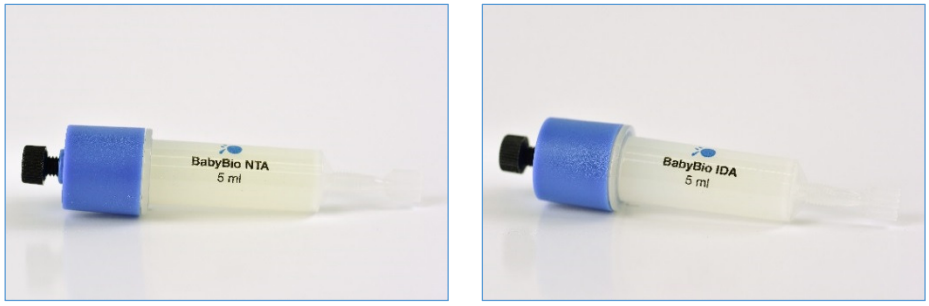
BabyBio NTA
BabyBio IDA

The ready-to-use BabyBio™ NTA and BabyBio IDA columns are prepacked with WorkBeads™ 40 NTA and WorkBeads 40 IDA resins, respectively, and are available in two column sizes, 1 ml and 5 ml. These columns can be charged with several divalent or trivalent transition metal ions (e.g., Ni^{2+}, Co^{2+}, Cu^{2+}, Zn^{2+}, Fe^{3+} or Ga^{3+}) to obtain Immobilized Metal Ion Affinity Chromatography (IMAC) columns with different selectivity for polyhistidine-tagged (His-tagged) proteins or other metal ion binding proteins. Columns charged with Fe^{3+} and Ga^{3+} ions can be used for purification of phosphorylated peptides and proteins. Metal ion charged BabyBio columns can be used to purify up to 70 mg and 350 mg protein using respectively a 1 ml or 5 ml column. The columns can also be used in their uncharged state for removal of the above mentioned metal ions from solution.

- Prepacked ready-to-use columns fast and reliable
- Prepared columns ready to be charged with the metal ion of choice
- High binding capacity and purity



Short protocol

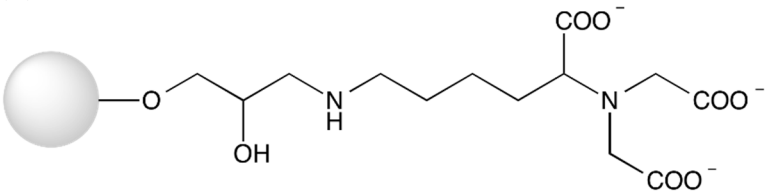
This short protocol includes both charging the resin with metal ions and preforming a protein purification using IMAC. Recommended buffers are listed in Table 3 and recommended metal salts for charging are listed in Table 2. Detailed instructions and recommendations for optimization are provided later in this instruction.

1. Connect the column to the chromatography system, syringe or pump.
2. Wash the column with 5 column volumes (CV) deionized water.
3. Charge the column by applying 2 CV 50 mM metal solution in deionized water.
4. Wash the column with 10 CV deionized water.
5. Equilibrate the column using 10 CV binding buffer.
6. Apply a clarified sample in the pH range 7 - 8.5. The sample should contain 10 mM imidazole.
7. Wash the column with 20 - 30 CV washing buffer.
8. Elute the target protein.
   Alternative 1: Desorb the target protein with 5 CV elution buffer.
   Alternative 2: For increased purity, gradient elution is recommended. For example, a gradient from 10 mM to 300 mM imidazole over 20 CV can be applied.
9. Wash the column with 5 CV deionized water to remove the elution buffer.
10. Equilibrate the column with 5 CV 20% ethanol for storage. Close the column using the included cap and plug.

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. WorkBeads resins are available with nitrilotriacetic acid (NTA) or iminodiacetic acid (IDA) chelating ligands as illustrated in Figure 1.

(A)



(B)

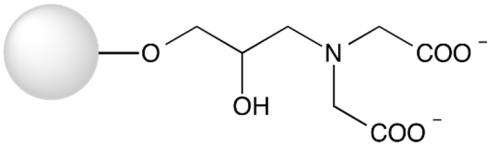


Figure 1. Structure of the chelating ligand used in WorkBeads 40 NTA (A) and WorkBeads 40 IDA (B) resins.

