



## INSTRUCTION

# Pre-charged WorkBeads 40 NTA and WorkBeads 40 IDA resins

These products comprise of WorkBeads™ 40 NTA and WorkBeads 40 IDA resins pre-charged with  $\text{Ni}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Cu}^{2+}$  or  $\text{Zn}^{2+}$  ions for use in Immobilized Metal Ion Affinity Chromatography (IMAC). The resins are designed for purification of poly-histidine tagged (His-tagged) proteins or other metal ion binding proteins. Metal ions have different affinities for several types of proteins which results in resins with slightly different selectivities.

- Pre-charged resins with different metal ions for optimal purity of the target protein
- Pre-charged resins for ease of use and time saving
- High binding capacity and flow properties



## 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, WorkBeads 40 Ni-IDA, WorkBeads 40 Co-IDA, WorkBeads 40 Cu-IDA and WorkBeads 40 Zn-NTA, 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.

## 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](mailto:complaints@bio-works.com)

## Short protocol

This short protocol is for the column packing of pre-charged WorkBeads 40 NTA and WorkBeads 40 IDA resins. Detailed instructions and recommendations for optimization are given later in this instruction.

### Packing

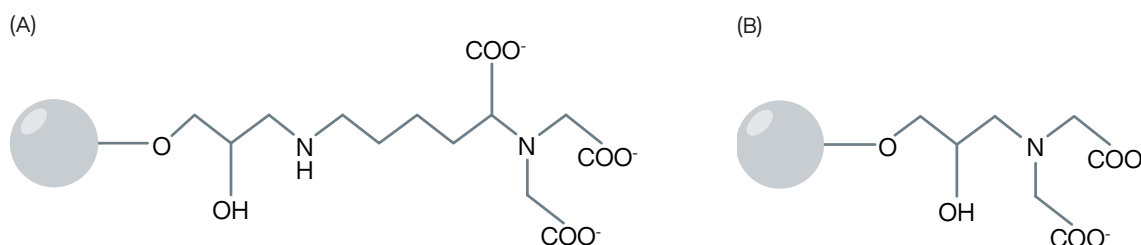
1. Make a slurry of the desired resin concentration in water.
2. Pour the slurry into the column.
3. Pack the resin with an appropriate flow rate.
4. Apply an axial compression of less than 2%.
5. Equilibrate the column with 20% ethanol for storage; or move to the section "Purification".

### Purification

1. Equilibrate the column using 10 column volumes (CV) binding buffer.
2. Apply a clarified sample in the pH range 7 – 8.5. The sample should contain 10 mM imidazole.
3. Wash the column using 20 – 30 CV washing buffer.
4. Elute the target protein.
  - Alternative 1, step gradient: Desorb the target protein with 5 CV elution buffer.
  - Alternative 2, linear gradient: For increased purity, linear gradient elution is recommended. For example, use a gradient from 10 mM to 300 mM imidazole over 20 CV.
5. Wash the column with 5 CV deionized water to remove the buffer salts.
6. Equilibrate with 10 CV 20% ethanol for storage.

## 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  $\text{Ni}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Cu}^{2+}$  and  $\text{Zn}^{2+}$ , 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.



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

IMAC is most common 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 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. WorkBeads 40 Ni-NTA is recommended as the primary choice for His-tagged protein purification and, in most cases, will give excellent results. For more difficult purifications, a screening is recommended with the eight different pre-charged WorkBeads IMAC resins available 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 pre-packed in GoBio Mini 1 mL and 5 mL columns.

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 resin 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 is done easily by using the recommended binding buffer.

Elution with a continuously decreasing pH gradient is an alternative to imidazole and after optimization with a pH step gradient could be 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 to a release of the protein. However, it is important to consider the target protein stability at low pH.

## Column packing

### Columns with i.d. $\leq$ 10 mm

WorkBeads resins are cross-linked using a proprietary method that results in a very rigid resin that tolerate pressures of several bars and consequently can run at high flow rates. Follow both these general advices when packing a column and the column manufacturer's specific instructions. Preferably, use a column with an adjustable adaptor. In some instances, a packing reservoir or column extension may be needed.

