BabyBio Ni-NTA™

BabyBio Ni-NTA 1 ml and 5 ml ready-to-use columns for purification of His-tagged proteins in lab-scale, and for process development.

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
	100 × 1 ml	45 655 110
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

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.

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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. See later in this instruction for more details.

Instructions

Purification can usually be performed at room temperature, but can be done at 4°C at reduced flow rate if needed. Prepare the sample according to step 4 before starting.

1. Connection of the column
   Connect the column to a chromatography system using finger tight connectors (coned 10-32) for 1/16" o.d. tubing. Fill the system with water and make a drop-to-drop connection with the column to avoid air getting into the column. Perform all steps at 1 ml/min (BabyBio Ni-NTA 1 ml) or 5 ml/min (BabyBio Ni-NTA 5 ml).
2. Removal of storage solution
   When the column is delivered it contains a storage solution of 20% ethanol. This solution should be washed out before use. Wash the column with 3 column volumes (CV) of water. Avoid higher flow rate before the storage solution has been removed to avoid overpressure due to the high viscosity of the 20% ethanol solution.
3. Equilibration
   Equilibrate the column with 10 CV Binding buffer (50 mM Na-phosphate, 300 mM NaCl, 10 mM imidazole, pH 8.0[a]). Other neutral buffers, with at least 10 mM imidazole, can also be used.
4. Sample application
   Clarify the sample by filtration using centrifugation at 10,000-20,000 × g for 15-30 minutes. It is recommended to also pass the sample through a 0.22-0.45 µm filter to remove any remaining particles. If the sample contains only small amounts of particles it may be enough to only perform filtration. Sample should have a pH between 5 and 8. Apply the sample at 0.5-1 ml/min (BabyBio Ni-NTA 1 ml) or 2-4 ml/min (BabyBio Ni-NTA 5 ml).

5. Wash
   Remove unbound impurities by washing the column with 10-20 CV of Washing buffer (50 mM Na-phosphate buffer, 300 mM NaCl, 20 mM imidazole, pH 8.0) or until desired A_{280} absorbance of the wash fractions (e.g., 0.01-0.02).
6. Elution
   Desorb the target protein with 5 CV Elution buffer (5 CV 50 mM Na-phosphate buffer, 300 mM NaCl, 500 mM imidazole, pH 8.0).
7. Removal of the elution buffer
   Wash with 5 CV deionised water to remove the salts of the elution buffer.
8. Equilibrate with 10 CV 20% ethanol for storage. Close the column using the included caps.

[a] To avoid bacterial growth and poor column performance, use only freshly prepared and filtered buffers.

Optimization

Selection of column

BabyBio Ni-NTA 1 ml can be used for purification of up to 50 mg proteins. Although, the medium has a binding capacity (static) of up to 70 mg/ml, the max binding capacity for BabyBio Ni-NTA columns are lower depedning on the column dimensions and the typical flow rate used for purification. Scale-up can be done by using larger column, the BabyBio Ni-NTA 5 ml. Scale-up can also be done by combining up to 5 columns in series. This will increase the capacity accordingly. The BabyBio columns can easily be connected together without accessories. With several columns connected in series the upper columns will be exposed to higher internal pressure. It may be necessary to decrease the flow rate to avoid passing the maximum hardware pressure over the top column. The pressure across each column bed will be the same for all columns.

By connection of columns in series any column volume from 1 ml up to 25 ml can be obtained in this way, corresponding to purification capacity of up to at least 1000 mg His-tagged protein. For larger scale packing a bigger column from bulk media is recommended. Column size should be selected based on estimated amount of target protein in each run. A test purification with a defined small volume of the sample on a BabyBio Ni-NTA 1 ml column can be used to estimate the concentration of the target in the sample.

Optimization of binding

Binding of His-tagged proteins to BabyBio Ni-NTA is favoured under the folowing conditions.

