Bio-Works

INSTRUCTION

GoBio Mini charged NTA

GoBio Mini Ni-NTA, GoBio Mini Co-NTA, GoBio Mini Cu-NTA and GoBio Mini Zn-NTA

The ready-to-use GoBio™ Mini NTA columns are prepacked with metal ion charged WorkBeads™ 40 NTA resins. They are available in two column sizes: 1 mL and 5 mL. The resins are charged with either Ni^{2+}, Co^{2+}, Cu^{2+} or Zn^{2+} ions for quick and convenient purification of polyhistidine-tagged (His-tagged) proteins by using immobilized metal ion affinity chromatography (IMAC). The GoBio Mini columns can be used to purify up to 70 mg and 350 mg protein using a 1 mL or 5 mL column.

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

Intended use

WorkBeads resins are developed and supported for both research and production-scale chromatography. WorkBeads resins are produced according to ISO 9001:2015, and Regulatory Support Files (RSF) are available to assist the process validation and submissions to regulatory authorities.

The GoBio™ prepacked column family has been developed for convenient, reproducible, and rapid results and can be used for small scale purification and all the way up to process development and full-scale manufacturing.

Safety

Please read the Safety Data Sheets (SDS) for WorkBeads 40 Ni-NTA, WorkBeads 40 Co-NTA WorkBeads 40 Cu-NTA, WorkBeads 40 Zn-NTA, and the safety instructions for any equipment to be used.

Note: Nickel and cobalt salts are considered to be allergenic and potentially carcinogenic. Use recommended safety equipment.

Unpacking and inspection

Unpack the shipment as soon as it arrives and inspect it for damage. Promptly report any damage or discrepancies to complaints@bio-works.com

Short Protocol

This general short protocol is for the use of metal ion charged GoBio Mini NTA columns. Detailed instructions and recommendations for optimization are provided later in this instruction. Recommended buffers are listed in Table 2.

1. Connect the column to the chromatography system, syringe or pump.
2. Equilibrate the column using 10 column volumes (CV) binding buffer.
3. Apply a clarified sample in the pH range 7 – 8.5. The sample should contain 10 mM imidazole.
4. Wash the column using 20 – 30 CV washing buffer.
5. 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.

   After the elution re-equilibrate the column with 10 CV binding buffer.
6. Wash the column with 5 CV deionized water to remove the elution buffer.
7. 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) chelating ligands as illustrated in Figure 1.

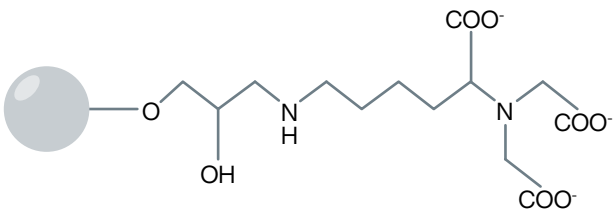

Figure 1. Structure of the chelating ligand used in WorkBeads 40 NTA resins.

IMAC is commonly used for the 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 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. GoBio Mini Ni-NTA columns are recommended as the primary choice for His-tagged protein purification and usually will give excellent results.

Imidazole is recommended for elution. This is the most common 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 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 (pKa 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, it may be 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 (GoBio Mini 1 mL column) or 5 mL/min (GoBio Mini 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 GoBio Mini 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 GoBio Mini 1 mL columns or 10 mL/min for GoBio Mini 5 mL columns before the storage solution has been removed to avoid overpressure due to high viscosity of the 20% ethanol solution.

4. Equilibrate the column

Equilibrate the column with 5 – 10 CV of binding buffer, see Table 2 for recommended buffers. Other neutral buffers, with at least 10 mM of imidazole, can also be used.

Table 2. Recommended buffers for purification.

Buffer	Composition
Binding buffer	50 mM sodium phosphate buffer, 300 mM NaCl, 10 mM imidazole, pH 8.0
Washing buffer	50 mM sodium phosphate buffer, 300 mM NaCl, 20 – 100 mM imidazole, pH 8.0
Elution buffer	50 mM sodium 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.

5. Apply the sample

Apply the sample at 0.5 – 1 mL/min for the GoBio Mini 1 mL or 2 – 5 mL/min for the GoBio Mini 5 mL columns. A too high flow rate may reduce the yield.

6. 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.

7. 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.

8. Re-equilibrate

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

9. Remove the elution buffer

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

10. Column storage

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

Purification additives

GoBio Mini IMAC 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

Pre-charged GoBio Mini NTA 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 GoBio Mini 1 mL column can easily be done by using a GoBio Mini 5 mL column and applying a five times larger sample volume. GoBio Mini columns can be connected in series with a maximum of five columns (column stacking). This will increase the capacity accordingly. By connecting GoBio Mini 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.