**Note:** Always make sure that the maximum pressure of the column hardware is not exceeded. Backpressure caused by the chromatography system components connected downstream of the column may reduce the maximum flow that can be used. Wear eye protection.

For laboratory scale columns, we recommend a so-called single step flow packing procedure since the contribution of the wall support is significant. A single step flow packing is fast and easy to implement. The wall support phenomena are described in the next section.

#### 1. Wash the resin

Most WorkBeads resins are supplied in 20% ethanol. To avoid undue backpressure when packing, wash the desired amount of resin with several column volumes (CV) of deionized water or packing buffer before packing.

#### 2. Make a slurry

Add packing solution to the washed resin to obtain a 40% to 60% slurry concentration. Make approximately 15% extra slurry to compensate for the compression during packing. The amount of slurry to be prepared can be calculated according to:

$$\text{Slurry volume} = \frac{\text{bed volume} \times 100}{\% \text{ slurry}} \times 1.15$$

The concentration of slurry can be determined using different methods. One such method is the centrifugation method: Transfer homogenized slurry to a graded centrifuge test tube. Centrifuge for 3 minutes at 3000 rpm and determine the relation between total volume and resin volume. If the relation is not within desired range, modify the slurry concentration of the resin to be packed by either adding or removing packing solution.

**Note:** If there is a limitation on the total slurry volume, slurry concentrations up to 70% can be used to compensate the lower volume.

**Note:** Different packing buffers can be used, such as 0.4 M NaCl or 20% ethanol. Some resins are better packed in packing buffer with higher conductivity.

### 3. Pour the slurry into the column

Pour the slurry slowly down the side of the column to avoid formation of air bubbles. Preferably, use a packing reservoir or an extra column tube to extend the column volume to accommodate the entire slurry volume during packing. If no packing adaptor is available, packing can be done by stepwise additions and packing. Although not recommended this will give acceptable results for most applications.

### 4. Pack the bed

Pack the resin with a downward flow higher than the intended operational flow. We recommend 600 cm/h for columns up to 10 mm i.d. Make sure the packing flow rate does not exceed the maximum pressure of the column hardware or the resin. The operational flow should not be more than 75% of the packing flow rate.

$$\text{Volumetric flow rate (ml/min)} = \frac{\text{Linear flow (cm/h)}}{60} \times \text{column cross sectional area (cm}^2\text{)}$$

$$\text{Column cross sectional area (cm}^2\text{)} = \frac{\pi \times d^2}{4}$$

d = column inner diameter in cm

After flow compression, and before mechanical compression, the bed height can be adjusted (i.e., using a spatula) so that the exact bed height is achieved, but do this with care since it is important not to disturb the flow compressed bed left in the column.

### 5. Close the column

When the bed height is constant mark the bed height on the column. Stop the flow and remove the packing reservoir or extra tube. Note that the bed height will increase temporarily when the flow is stopped. If needed, adjust the bed height by removing excess resin but be careful not to remove too much. Gently fill the column with packing solution to its rim without disrupting the packed bed. Insert the adjustable adapter on top of the packed bed. Apply an axial compression of 1 – 2% of the final bed height by lowering the adapter into the packed bed.

### 6. Apply a flow (conditioning of bed)

Apply a flow of 450 cm/h and check for any gap formation above the surface of the resin bed. If a gap is observed, stop the flow and adjust the adaptor to eliminate the gap.

### Columns with i.d. $\geq$ 10 mm

During scale up, the phenomena of wall support gradually is decreased/diminished. Wall support means that the column wall supports the resin bed and gives it better flow properties. This mainly occurs when the column inner diameter is smaller, i.e.  $\leq$  10 mm. To accommodate for the lack of wall support in wider columns, we recommend a two-step flow packing procedure. The bed should first be consolidated at a low flow rate to ensure the bed is optimally settled (60 cm/h for at least 1.5 CV). After the initial consolidation step, the bed is further compressed at a higher flow rate (300 – 450 cm/h depending on restrictions for at least 1.5 CV). When the bed has been flow-packed, a mechanical compression of 10 – 20% can be performed.

