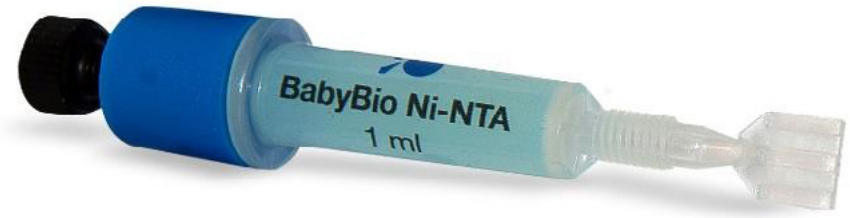
interchim®

FT-WorkBeads IMAC

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

Bio-Works



These products comprise of WorkBeads™40 NTA and WorkBeads 40 IDA resins charged with $Ni2^+$, $Co2^+$, $Cu2^+$, or $Zn2^+$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 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 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: $Ni2^+$, $Co2^+$, $Cu2^+$ or $Zn2^+$ as denoted in their names.

Table 1. Main characteristics of WorkBeads 40 Ni-NTA, WorkBeads40Co-NTA, WorkBeads 40 Cu-NTA and WorkBeads40Zn-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			
Average particle size[1](DV50)	45µm			
Chelating ligand	Nitrilotriacetic acid (NTA)			
Metal ion	Nickel(II)	Cobalt(II)	Cupper(II)	Zink(II)
Metal ion capacity for the chelating ligand[2]	NA	NA	50-60µmol $Cu2^+$/mL resin	NA
Dynamic binding capacity[3]	> 60mg His6-GFP/mL resin	NA	NA	NA
Max flow rate (20cm bed height and 5bar)	600cm/h	600cm/h	600cm/h	600cm/h
Chemical stability	Compatible with all standard aqueous buffers used for protein purification, 8M urea, 6M guanidine-HCl, non-ionic detergents, 20% ethanol. Chelating substances (e.g. EDTA) will strip off the metal ions. Stripped resin:10mM HCl (pH 2), 10mM NaOH (pH 12),10mM sodium citrate-HCl (pH 3)			
pH stability	7-9 (working range) 2-12 cleaning (stripped resin)			
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. 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 NIDA 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			
Average particle size[1](DV50)	45µm			
Chelating ligand	IminoDiacetic Acid (IDA)			
Metal ion	Nickel(II)	Cobalt(II)	Cupper(II)	Zink(II)
Metal ion capacity for the chelating ligand[2]	NA	NA	50-60µmol $Cu2^{+}$/mL resin	NA
Dynamic binding capacity[3]	> 60mg His6-GFP/mL resin	NA	NA	NA
Max flow rate (20cm bed height and 5bar)	600cm/h	600cm/h	600cm/h	600cm/h
Chemical stability	Compatible with all standard aqueous buffers used for protein purification, 8M urea, 6M guanidine-HCl, non-ionic detergents, 20% ethanol. Chelating substances (e.g. EDTA) will strip off the metal ions. Stripped resin:10mM HCl (pH 2), 10mM NaOH (pH 12),10mM sodium citrate-HCl (pH 3)			
pH stability	7-9 (working range) 2-12 cleaning (stripped resin)			
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. The binding capacity is determined using a BabyBio Ni-IDA 1 ml. The binding capacity is dependent on the size of the target protein, and on the competition of impurities.

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 Ni2+, Co2+, Cu2+and Zn2+, immobilized via a metal chelating ligand on the chromatography resin. IMAC is commonly used for purification of recombinant His-tagged proteins. A 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 (pKaapprox.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.

Applications

Columns are excellent for swifter purification of His-tagged proteins.

1. Installation of the column
2. Removal of storage solution
3. Equilibrate the column using 10 column volumes (CV) of 50mM Na-phosphate buffer, 300mM NaCl, 10mM imidazole, pH 8.0 (Binding buffer).
4. Apply a clarified sample under neutral conditions (pH 7.5-9.0). The sample should contained 10 mM imidazole.
5. Wash using 10-20 CV 50 mM Na-phosphate buffer, 300 mM NaCl, 20 mM imidazole, pH 8.0 (Washing buffer).
6. Elute with 5 CV 50 mM Na-phosphate buffer, 300 mM NaCl, 500 mM imidazole, pH 8.0 (Elution buffer).
7. Wash with 5 CV water to remove the elution buffer.
8. Equilibrate with 10 CV 20% ethanol for storage. Close the column using the included caps.

Cleaning

Samples containing small amounts of impurities tend to adsorb to the column by unspecific interactions. Collecting such material may reduce the performance over time. It is therefore important to clean column regularly. This can should be done by stripping off the Ni_{2+} with EDTA and washing with 100mM NaOH, and recharging with fresh Ni_{2+} ions.

Instruction of use

Short protocol

1. Equilibrate the column using 10 column volumes (CV) of 50 mM Na-phosphate buffer, 300 mM NaCl, 10 mM imidazole, pH 8.0 (Binding buffer).
2. Apply a clarified sample under neutral conditions (pH 7.5-9.0). The sample should contain 10 mM imidazole.
3. Wash using 10-20 CV 50 mM Na-phosphate buffer, 300 mM NaCl, 20 mM imidazole, pH 8.0 (Washing buffer).
4. Elute with 5 CV 50 mM Na-phosphate buffer, 300 mM NaCl, 500 mM imidazole, pH 8.0 (Elution buffer).
5. Wash with 5 CV water to remove the elution buffer.
6. Equilibrate with 10 CV 20% ethanol for storage. Close the column using the included caps.

Optimization may be needed for optimal purification results.

Ordering Information

Product name	Pack size	Article number
WorkBeads 40 Ni-NTA	25mL	40651001
	150mL	40651003
	1L	40651010
WorkBeads 40 Co-NTA 25mL	25mL	40651401
	150mL	40651403
	1L	40651410
WorkBeads 40 Cu-NTA	25mL	40651301
	150mL	40651303
	1L	40651310
WorkBeads 40 Zn-NTA	25mL	40651501
	150mL	40651503
	1L	40651510
WorkBeads 40 Ni-IDA	25mL	40651001
	150mL	40651003
	1L	40651010
WorkBeads 40 Co-IDA	25mL	40650401
	150mL	40650403
	1L	40650410
WorkBeads 40 Cu-IDA	25mL	40650301
	150mL	40650303
	1L	40650310
WorkBeads 40 Zn-IDA	25mL	40650501
	150mL	40650503
	1L	40650510