BabyBio NTA His-tag Screening kit
BabyBio IDA His-tag Screening kit

BabyBio™ His-tag Screening kits contain columns prepacked with WorkBeads™ IDA and WorkBeads NTA charged with Ni^{2+}, Co^{2+}, Cu^{2+} or Zn^{2+} ions. The kits are excellent tools for screening combinations of metal ions and chelating ligand (NTA or IDA) to optimize purity and yield when purifying polyhistidine-tagged (His-tagged) proteins. Other native proteins containing histidine, cysteine and tryptophan residues may also bind and can therefore be purified using these columns. The selected column can be used to purify up to 70 mg and 350 mg protein respectively using a 1 ml or 5 ml column.

- Pre-charged columns with different metal ions for easy screening for optimal purity
- Ready-to-use columns for fast results
- High binding capacity and purity

Resin description

BabyBio columns provided in the screening kits are prepacked with resins based on cross-linked agarose. The columns are excellent for research scale purification and selectivity screening in process development.

The resins packed in BabyBio His-tag Screening kit columns are WorkBeads 40 NTA or WorkBeads 40 IDA which contain immobilized chelating ligands based on nitrilotriacetic acid (NTA) or iminodiacetic acid (IDA) respectively. The resins are pre-charged with either Ni^{2+}, Co^{2+}, Cu^{2+} or Zn^{2+} metal ions.

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

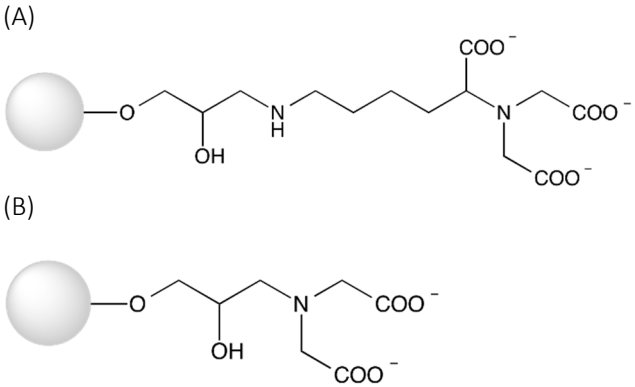

Figure 1. Structure of the chelating ligands used in WorkBeads 40 NTA (A) and WorkBeads 40 IDA (B) resins.

The main characteristics of BabyBio His-tag Screening kit columns are shown in Table 1. For more details, please see instructions IN 45 700 010.

Table 1. Main characteristics of BabyBio His-tag Screening kit 1 ml and 5 ml columns.

	BabyBio: Ni-NTA, Co-NTA, Cu-NTA, Zn-NTA	**BabyBio: Ni-IDA. Co-IDA, Cu-IDA, Zn-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 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
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)
Metal ion	Ni^{2+}, Co^{2+}, Cu^{2+} or Zn^{2+}	Ni^{2+}, Co^{2+}, Cu^{2+} or Zn^{2+}
Static binding capacity[2]	70 mg His-tagged protein/ml resin	NA
Dynamic binding capacity[2]	50 mg His-tagged protein/ml resin	NA
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		
BabyBio 1 ml	1 ml/min (150 cm/h)	1 ml/min (150 cm/h)
BabyBio 5 ml	5 ml/min (225 cm/h)	5 ml/min (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	7 - 9 (working range) 2 - 12 cleaning (stripped resin) Do not keep the resin at low pH for prolonged time.	7 - 9 (working range) 2 - 12 cleaning (stripped resin) 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. 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 with other substances.
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).

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).

Applications

BabyBio IMAC columns supplied in the screening kits can easily be used for fast purifications of His-tagged proteins or native proteins containing histidine, cysteine or tryptophan residues. BabyBio Ni-NTA 1 ml and 5 ml columns can be used to purify up to 70 mg or 350 mg of proteins, respectively. Similar capacities can be expected for the other BabyBio IMAC columns. The purity obtained depends on several factors. A sample including impurities that can bind to the resin may reduce the purity of the target protein. Proteins expressed in *E. coli* are usually easier to purify than proteins expressed in eukaryotic systems (e.g., yeast or mammalian cells). The purification result also depends on the structure of the chelating ligand and the nature of the immobilized metal ion. The large selection of BabyBio IMAC columns offers many possibilities, choosing between two different ligands, NTA or IDA, charged with either Ni^{2+}, Co^{2+}, Cu^{2+} or Zn^{2+} metal ions. BabyBio Ni-NTA is recommended as the starting point for His-tagged protein purification as it in many cases will give excellent purification results. For more difficult purifications, a screening is often recommended with the different BabyBio IMAC columns to find the optimal combination of ligand and metal ion. For very high purity requirements, it is common to add a second purification step to remove the final impurities and for buffer exchange and salt removal. This can be done by using size exclusion chromatography (SEC/gel filtration), such as WorkBeads SEC resins.