The optimal mechanical compression to apply will depend on the dimensions and resin type, therefore it is essential to evaluate the packed column prior to use (asymmetry and plate number tests as described below).

**Note:** After flow compression, and before mechanical compression, the bed height can be adjusted (i.e., using a spatula) so that the exact bed height is achieved but this is not to recommend at larger scales.

There is a guide available for large-scale packing,  
<https://www.bio-works.com/packing-guide-download>

## Evaluation of the packed column

Test the column efficiency to check the quality of packing. Testing should be done after packing, at regular intervals during the usage of the column or when separation performance is seen to deteriorate.

The best method of expressing the efficiency of a packed column is in terms of the height equivalent to a theoretical plate (HETP) and the asymmetry factor ( $A_s$ ). These values are easily determined by applying a sample such as 1% acetone solution to the column.

For optimal results, the sample volume should be 2.5% of the column volume (CV) and the flow rate 30 cm/h.

If an acceptance limit is defined in relation to column performance, the column plate number can be used as one of the acceptance criteria for column use.

**Note:** The calculated number of plates will vary according to the test conditions and should only be used as a reference value. Keep test conditions and equipment constant so that results are comparable. Changes of for example solute, solvent, eluent, sample volume, flow velocity, liquid pathway, temperature, etc. will influence the results.

### Measuring HETP and $A_s$

Calculate HETP and  $A_s$  from the UV curve (or conductivity curve).

$$\text{HETP} = \frac{L}{N}$$

$$N = 5.54 \times \left( \frac{V_R}{W_h} \right)^2$$

$L$  = bed height (cm)

$N$  = number of theoretical plates

$V_R$  = volume eluted from the start of sample application to the peak maximum

$W_h$  = peak width measured as the width of the recorded peak at half of the peak height

$V_R$  and  $W_h$  are in the same units

The concept of reduced plate height is often used for comparing column performance. The reduced plate height,  $h$ , is calculated:

$$h = \frac{\text{HETP}}{d_{50v}}$$

$d_{50v}$  = Median particle size of the cumulative volume distribution (cm)

As a guideline, a value of  $< 3$  is very good.

The peak must be symmetrical, and the asymmetry factor as close to 1 as possible. (A typical acceptable range is  $0.7 < A_s < 1.3$ ). A change in the shape of the peak is usually the first indication of bed deterioration.

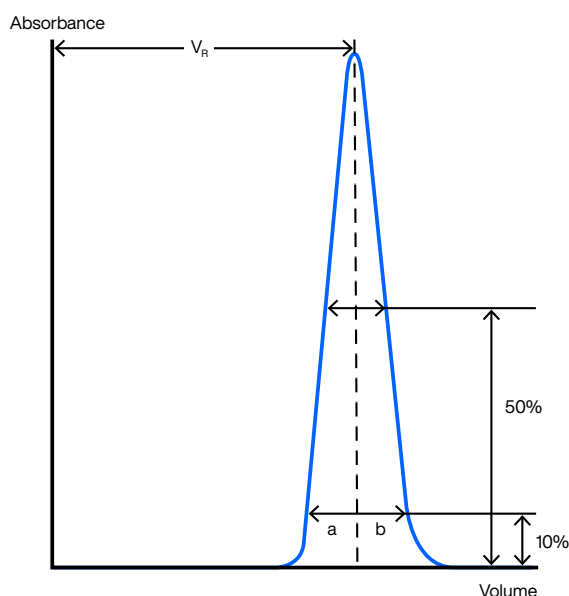
Peak asymmetry factor calculation:

$$A_s = \frac{a}{b}$$

a = ascending part of the peak width at 10% of peak height

b = descending part of the peak width at 10% of peak height

Figure 2 on next page shows a UV trace for acetone in a typical test chromatogram from which the HETP and  $A_s$  values are calculated.



**Fig 2.** A typical test chromatogram showing the parameters used for HETP and  $A_s$  calculations.

## Purification

The pre-charged WorkBeads 40 NTA and WorkBeads 40 IDA resins are excellent for purification of His-tagged proteins. Recommended buffers for purification of His-tagged proteins are shown in Table 1.