IMAC is commonly used for the 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 unbound material will pass through the column. Bound proteins are desorbed by stepwise or gradient elution using a competing agent, or by applying a low pH buffer. BabyBio Ni-NTA columns are recommended as the primary choice for His-tagged protein purification and usually will give excellent results. For more difficult purifications, a screening is recommended using the eight available different pre-charged WorkBeads IMAC resins are recommended to find the optimal combination of ligand and metal ion, see *Related products*. Bio-Works also offer two different Screening kits with pre-charged WorkBeads IMAC resins prepacked in BabyBio 1 ml and 5 ml columns.

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 ligand in order to prevent non-specific binding of endogenous proteins that may bind via histidine clusters for example. This can easily be done using the recommended binding buffer.

Elution with a continuously decreasing pH gradient is an alternative to imidazole and after optimization a pH step gradient may be more appropriate for scale-up. 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. It is important to consider the target protein stability at low pH.

Instructions

Purification can be carried out at room temperature or at temperatures down to 4°C. Operation at a low temperature may require a reduced flow rate due to the increased viscosity of the buffer. All steps can be carried out with a syringe, a peristaltic pump or a chromatography system. If the chromatography system has a pressure limit functionality, set the maximum pressure over the column to 3 bar (remember to take the system fluidics contribution to the pressure into account).

1. Prepare the sample

After cell disruption or extraction, clarify the sample by centrifugation at 10 000 - 20 000 × *g* for 15 - 30 minutes. It is generally recommended also to pass the sample through a 0.22 - 0.45 µm filter (e.g., a syringe filter) to avoid inadvertently applying any remaining particles onto the column. If the sample contains only small amounts of particles, centrifugation may be omitted and it is enough only to carry out filtration. Application of a sample that has not been properly clarified may reduce the performance and lifetime of the column. The sample should be applied under conditions similar to those of the binding buffer. Add imidazole to the sample to have the same concentration as in the binding buffer.

2. Connect the column

Cut off or twist off the end at the outlet of the column, see Figure 2. **Note:** It is of high importance to cut off the tip at the very end of the cone, preferable using a scalpel. Incorrect removal of the end piece will affect the performance of the column.

Connect the column to your equipment using the recommended connectors shown in Table 1. Fill the equipment with deionized water or buffer and make drop-to-drop connection with the column to avoid getting air into the column. Carry out all steps, except for sample application, at 1 ml/min (BabyBio 1 ml column) or 5 ml/min (BabyBio 5 ml column).

Figure 2. Removal of the cut-off end at the column outlet should be done by cutting or by twisting (A) not bending (B).

Table 1. Recommended connectors for coupling BabyBio columns to the equipment of choice.

Equipment	Accessories for connection
Syringe	Female luer or male coned 10 - 32 threads
Chromatography system	Fingertight connectors (coned 10 - 32 threads) for 1/16” o.d. tubing

3. Remove the storage solution

The column contains 20% ethanol on delivery. This storage solution should be washed out before use. Wash the column with 5 CV deionized water or buffer. Avoid flow rates higher than 2 ml/min for BabyBio 1 ml columns or 10 ml/min for BabyBio 5 ml columns before the storage solution has been removed to avoid overpressure due to high viscosity of the 20% ethanol solution.

4. Charge the resin

Charge the resin with the metal ion of choice by applying approximately 2 CV 50 mM metal ion solution in deionized water. See recommended salts in Table 2.

Table 2. Recommended metal ions salts for charging. Other metal salts can possibly be used.

Metal ion immobilized	Metal salt recommended
Ni^{2+}	50 mM Nickel(II) sulfate
Co^{2+}	50 mM Cobalt(II) sulfate
Cu^{2+}	50 mM Copper(II) sulfate
Zn^{2+}	50 mM Zinc(II) sulfate
Ga^{3+}	50 mM Gallium(III) nitrate
Fe^{3+}	50 mM Iron(III) sulfate

5. Remove the unbound metal ions

Remove the excess of the metal ion solution by washing the column with 10 CV deionized water.

6. Equilibrate the column

Equilibrate the column with 5 - 10 CV of binding buffer (see Table 3 for recommended buffers). Other neutral buffers, with at least 10 mM of imidazole, can also be used.

Table 3. Recommended buffers for purification. Other buffers can be used.

Buffer	Composition
Binding buffer	50 mM Na-phosphate buffer, 300 mM NaCl, 10 mM imidazole, pH 8.0
Washing buffer	50 mM Na-phosphate buffer, 300 mM NaCl, 20 - 100 mM imidazole, pH 8.0
Elution buffer	50 mM Na-phosphate buffer, 300 mM NaCl, 300 mM imidazole, pH 8.0

Note: To avoid bacterial growth and poor column performance, use only freshly prepared and filtered buffers.