GoBio Mini columns can be connected 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 3. Remember to take the system fluidics contribution to the pressure into account.

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

No. of columns in series	Max pressure GoBio Mini 1 mL (bar)	Max pressure GoBio Mini 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 GoBio Mini 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 use a single column either by packing a column with bulk resin or for convenience and reproducibility use a prepacked column from the GoBio prepacked column family, as the limitations of column stacking will then impact chromatographic performance.

These pre-charged IMAC resins are all available in different prepacked GoBio column formats, such as GoBio Prep 16x100 (20 mL), GoBio Prep 26x100 (53 mL) and GoBio Prep columns starting from 1 L, see “Ordering information”.

To find out more about Bio-Works chromatography products 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 these 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 provides increased purity compared to step elution, but step elution may be desired to obtain the highest possible concentration of the target protein and when scaling up. 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.

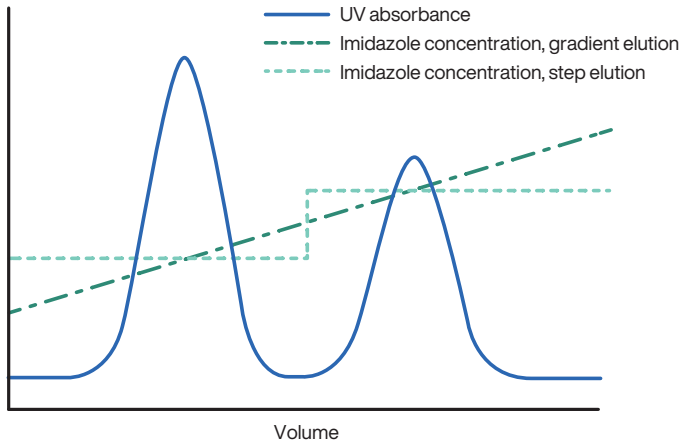

Figure 3. Optimization of step elution with imidazole. A test run with linear gradient elution gives information about suitable imidazole concentrations to be used in step elution.

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 can be used before analysis and/or after purification with for example ion exchange chromatography. This can be carried out quickly and easily in lab-scale using GoBio Mini Dsalt 1 mL, GoBio Mini Dsalt 5 mL, GoBio Prep 16x100 Dsalt (20 mL) and GoBio Prep 26x100 Dsalt (53 mL) columns depending on sample volumes, see “Related products”. These columns are also very useful alternatives to dialysis or when samples need to be processed rapidly to avoid degradation. For larger sample volumes prepacked GoBio Prod columns starting from 1 L are available or diafiltration can be used.

Additional purification

His-tagged protein purification on GoBio Mini pre-charged IMAC 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 40/100 SEC, WorkBeads 40/100 0SEC and WorkBeads 40/10 000 SEC resins facilitate the purification of target proteins of different size. WorkBeads 40S, WorkBeads 40Q, WorkBeads 40 DEAE and WorkBeads 40 TREN resins are excellent for ion exchange chromatographic purification. All these resins are also available in ready-to-use GoBio prepacked columns in several different sizes.

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Maintenance of the column

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)

Note: Nickel and cobalt salts are considered to be allergenic and potentially carcinogenic. Use recommended safety equipment.

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.

Note: GoBio Mini IMAC columns pre-charged with metal ions are produced under controlled conditions. Restoring full functionality after cleaning and recharging with metal ions is the responsibility of the user and is not guaranteed by the manufacturer.

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.

Product information

	GoBio Mini: Ni-NTA, Co-NTA, Cu-NTA, Zn-NTA
Target substances	His-tagged proteins, proteins containing histidine cysteine and/or tryptophan amino acid side chains
Resins	WorkBeads 40 Ni-NTA WorkBeads 40 Co-NTA WorkBeads 40 Cu-NTA WorkBeads 40 ZN-NTA
Matrix	Rigid, highly cross-linked agarose
Average particle size (D_{V50})[1]	45 µm
Ligand	Nitrilotriacetic acid (NTA)
Metal ion	Ni^{2+}, Co^{2+}, Cu^{2+} or Zn^{2+}
Static binding capacity[2]	70 mg His-tagged protein/mL resin
Dynamic binding capacity[2]	50 mg His-tagged protein/mL resin
Column volume	1 mL 5 mL
Column dimension	7 × 28 mm (1 mL) 13 × 38 mm (5 mL)
Recommended flow rate[3] GoBio Mini 1 mL GoBio Mini 5 mL	 0.25 – 1 mL/min (37 – 150 cm/h) 1.25 – 5 mL/min (56 – 225 cm/h)
Maximum flow rate[4] GoBio Mini 1 mL GoBio Mini 5 mL	 5 mL/min (780 cm/h) 20 mL/min (900 cm/h)
Maximum back pressure	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)[5] 2 – 12 cleaning (stripped column) Do not keep the resin at low pH for prolonged time
Storage	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 GoBio Mini Ni-NTA 1 mL. The binding capacity is dependent on the size of the target protein, and on the competition with impurities.
3 Optimal flow rate during binding is depending on the sample. During column wash and elution, a flow rate of 1 mL/min and 5 mL/min can be used for 1 mL and 5 mL columns, respectively. Note: The maximum pressure the packed bed can withstand depends on the sample/liquid viscosity and chromatography resin characteristics. The pressure also depends on the tubing used to connect the column and the system restrictions after the column outlet.
4 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).
5 This is the most common pH range for purification of His-tagged proteins.