Purification can be carried out at room temperature or at temperatures down to 4°C. Operation at a low temperature may require reduced flow rate due to increased viscosity. Prepare the sample according to sample preparation below before starting. Equilibrate the column with 5 – 10 CV equilibration buffer before use.

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

**Table 1.** Recommended buffers for purification of His-tagged proteins.

Buffer	Composition
Binding buffer <sup>1</sup>	50 mM sodium phosphate buffer, 300 mM NaCl, 10 mM imidazole, pH 8.0
Washing buffer <sup>1</sup>	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

<sup>1</sup> The imidazole concentration may have to be optimized. A too high concentration may elute the target during washing. An imidazole concentration just below the concentration where the target proteins are still bound will prevent impurities to bind. This is an ideal washing buffer.

## Sample preparation

After cell disruption or extraction, clarify the sample by centrifugation at 10 000 – 20 000 × g for 15 – 30 minutes. It is generally also recommended to pass the sample through a 0.22 – 0.45 µm filter, e.g., a syringe filter, to avoid transferring any remaining contaminating particles onto the column. Large sample volumes may be clarified by filtration through depth filters or by tangential flow filtration, which may be cheaper and more efficient than investing in a large-scale centrifuge. Application of a sample that has not been properly clarified may reduce the performance and lifetime of the packed column.

**Note:** Add imidazole to the sample in the same concentration as in the binding buffer.

## Standard purification

After sample application, remove unbound and loosely bound impurities by washing the column with 20 – 30 CV washing buffer or until desired A280 nm absorbance of the wash fractions (e.g., 0.01 – 0.02) is obtained. Binding buffer can be used instead of washing buffer if the target protein binding is weak. However, this may decrease the final purity. Binding buffer can also be an option when using gradient elution since most of the impurities will elute earlier than the His-tagged target protein. Elute the target protein by either desorption of the target protein with 5 CV elution buffer or for high purity, gradient elution is recommended. For example, use a gradient from 10 mM imidazole to 300 mM imidazole over 20 CV.

## Purification additives

A multitude of additives can be used in IMAC, 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 can be done, although significant further development may be required to obtain native protein structure.

**Note:** The metal pre-charged WorkBeads 40 NTA and WorkBeads 40 IDA may be compatible with normal use of reducing agents, but if reducing and/or chelating agents are included in the cell culture medium and/or in the buffers we recommend using WorkBeads NiMAC resin or prepacked GoBio NiMAC columns that are resistant with up to 20 mM DTT and 20 mM EDTA without losing binding capacity, see “Related products”.

## Optimization

The following section will give tips on some parameters that can be tuned to get the optimal conditions for increased purity.

### Optimization of binding

#### Low imidazole concentration

The sample and the binding buffer should contain a low concentration of imidazole (not below 10 mM) to reduce unwanted binding of host cell proteins, and to avoid pH affects that may interfere with 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. A lower pH protonates the histidine residues ( $pK_a$  approx. 6) and causes desorption of bound proteins.

### 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. It may be useful to lower the flow rate under some circumstances (for some proteins or sample compositions, or at low temperature).

### Addition of a denaturing agent

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

## Optimization of washing and elution

### Washing

A continuously decreasing UV signal is an indication of unbound material being washed out. The amount of washing buffer applied should be continued until the UV signal is stable and is 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. Those cases allow more stringent washing conditions (higher concentration of imidazole), which can give higher purity. The washing step can also be optimized by increasing the imidazole concentration in an additional washing step. Note that if the imidazole concentration is too high it may cause elution of the target protein.