7. Apply the sample

Apply the sample at 0.5 - 1 ml/min for the BabyBio 1 ml or 2 - 5 ml/min for the BabyBio 5 ml columns. A too high flow rate may reduce the yield.

8. Wash

After sample application, remove unbound impurities by washing the column with 20 - 30 CV of washing buffer or until desired $A_{280\ nm}$ absorbance of the wash fractions (e.g., 0.01 - 0.02) is obtained. The binding buffer can be used instead of the washing buffer if the target protein binding is weak. However, this may decrease the final purity. If a gradient elution is planned, the binding buffer may be used for washing since most of the impurities will be eluted earlier than the His-tagged protein during the elution.

9. Elute

Alternative 1: Desorb the target protein with 5 CV elution buffer.
Alternative 2: For increased purity, gradient elution is recommended. For example, a gradient from 10 mM to 300 mM of imidazole over 20 CV can be applied.

10. Re-equilibrate

Before the next purification, re-equilibrate the column with 10 CV binding buffer.

11. Remove the elution buffer

Wash the column with 5 CV deionized water to remove the salts of the elution buffer.

12. Column storage

Equilibrate the column with 5 CV 20% ethanol for storage. Close the column using the cap and plug (included).

Purification additives

Metal ion charged BabyBio NTA and BabyBio IDA columns are compatible with a multitude of additives, including various buffer substances, salts, detergents and stabilizers. Integral membrane proteins can be purified in the presence of detergents. Denaturing agents such as guanidine-HCl or urea can be used, although they may denature the target protein. Proteins expressed as inclusion bodies often have an incomplete folding. Dissolution of the inclusion body followed by IMAC purification in the presence of a denaturing agent, and finally renaturation is sometimes done, although significant further development may be required to obtain native protein structure.

Note: The use of chelating substances and reducing agents should be avoided. If needed, Tris(2-carboxyethyl)phosphine (TCEP) is recommended as reducing agent.

Scale-up

Metal ion charged BabyBio NTA and BabyBio IDA 1 ml columns are commonly used for purification of up to 50 mg of protein sample, but a capacity of up to 70 mg/ml is often possible. However, this depends on the properties (mainly size) of the target protein. The capacity is also dependent on the sample composition and conditions used for the purification. Scale-up from a BabyBio 1 ml column can easily be done by using a BabyBio 5 ml column and applying a sample volume five times larger. BabyBio columns can be connected in series with a maximum of five columns (column stacking). This will increase the capacity accordingly. By connecting BabyBio columns in series, column volumes from 1 ml to 25 ml can be obtained. This means a binding capacity of 1000 mg of His-tagged protein can be achieved.

BabyBio columns can be connected together easily without accessories. The pressure drop across each column bed will be the same as for a single column, but the upstream columns will be exposed to a higher internal pressure since it is affected by the added pressure drops across the downstream columns. It may therefore be necessary to decrease the flow rate accordingly to avoid reaching the maximum pressure limit in the first column. If possible, the maximum pressure of the chromatography system should be set according to Table 4. Remember to take the system fluidics contribution to the pressure into account.

Table 4. Recommended maximum pressure settings for BabyBio columns connected in series. Notice that the maximum pressure over each column is always 3 bar.

Number of columns in series	Max pressure BabyBio 1 ml (bar)	Max pressure BabyBio 5 ml (bar)
1	3.0	3.0
2	6.0	6.0
3	9.0	9.0
4	12	10[1]
5	15	10[1]

1 The maximum pressure is defined by the column hardware maximum pressure.

The column size should be selected based on the estimated amount of protein to be purified. A test run with a defined small volume of sample on a BabyBio 1 ml column should be used to estimate the concentration of the target protein in the sample. A general recommendation is to use 70 - 80% of the column binding capacity. For large sample volumes with low concentrations of the target protein, it may be suitable to use a larger column than the calculated one to allow higher sample flow rates, and consequently shorter application time. For example, using a 5 ml column instead of a 1 ml column allows a flow rate five times higher due to the larger cross-section of the column. Have in mind that too high flow rate may reduce the binding capacity.

For columns larger than 20 ml, it is recommended to pack a single column using bulk resin as the limitations of column stacking will then impact chromatographic performance. To find out more about Bio-Works bulk chromatography resins, please visit www.bio-works.com

Optimization

The following paragraphs will give indications on some parameters that can be tuned to find the optimal conditions for the purification.

