 001
WorkBeads 40 IDA	25 ml	40 601 001

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+}

Ordering information

Product name	Pack size	Article number
WorkBeads 40 Ni-NTA	25 ml	40 651 001
	150 ml	40 651 003
	1 L	40 651 010
WorkBeads 40 Co-NTA	25 ml	40 651 401
	150 ml	40 651 403
	1 L	40 651 410
WorkBeads 40 Cu-NTA	25 ml	40 651 301
	150 ml	40 651 303
	1 L	40 651 310
WorkBeads 40 Zn-NTA	25 ml	40 651 501
	150 ml	40 651 503
	1 L	40 651 510
WorkBeads 40 Ni-IDA	25 ml	40 650 001
	150 ml	40 650 003
	1 L	40 650 010
WorkBeads 40 Co-IDA	25 ml	40 650 401
	150 ml	40 650 403
	1 L	40 650 410
WorkBeads 40 Cu-IDA	25 ml	40 650 301
	150 ml	40 650 303
	1 L	40 650 310
WorkBeads 40 Zn-IDA	25 ml	40 650 501
	150 ml	40 650 503
	1 L	40 650 510

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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