Principle

IMAC utilizes the affinity of histidine, cysteine and tryptophan amino acid side chains on the protein surface for binding to 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 column 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 for example histidine clusters. This is easily done by using the recommended binding buffer.

For more detailed description of the IMAC principle, see instructions IN 45 700 010.

Purification of His-tagged proteins

An example of purification of a recombinant His_6-tagged Green Fluorescent Protein (His_6-GFP) expressed in *E.coli* on eight different pre-charged BabyBio IMAC columns is shown in Figure 2. The purity was checked by SDS-PAGE, shown in Figure 3.

Sample:	2 ml His_6-GFP in binding buffer
Columns:	Pre-charged BabyBio IMAC columns 1 ml
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
Gradient:	0 to 100% elution buffer in 15 column volumes (CV)
Flow rate:	0.5 ml/min (78 cm/h)

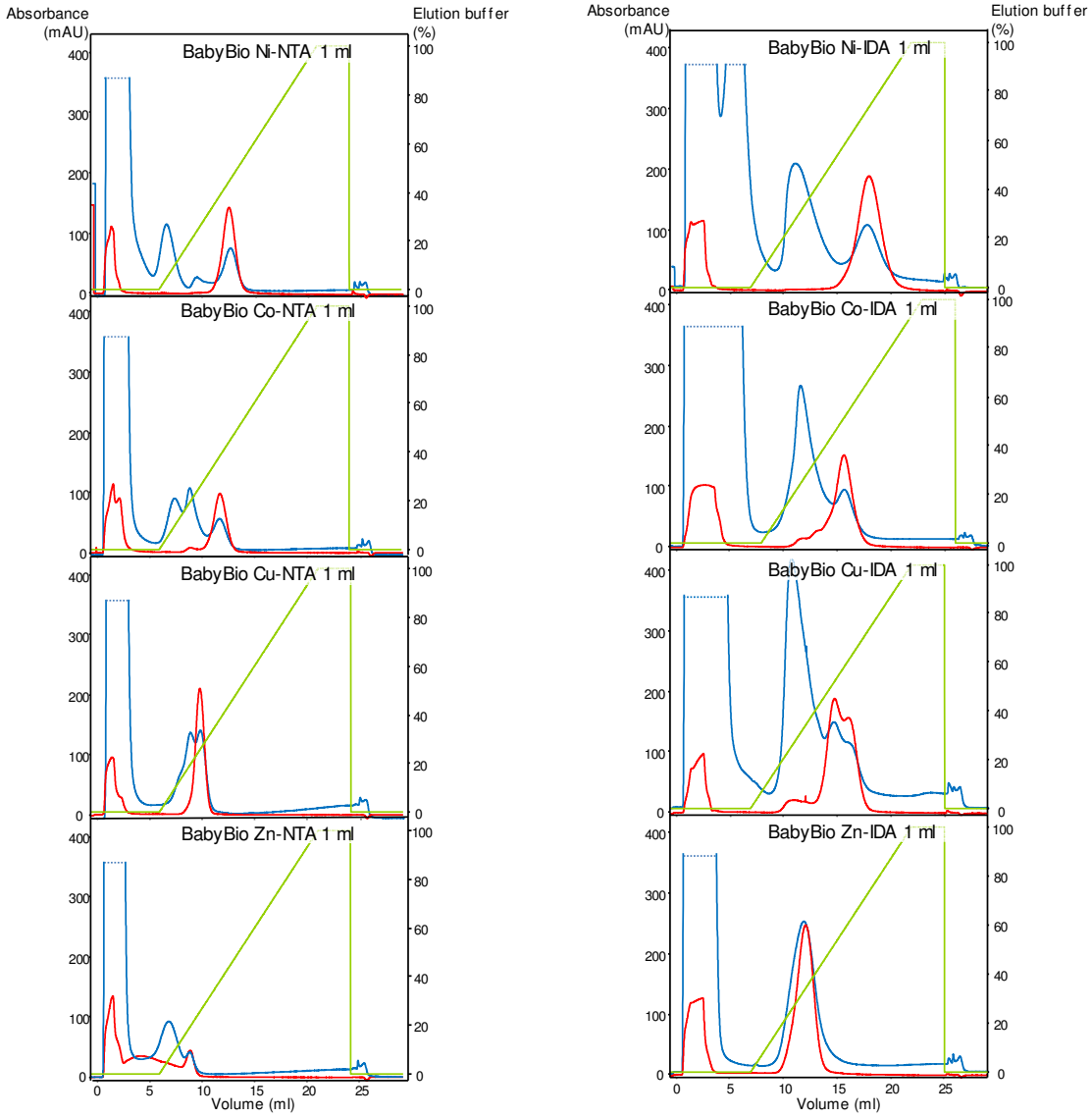

Figure 2. Chromatogram showing comparisons of purifications of clarified His_6-GFP on BabyBio NTA 1 ml and BabyBio IDA 1 ml charged with Ni^{2+}, Co^{2+}, Cu^{2+} and Zn^{2+} ions. The blue and red lines correspond to the absorbance signal at 280 nm and 490 nm, respectively, and the green line to the percentage of elution buffer.

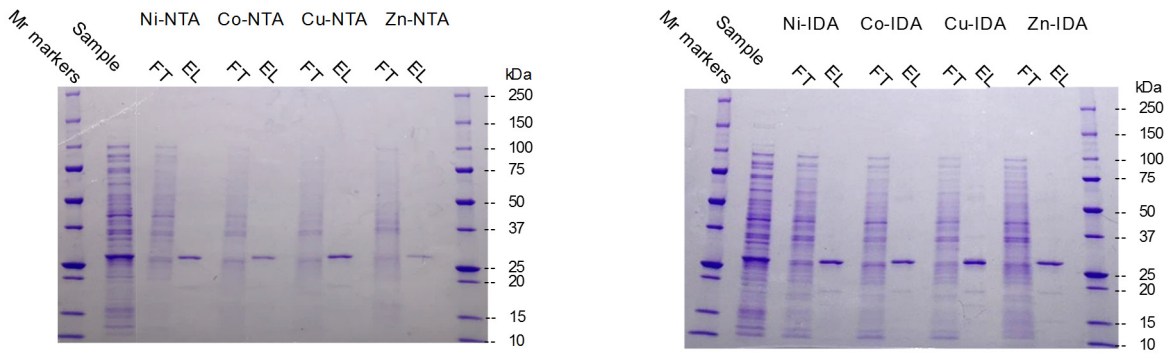

Figure 3. SDS-PAGE analysis of purified His_6-GFP from the previous chromatograms. FT = flow through and EL = eluted protein.

Scale-up

Scale-up can conveniently be carried out from a 1 ml column to a 5 ml column. If increased capacity is required several columns can be coupled in series (column stacking). Note that the backpressure will increase proportionally to the resin bed height (up to a maximum of 5 columns).

Further scale-up can be done by packing bulk WorkBeads IMAC resins in larger columns (see *Related products*). For more detailed description, please see instructions IN 45 700 010.