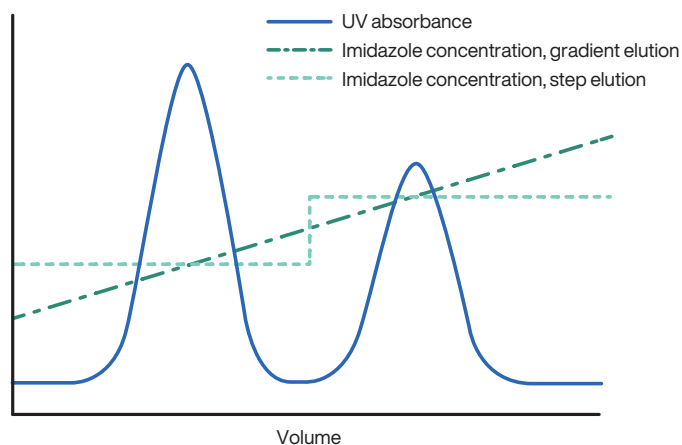
300 – 500 mM NaCl is usually included in the elution buffer to reduce electrostatic interactions. In rare cases it may be worthwhile to optimize the ionic strength. Other parameters such as pH and additives can be considered for optimization of the purity and stability of the purified target protein.

### Elution

Elution can be carried out using a high imidazole concentration, 300 mM imidazole is usually sufficient. A stronger binding may require higher imidazole concentrations for elution. Aggregates of His-tagged proteins can bind via multiple tags thus increasing the affinity. Optimization of the imidazole concentration may allow elution of the His-tagged protein without the aggregates.

The optimal imidazole concentration is dependent on purity and recovery requirements as well as properties of the target protein and the sample. Applying gradient elution gives often increased purity than step elution, but step elution may be necessary to obtain the highest possible concentration of the target protein. It is an important consideration when scaling-up. To optimize the imidazole concentration for step elution an initial linear gradient test run should be carried out to obtain suitable step elution conditions for purification of the sample, see Figure 3.

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



**Fig 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.



## Scale-up

After developing a chromatographic procedure in a small-scale column, e.g., 7 (i.d.) × 100 mm (bed height), WorkBeads resins can be packed into larger columns for scale-up. Large scale purification is often carried out in columns with bed heights of 100 – 300 mm.

### Scale-up principles

During scale-up the ratio between sample volume and column volume should be kept constant. The column volume is scaled up by increasing the column diameter while keeping the bed height the same (e.g., 100 – 200 mm). The linear flow rate should remain the same while the volumetric flow rate increases. The volumetric flow rate for each column can be calculated according to:

$$\text{Volumetric flow rate} = \frac{\text{Linear flow rate (cm/h)} \times \text{Column cross sectional area (cm}^2\text{)}}{60}$$

### Flow

The concepts of volumetric flow, linear flow rate and residence time is important when doing scale-up in chromatography. Volumetric flow is measured in mL/min or L/min, linear flow in cm/h and residence time in minutes. The relationship between these metrics is:

$$\text{Linear flow rate (cm/h)} = \frac{\text{Volumetric flow (mL/min)} \times 60}{\text{Column cross sectional area (cm}^2\text{)}}$$

$$\text{Residence time (minutes)} = \frac{\text{Column bed height (cm)} \times 60}{\text{Linear flow rate (cm/h)}}$$

In the initial process development it is common to use a small column, e.g., 7 × 100 mm, to save sample, buffers and time. This column has a shorter bed height than the final column, which may have a bed height of 200 mm or more. The flow rate for the larger column can be calculated from the flow established on the small column, using the equation above by keeping the residence time of the small column the same for the larger column. This will allow an increase of the linear flow in proportion to the increase in bed height between the columns see Table 2 for examples. If the column bed heights are constant during scale-up the linear flow rate should be also constant (as well as the residence time).

**Table 2.** Example of scale-up parameters

Column dimension	Residence time (minutes)	Linear flow rate (cm/h)	Volumetric flow rate (mL/min)
16x100	4	150	5.0
26x100	4	150	13.3
80x200	8	150	126
130x200	8	150	332
200x200	8	150	785
240x200	8	150	1131
330x250	10	150	2138

## Additional purification steps

His-tagged protein purification on WorkBeads IMAC resins frequently gives high purity in a single purification step. For very high purity requirements, it can be necessary to add a second purification step. The additional purification step is to remove remaining proteins and/or impurities from the sample. In research-scale purification, size exclusion chromatography (SEC) is often a good polishing step since it removes impurities, the imidazole used for elution and potential

aggregates of the target protein. Size exclusion chromatography can be done using WorkBeads 40/100 SEC, WorkBeads 40/1000 SEC and WorkBeads 40/10 000 SEC resins having different separation ranges. Ion exchange chromatography is suitable for both research scale purification and process scale. WorkBeads 40S, WorkBeads 40Q, WorkBeads 40 DEAE and WorkBeads 40 TREN resins provide different selectivities for ion exchange chromatography.