- Neutral pH.
- Preferably pH 7.5-8.5. A lower pH protonates the histidine residues of the tag, causing elution.
- Low imidazole concentration.
- The sample and Binding buffer should contain a low concentration of imidazole (not below 10 mM) to avoid pH effects that may interfere with protein binding.
- Low flow rate.
- Binding of His-tagged proteins to BabyBio Ni-NTA is a rather fast mechanism, and high flow rate will usually not effect the yield when moderate loadings are done. It may be useful to lower the flow rate under some circumstances (for some proteins or sample compositions, or at low temperature).

Optimization of washing and elution

The purity of the target protein can be optimised by selecting suitable imidazole concentration in the different steps. A recommended optimization is to apply an extra purification step (see Polishing below) based on another chromatographic technique such as size-exclusion or ion-exchange chromatography.

- The binding conditions can be optimized to reduce the binding of protein impurities from the sample by increasing the imidazole concentration above 10 mM. (Too high imidazole concentration and the His-tagged protein will not bind). Instead it is often easier to include a wash step after binding.
- Washing is performed using a slightly elevated imidazole concentration. (Too high will cause elution of the His-tagged protein together with the impurities). Note that the affinity of His-tagged proteins for the column may be different, sometimes allowing extra stringent washing conditions.
- Elution can be performed using a high imidazole concentration (500 mM or more). There are very few impurities binding stronger than a His-tagged protein. Aggregates of the His-tagged protein may bind via multiple tags thus increasing the affinity. Optimization of the imidazole concentration may allow elution of the His-tagged protein without the aggregates.
- Elution using a gradient of imidazole is a useful alternative to optimized step elution purifications. It can also be used to find suitable conditions for binding, washing and elution. A gradient test run can be performed before purification of the sample.
- Other parameters such as ionic strength, pH and additives might be needed to optimize. However often not needed.

Additional purification (Polishing)

His-tagged protein purification on BabyBio Ni-NTA give high purity in a single step. For very high requirement on purity it may be necessary to add a second purification step. This polishing step is used to remove remaining impurities from the sample. In fact, an added polishing step may allow omission of optimization of the first purification step. The polishing purification step can be based on several chromatographic techniques:

Size-exclusion chromatography (gel filtration)

This technique is based on separation of substances according to size. Large substances are eluted before small. Dimers or aggregates of the target protein and impurities with different sizes can be removed. Buffer exchange can be done by equilibrating the column with desired buffer before applying the sample. This technique is simple to set up and is recommended for high purity demands in lab scale purification. Optimization is often not required for significant purification, but may sometimes be worthwhile. The technique is often less useful for bioprocess scale applications due to dilution effects and low capacity.

Ion exchange chromatography

This technique is based on separation of substances according to charge. The ion strength of the sample must be low enough to allow binding. The pH value affect the charge of proteins and may have to be optimized to allow binding of the target protein, and to allow separation from impurities. It is often required to perform buffer exchange of the sample. This can be done using BabyBio Dsalt 1 ml or 5 ml columns, by dialysis or other techniques.

Maintenance of the column

Storage

Between use wash the column using 20% ethanol, and close it using lids at the inlet and outlet. Store the columns at +2 to +25°C.

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 common to make regular cleaning of the column. This must be done by stripping off the Ni^{2+} ions, cleaning, and recharging with fresh Ni^{2+} ions.

1. Wash with 5 CV water
2. 10 CV 50 mM Na_2-EDTA, 500 mM NaCl, pH 8
3. 10 CV 100 mM NaOH
4. 10 CV water
5. 2 CV 50 mM $NiSO_4$ (Nickel solutions are poisonous, use gloves)
6. 10 CV water
7. 10 CV 20% ethanol (if being stored)

Intended use

BabyBio Ni-NTA is intended for research and for process development. BabyBio Ni-NTA shall not be used for preparation of material for clinical or diagnostic purposes.

Safety

Please read the MSDS for BabyBio, and the safety instructions for any equipment to be used. Note that maximum back pressure BabyBio Ni-NTA is 0.3 MPa, 3 bar, 43 psi.

Media Description

Target substance	His-tagged proteins
Medium	WorkBeads Ni-NTA
Matrix	Rigid, highly cross-linked agarose
Average particle size	45 µm
Ligand	Nitrilotriacetic acid (NTA)
Coupling chemistry	Bromohydrin
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[2]	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] 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).

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