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 bound impurities may reduce the performance of the packed 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 using 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) 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 microorganisms to be removed, and needs to be evaluated for each case.

Equipment

Prepacked BabyBio His-tag Screening kit columns can be used with most standard liquid chromatography equipment. Purification can also be carried out using a syringe connected to the column by a luer or a std HPLC connector.

Storage

Equilibrate the columns in 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 Dsalt 5 ml	5 ml x 5	45 360 107
BabyBio Ni-NTA 1 ml	1 ml x 5	45 655 103
BabyBio Co-NTA 1 ml	1 ml x 5	45 655 133
BabyBio Cu-NTA 1 ml	1 ml x 5	45 655 123
BabyBio Zn-NTA 1 ml	1 ml x 5	45 655 143
BabyBio Ni-IDA 1 ml	1 ml x 5	45 655 003
BabyBio Co-IDA 1 ml	1 ml x 5	45 655 033
BabyBio Cu-IDA 1 ml	1 ml x 5	45 655 023
BabyBio Zn-IDA 1 ml	1 ml x 5	45 655 043
BabyBio S 5 ml	5 ml x 5	45 200 107
BabyBio Q 5 ml	5 ml x 5	45 100 107
BabyBio DEAE 5 ml	5 ml x 5	45 150 107
OptioBio 40S 10x100	7.9 ml x 1	55 420 011
OptioBio 40Q 10x100	7.9 ml x 1	55 410 011
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	40 651 504
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
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

Ordering information

Product name	Pack size	Article number
BabyBio NTA His-tag Screening kit 1 ml[1]	1 ml x 4	45 700 101
BabyBio NTA His-tag Screening kit 5 ml[1]	5 ml x 4	45 700 102
BabyBio IDA His-tag Screening kit 1 ml[1]	1 ml x 4	45 700 001
BabyBio IDA His-tag Screening kit 5 ml[1]	5 ml x 4	45 700 002

1. Includes one column each charged with Ni^{2+}, Co^{2+}, Cu^{2+} or Zn^{2+}

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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