GoBio prepacked column family

GoBio prepacked column family is developed for convenient, reproducible and fast results and includes columns with different sizes and formats.

GoBio Mini 1 mL and GoBio Mini 5 mL for small scale purification and screening using a shorter packed bed.

GoBio Screen 7x100 (3.8 mL) for reproducible process development including fast and easy optimization of methods and parameters.

GoBio Prep 16x100 (20 mL) and GoBio Prep 26x100 (53 mL) for lab-scale purifications and scaling up.

GoBio Prep 16x600 (120 mL) and GoBio Prep 26x600 (320 mL) for preparative lab-scale size exclusion chromatography.

GoBio Prod 80x200 (1 L), GoBio Prod 130x200 (2.7 L), GoBio Prod 200x200 (6 L), GoBio Prod 240x200 (9 L) and GoBio Prod 330x250 (21.4 L) for production-scale purifications.

Related products

Product name	Pack size[1]	Article number
Prepacked columns		
GoBio Mini Dsalt 1 mL	1 mL × 5	45 360 101
GoBio Mini Dsalt 5 mL	5 mL × 5	45 360 107
GoBio Screen 7x100 Ni-NTA[2]	3.8 mL × 1	55 651 001
GoBio Screen 7x100 Co-NTA[2]	3.8 mL × 1	55 651 401
GoBio Screen 7x100 Cu-NTA[2]	3.8 mL × 1	55 651 301
GoBio Screen 7x100 Zn-NTA[2]	3.8 mL × 1	55 651 501
GoBio Prep 16x100 Dsalt[2]	20 mL × 1	55 700 021
GoBio Prep 26x100 Ni-NTA[2]	53 mL × 1	55 651 031
GoBio Prep 26x100 Co-NTA[2]	53 mL × 1	55 651 431
GoBio Prep 26x100 Cu-NTA[2]	53 mL × 1	55 651 331
GoBio Prep 26x100 Zn-NTA[2]	53 mL × 1	55 651 531
GoBio Prep 26x100 Dsalt	53 mL × 1	55 700 031
Bulk resins		
WorkBeads 40 Ni-NTA	25 mL 150 mL	40 651 001 40 651 003
WorkBeads 40 Co-NTA	25 mL 150 mL	40 651 401 40 651 403
WorkBeads 40 Cu-NTA	25 mL 150 mL	40 651 301 40 651 303
WorkBeads 40 Zn-NTA	25 mL 150 mL	40 651 501 40 651 503
WorkBeads Dsalt	300 mL	40 360 003
Accessories		
Column plug male 1/16”	10	70 100 010
Column cap female 1/16”	10	70 100 020

[1] All different pack sizes are available on www.bio-works.com
[2] Packed on request.

Ordering information

Product name	Pack size[1]	Article number
GoBio Mini Ni-NTA 1 mL	1 mL × 1 1 mL × 5 1 mL × 10	45 655 101 45 655 103 45 655 104
GoBio Mini Ni-NTA 5 mL	5 mL × 1 5 mL × 5 5 mL × 10	45 655 105 45 655 107 45 655 108
GoBio Mini Co-NTA 1 mL	1 mL × 1 1 mL × 5 1 mL × 10	45 655 131 45 655 133 45 655 134
GoBio Mini Co-NTA 5 mL	5 mL × 1 5 mL × 5 5 mL × 10	45 655 135 45 655 137 45 655 138
GoBio Mini Cu-NTA 1 mL	1 mL × 1 1 mL × 5 1 mL × 10	45 655 121 45 655 123 45 655 124
GoBio Mini Cu-NTA 5 mL	5 mL × 1 5 mL × 5 5 mL × 10	45 655 125 45 655 127 45 655 128
GoBio Mini Zn-NTA 1 mL	1 mL × 1 1 mL × 5 1 mL × 10	45 655 141 45 655 143 45 655 144
GoBio Mini Zn-NTA 5 mL	5 mL × 1 5 mL × 5 5 mL × 10	45 655 145 45 655 147 45 655 148

[1] Packed on request.

Orders: sales@bio-works.com or contact your local distributor.

For more information about local distributor and products visit www.bio-works.com or contact us at info@bio-works.com

bio-works.com

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