All these resins are available as ready-to-use prepacked columns in the GoBio column family.

## Desalting and buffer exchange

Buffer exchange or desalting of a sample can be used before analysis and/or after purification. 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) prepacked columns depending on sample volumes. GoBio Prod prepacked columns starting from 1 L are available for larger sample volumes, see “Related products”.

These columns are very useful alternatives to dialysis for larger sample volumes or when samples need to be processed rapidly to avoid degradation.

Pre-swollen WorkBeads Dsalt is also available in bulk for packing column format of choice.

To find out more about Bio-Works' chromatography products visit [www.bio-works.com](http://www.bio-works.com)

## Maintenance

### Cleaning and recharging with metal ions

Small amounts of impurities can be found in some samples, which tend to adsorb to the resin by unspecific interactions. This may reduce the resin performance. It is therefore common to strip off the metal ions, followed by 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<sub>2</sub>-EDTA, 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.

**Note:** WorkBeads NTA and IDA resins 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

Store at 2 to 25°C in 20% ethanol.

**Note:** Use a reduced flow rate during equilibration with 20% ethanol, maximum 50% of the maximum flow rate.

## Product description NTA based resins

	WorkBeads 40 Ni-NTA	WorkBeads 40 Co-NTA	WorkBeads 40 Cu-NTA	WorkBeads 40 Zn-NTA
Target substance	His-tagged proteins, proteins containing histidine cysteine and/or tryptophan amino acid side chains			
Matrix	Highly cross-linked agarose	Highly cross-linked agarose	Highly cross-linked agarose	Highly cross-linked agarose
Average particle size ( $D_{V50}$ ) <sup>1</sup>	45 µm	45 µm	45 µm	45 µm
Chelating ligand	Nitrilotriacetic acid (NTA)	NTA	NTA	NTA
Metal ion	Nickel (II)	Cobalt (II)	Copper (II)	Zink (II)
Metal ion capacity for the chelating ligand <sup>2</sup>	NA	NA	50 – 60 µmol Cu <sup>2+</sup> /mL resin	
Dynamic binding capacity <sup>3</sup>	> 60 mg His <sub>6</sub> -GFP/mL resin	NA	NA	NA
Max flow rate (20 cm bed height and 5 bar)	600 cm/h	600 cm/h	600 cm/h	600 cm/h
Chemical stability	Compatible with all standard aqueous buffers used for protein purification, 8 M urea, 6 M guanidine-HCl, non-ionic detergents, 20% ethanol. Chelating substances (e.g., EDTA) will strip off the metal ions. Stripped resin: 10 mM HCl (pH 2), 10 mM NaOH (pH 12), 10 mM sodium citrate-HCl (pH 3).			
pH stability	7 – 9 (working range) <sup>4</sup> 2 – 12 (cleaning, stripped resin)	7 – 9 (working range) <sup>4</sup> 2 – 12 (cleaning, stripped resin)	7 – 9 (working range) <sup>4</sup> 2 – 12 (cleaning, stripped resin)	7 – 9 (working range) <sup>4</sup> 2 – 12 (cleaning, stripped resin)
Storage	2 to 25 °C	2 to 25 °C	2 to 25 °C	2 to 25 °C

<sup>1</sup> The median particle size of the cumulative volume distribution.

<sup>2</sup> Metal ion capacity is determined by frontal analysis at 50% breakthrough using copper solution.

<sup>3</sup> The binding capacity is determined using a GoBio Mini Ni-NTA 1 mL column. The binding capacity is dependent on the size of the target protein, and on the competition of Impurities.

<sup>4</sup> This is the most common pH range for purification of His-tagged proteins.

