BabyBio Ni-NTA™

BabyBio Ni-NTA ready to use mini column available in 1 ml and 5 ml that for quick, easy and convenient affinity purification of proteins carrying a polyhistidine tag (His).

- **Swifter purification of His-tagged proteins**
- **Higher binding capacity and purity in one step**
- **Simple and easy method giving reproducible results**

Media description

BabyBio Ni-NTA consists of Cube Biotech PureCube Ni-NTA Agarose developed for affinity purification of proteins carrying a His-tag. The base matrix is Workbeads, which is highly porous to allow for optimal protein interaction. Cross-linked agarose is physically strong and suitable for purification processes at high flow rates.

An NTA ligand is coupled to the agarose matrix and charged with nickel ions to obtain an affinity matrix with high binding capacity. The metal ion capacity is > 15 µeqv Ni^{2+}/mL. Possible metal ions are Co^{2+}, Zn^{2+}, Fe^{3+}, and Al^{3+}, resulting in different affinities, e.g. for zincfinger proteins or phosphorylated Cross-linked agarose is physically strong and suitable for purification processes at high flow rates. If required, the nickel ions could be removed from the bead using 5 column volumes 100 mM EDTA and 10 column volumes water. The medium can then be recharged with a different metal ion.

Column description

The BabyBio column body is made from biocompatible polypropylene, which does not significantly interact with biomolecules. The top and bottom filters are made from polyethylene. These ready to use columns are delivered with plugs in the inlet and a snap-off end at the outlet. A cap for the outlet is included for closing the column during storage. The columns can be connected a syringe, pump or chromatography system using fingertight fittings (coned 10-32) for 1/16” o.d. tubing

Bio-Works

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 50 mM Na-phosphate buffer, 300 mM NaCl, 10 mM imidazole, pH 8.0 (Binding buffer).
4. Apply a clarified sample under neutral conditions (pH 7.5-9.0). The sample should contain 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 100 mM NaOH, and recharging with fresh Ni^{2+} ions

Scale-up

Scale-up can conveniently be performed from a 1 ml column to a 5 ml column. Or by coupling the columns in series (note that the back pressure will increase).

Equipment

BabyBio Ni-NTA can generally be used together with most equipment available for chromatograpy.

Column characteristics

	BabyBio Ni-NTA
Target substance	His-tagged proteins
Medium	WorkBeads Ni-NTA
Ligand	Nitrilotriacetic acid (NTA) charged with Nickel ions
Static binding capacity[1]	70 mg His-tagged protein/ml medium
Dynamic binding capacity[1]	50 mg His-tagged protein/ml medium
Column volumes	1 ml 5 ml
Column dimensions	7 x 28 mm (1ml) 13 x 38 mm (5ml)
Recommended flow rate	1 ml/min (BabyBio Ni-NTA 1 ml) 5 ml/min (BabyBio Ni-NTA 5 ml)
Max flow rate[1]	5 ml/min (BabyBio Ni-NTA 1 ml) 20 ml/min (BabyBio Ni-NTA 5 ml)
Maximum back pressure	0.3 MPa, 3 bar, 43 psi
Chemical stability	Compatible with all standard aqueous buffers used for protein purification. 20% ethanol. Chelating substances (e.g, EDTA will strip off the Ni^{2+} ions) Stripped column: 10 mM HCl (pH 2), 10 mM NaOH (pH 12), 0.1 M sodium citrate-HCl (pH 3), 6 M guanidine-HCl. Should not be stored at low pH for prolonged time.
Recommended working range pH Stability	7-9 short term 2-12 cleaning (stripped column)
Storage	+2°C to +25°C in 20% ethanol

[1] Determined by purification of 6xHis-tagged GFP protein from *E. coli* cleared lysates, and quantified via spectrophotometry. The binding capacity depend on the size of the target protein, and on the competition from impurities.

[2] Aqueous buffers at 20°C. Decrease the max flow if the liquid has a higher viscosity. Higher viscosities can be caused by low temperature (use max flow/2 at 4°C), or by additives (e.g, use max flow/2 for 20% ethanol).

Ordering information

Product name	Pack size	Article number
BabyBio Ni-NTA 1 ml	1 × 1 ml	45 655 101
	2 × 1 ml	45 655 102
	5 × 1 ml	45 655 103
	10 × 1 ml	45 655 104
BabyBio Ni-NTA 5 ml	1 × 5 ml	45 655 105
	2 × 5 ml	45 655 106
	5 × 5 ml	45 655 107
	10 × 5 ml	45 655 108
	100 × 5 ml	45 655 109
	100 × 1 ml	45 655 110

Order direct on info@bio-works.net or through your local distributor.

Bio-Works

Bio-Works Technologies AB
Virdings allé 18
754 50 Uppsala
Sweden

www.bio-works.net
info@bio-works.net