Optimization of the binding

Low imidazole concentration

The sample and the binding buffer should contain a low concentration of imidazole, but not below 10 mM, to reduce unwanted binding of host cell proteins and to avoid pH effects that may interfere with the protein binding. Keep in mind that if the imidazole concentration is too high the His-tagged protein will not bind at all. Use high quality imidazole which has little or no absorbance at 280 nm.

Slightly basic pH

Binding of His-tagged proteins is preferably carried out at pH 7 - 8.5. At lower pH the histidine residues will be protonated (pK_a approx. 6) and will not bind to the column.

Tuning the flow rate

Binding of His-tagged proteins to a metal chelating column is a rather fast mechanism, and a high flow rate will usually not affect the yield when moderate loadings are applied. At low temperature or for exotic protein or sample composition, it may be useful to lower the flow rate.

Addition of a denaturing agent

If the target protein is insoluble or present as inclusion bodies it can be dissolved by using a denaturing agent (e.g., 8 M urea or 6 M guanidine-HCl). The denaturing agent should be included in all buffers during the purification. The protein is usually denatured by the treatment. In some cases, subsequent renaturation is desired.

Optimization of washing and elution

Prolonged or harsher wash

A continuously decreasing UV signal is an indication of unbound material being washed out. The washing buffer applied should be continued, until the UV signal is stable and the same as for the washing buffer. The binding affinity for some His-tagged proteins may be very strong due to extra His-residues on the protein surface or to multimeric properties. In this instances use more stringent washing conditions (higher concentration of imidazole), which can give higher purity.

Increased imidazole concentration

An additional washing step with a higher imidazole concentration in the washing buffer can be tested. Note that if the imidazole concentration is too high it may cause premature elution of the target protein.

Additives

In IMAC, 300 - 500 mM NaCl is usually included in the eluents to reduce electrostatic interactions. In rare cases, it may be worthwhile to optimize the ionic strength. Other parameters such as pH and additives can be considered for optimization to increase the purity and stability of the target protein.

Optimizing elution conditions

Elution can be performed using a high imidazole concentration (but rarely higher than 300 mM). A stronger binding may require higher imidazole concentrations for elution. Aggregates of His-tagged protein bind via multiple tags, thus increasing the affinity. By optimizing the imidazole concentration, it is possible to elute the His-tagged protein separately from the aggregates.

Step elution

The optimal imidazole concentration is dependent on the purity and recovery requirements as well as the properties of the target protein and the sample. Applying gradient elution often gives increased purity than step elution, but step elution may be desired to obtain the highest possible concentration of the target protein and also when scaling up. In order to optimize the imidazole concentration for step elution an initial linear gradient test run should be performed to obtain suitable step elution conditions, see Figure 3.

Note: Remember to take the system dead volume into account when comparing the print out of the gradient and the trace.

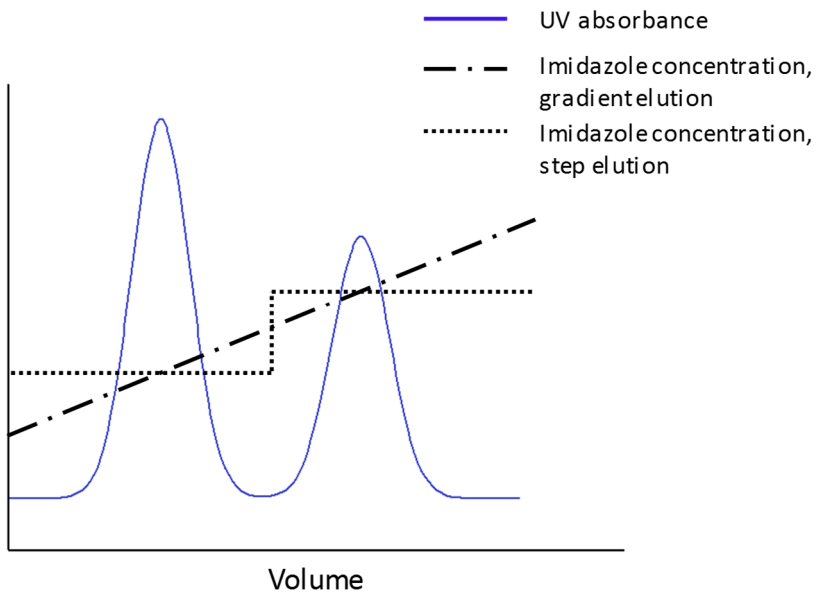


Figure 3. Optimization of step elution with imidazole. A test run with linear gradient elution gives information about suitable imidazole concentrations to be used in step elution.