## Product description IDA based resins

	WorkBeads 40 Ni-IDA	WorkBeads 40 Co-IDA	WorkBeads 40 Cu-IDA	WorkBeads 40 Zn-IDA
Target substance	His-tagged proteins, proteins containing histidine cysteine and/or tryptophan amino acid side chains			
Matrix	Highly cross-linked agarose	Highly cross-linked agarose	Highly cross-linked agarose	Highly cross-linked agarose
Average particle size <sup>1</sup> ( $D_{V50}$ )	45 µm	45 µm	45 µm	45 µm
Chelating ligand	Iminodiacetic acid (IDA)	IDA	IDA	IDA
Metal ion	Nickel (II)	Cobalt (II)	Copper (II)	Zink (II)
Metal ion capacity for the chelating ligand <sup>2</sup>	NA	NA	50 – 60 µmol Cu <sup>2+</sup> /mL resin	NA
Dynamic binding capacity <sup>3</sup>	> 60 mg His <sub>6</sub> -GFP/mL resin	NA	NA	NA
Max flow rate (20 cm Bed height and 5 bar)	600 cm/h	600 cm/h	600 cm/h	600 cm/h
Chemical stability	Compatible with all standard aqueous buffers used for protein purification, 8 M urea, 6 M guanidine-HCl, non-ionic detergents, 20% ethanol. Chelating substances (e.g., EDTA) will strip off the metal ions. Stripped resin: 10 mM HCl (pH 2), 10 mM NaOH (pH 12), 10 mM sodium citrate-HCl (pH 3).			
pH stability	7 – 9 (working range) <sup>4</sup> 2 – 12 (cleaning, stripped resin)	7 – 9 (working range) <sup>4</sup> 2 – 12 (cleaning, stripped resin)	7 – 9 (working range) <sup>4</sup> 2 – 12 (cleaning, stripped resin)	7 – 9 (working range) <sup>4</sup> 2 – 12 (cleaning, stripped resin)
Storage	2 to 25 °C	2 to 25 °C	2 to 25 °C	2 to 25 °C

<sup>1</sup> The median particle size of the cumulative volume distribution.

<sup>2</sup> Metal ion capacity is determined by frontal analysis at 50% breakthrough using copper solution.

<sup>3</sup> The binding capacity is determined using a GoBio Mini Ni-NTA 1 mL column. The binding capacity is dependent on the size of the target protein, and on the competition of Impurities.

<sup>4</sup> 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 <sup>1</sup>	Article number
<b>Prepacked columns</b>		
GoBio Mini NTA His-tag Screening kit 1 mL <sup>2</sup>	1 mL × 4	45 700 101
GoBio Mini NTA His-tag Screening kit 5 mL <sup>2</sup>	5 mL × 4	45 700 102
GoBio Mini IDA His-tag Screening kit 1 mL <sup>2</sup>	1 mL × 4	45 700 001
GoBio Mini IDA His-tag Screening kit 5 mL <sup>2</sup>	5 mL × 4	45 700 002
GoBio Mini Ni-NTA 1 mL	1 mL × 5	45 655 103
GoBio Mini Ni-NTA 5 mL	5 mL × 5	45 655 107
GoBio Mini Co-NTA 1 mL	1 mL × 5	45 655 133
GoBio Mini Co-NTA 5 mL	5 mL × 5	45 655 137
GoBio Mini Cu-NTA 1 mL	1 mL × 5	45 655 123
GoBio Mini Cu-NTA 5 mL	5 mL × 5	45 655 127
GoBio Mini Zn-NTA 1 mL	1 mL × 5	45 655 143
GoBio Mini Zn-NTA 5 mL	5 mL × 5	45 655 147
GoBio Mini Ni-IDA 1 mL	1 mL × 5	45 655 003
GoBio Mini Ni-IDA 5 mL	5 mL × 5	45 655 007
GoBio Mini Co-IDA 1 mL	1 mL × 5	45 655 033
GoBio Mini Co-IDA 5 mL	5 mL × 5	45 655 037
GoBio Mini Cu-IDA 1 mL	1 mL × 5	45 655 023
GoBio Mini Cu-IDA 5 mL	5 mL × 5	45 655 027
GoBio Mini Zn-IDA 1 mL	1 mL × 5	45 655 043
GoBio Mini Zn-IDA 5 mL	5 mL × 5	45 655 047
GoBio Mini NiMAC 1 mL	1 mL × 5	45 655 313
GoBio Mini NiMAC 5 mL	5 mL × 5	45 655 317
GoBio Mini Dsalt 1 mL	1 mL × 5	45 360 103
GoBio Mini Dsalt 5 mL	5 mL × 5	45 360 107
GoBio Screen 7x100 Ni-NTA <sup>3</sup>	3.8 mL × 1	55 651 001