Extra purification step

Optimization of the overall purification process by tuning the binding, washing and/or elution steps, is a possibility. However, an extra purification step based on another chromatography technique is recommended (see *Additional purification*).

Desalting and buffer exchange

Buffer exchange or desalting of a sample is often necessary before analysis and/or after chromatography steps such as IMAC purification. This can be carried out quickly and easily using BabyBio Dsalt 1 ml or 5 ml columns (see *Related products*). BabyBio Dsalt columns are also useful for the dialysis of larger sample volumes or when samples need to be processed rapidly, to avoid degradation.

Additional purification

His-tagged protein purification on metal ion charged BabyBio columns gives high purity in a single step. For very high purity requirements, it can be necessary to add a second purification step. The additional purification step is used to remove remaining proteins and/or impurities from the sample. WorkBeads 40/1000 SEC, WorkBeads 40/100 SEC and WorkBeads 40/10 000 SEC resins facilitate the purification of target proteins of different size. WorkBeads 40S, WorkBeads 40Q and WorkBeads 40 DEAE resins are excellent for ion exchange chromatographic purification. These resins are also available as ready-to-use BabyBio Q, BabyBio S and BabyBio DEAE columns, as well as prepacked OptioBio 40S 10x100 and OptioBio 40Q 10x100 columns with 7.9 ml column volume.

To find out more about Bio-Works chromatography resins for additional purification, please visit www.bio-works.com

Maintenance of the column

Unpacking and inspection

Unpack the shipment as soon as it arrives and inspect it for damage. Promptly report any damage or discrepancies to your local supplier.

Cleaning and recharging with metal ions

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.

It is important to strip off the metal ions, before cleaning and then recharge the resin with fresh metal ions. If the resin is packed in a column; stripping, cleaning and recharging the resin can be carried out as followed:

Wash with:

1. 5 CV deionized water
2. 10 CV 50 mM Na_2EDTA, pH 8.0
3. 10 CV 100 mM NaOH
4. 10 CV deionized water
5. 2 CV 50 mM metal salt solution in deionized water
6. 10 CV deionized water
7. 10 CV 20% ethanol (for storage)

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.

Storage

Equilibrate the column in 20% ethanol and close it securely using the included plug and cap. Store the column at 2 to 25°C.

Additional information

Intended use

BabyBio columns are intended for research and process development only. BabyBio columns shall not be used for preparation of material for clinical or diagnostic purposes.

Safety

Please read the associated Safety Data Safety (SDS) for BabyBio columns, and the safety instructions for any equipment to be used. Nickel and cobalt salts are considered to be allergenic and potentially carcinogenic. Use recommended safety equipment. Note that the maximum backpressure of BabyBio NTA and BabyBio IDA columns is 0.3 MPa (3 bar, 43 psi).

Product information

	BabyBio NTA	**BabyBio 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 NTA	WorkBeads 40 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)
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	2 - 12 cleaning (stripped column) Do not keep the resin at low pH for prolonged time	2 - 12 cleaning (stripped column) 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. 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).

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 NTA His-tag Screening kit 5 ml[2]	5 ml x 4	45 700 102
BabyBio IDA His-tag Screening kit 1 ml[2]	1 ml x 4	45 700 001
BabyBio IDA His-tag Screening kit 5 ml[2]	5 ml x 4	45 700 002
BabyBio Dsalt 5 ml	5 ml x 5	45 360 107
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 NTA	25 ml	40 602 001
WorkBeads 40 IDA	25 ml	40 601 001
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
2. Includes one column each charged with Ni^{2+}, Co^{2+}, Cu^{2+} or Zn^{2+}

Ordering information

Product name	Pack size	Article number
BabyBio NTA 1 ml	1 ml x 1	45 655 111
	1 ml x 2	45 655 112
	1 ml x 5	45 655 113
	1 ml x 10	45 655 114
BabyBio NTA 5 ml	5 ml x 1	45 655 115
	5 ml x 2	45 655 116
	5 ml x 5	45 655 117
	5 ml x 10	45 655 118
BabyBio IDA 1 ml	1 ml x 1	45 655 011
	1 ml x 2	45 655 012
	1 ml x 5	45 655 013
	1 ml x 10	45 655 014
BabyBio IDA 5 ml	5 ml x 1	45 655 015
	5 ml x 2	45 655 016
	5 ml x 5	45 655 017
	5 ml x 10	45 655 018

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

Bio-Works

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