Product name	Pack size <sup>1</sup>	Article number
GoBio Screen 7x100 Co-NTA <sup>3</sup>	3.8 mL × 1	55 651 401
GoBio Screen 7x100 Cu-NTA <sup>3</sup>	3.8 mL × 1	55 651 301
GoBio Screen 7x100 Zn-NTA <sup>3</sup>	3.8 mL × 1	55 651 501
GoBio Screen 7x100 Ni-IDA <sup>3</sup>	3.8 mL × 1	55 650 001
GoBio Screen 7x100 Co-IDA <sup>3</sup>	3.8 mL × 1	55 650 401
GoBio Screen 7x100 Cu-IDA <sup>3</sup>	3.8 mL × 1	55 650 301
GoBio Screen 7x100 Zn-IDA <sup>3</sup>	3.8 mL × 1	55 650 501
GoBio Prep 16x100 Ni-NTA <sup>3</sup>	20 mL × 1	55 651 021
GoBio Prep 26x100 Ni-NTA <sup>3</sup>	53 mL × 1	55 651 031
GoBio Prep 16x100 Co-NTA <sup>3</sup>	20 mL × 1	55 651 421
GoBio Prep 26x100 Co-NTA <sup>3</sup>	53 mL × 1	55 651 431
GoBio Prep 16x100 Cu-NTA <sup>3</sup>	20 mL × 1	55 651 321
GoBio Prep 26x100 Cu-NTA <sup>3</sup>	53 mL × 1	55 651 331
GoBio Prep 16x100 Zn-NTA <sup>3</sup>	20 mL × 1	55 651 521
GoBio Prep 26x100 Zn-NTA <sup>3</sup>	53 mL × 1	55 651 531
GoBio Prep 16x100 Ni-IDA <sup>3</sup>	20 mL × 1	55 650 021
GoBio Prep 26x100 Ni-IDA <sup>3</sup>	53 mL × 1	55 650 031
GoBio Prep 16x100 Co-IDA <sup>3</sup>	20 mL × 1	55 650 421
GoBio Prep 26x100 Co-IDA <sup>3</sup>	53 mL × 1	55 650 431
GoBio Prep 16x100 Cu-IDA <sup>3</sup>	20 mL × 1	55 650 321
GoBio Prep 26x100 Cu-IDA <sup>3</sup>	53 mL × 1	55 650 331
GoBio Prep 16x100 Zn-IDA <sup>3</sup>	20 mL × 1	55 650 521
GoBio Prep 26x100 Zn-IDA <sup>3</sup>	53 mL × 1	55 650 531
GoBio Prep 16x100 Dsalt <sup>3</sup>	20 mL × 1	55 700 021
GoBio Prep 26x100 Dsalt	53 mL × 1	55 700 031
GoBio Prod 80x200 Ni-NTA <sup>3</sup>	1 L × 1	55 651 042
GoBio Prod 130x200 Ni-NTA <sup>3</sup>	2.7 L × 1	55 651 062
GoBio Prod 200x200 Ni-NTA <sup>3</sup>	6 L × 1	55 651 072
GoBio Prod 240x200 Ni-NTA <sup>3</sup>	9 L × 1	55 651 082
GoBio Prod 330x250 Ni-NTA <sup>3</sup>	21.4 L × 1	55 651 093
GoBio Prod 80x200 Co-NTA <sup>3</sup>	1 L × 1	55 651 442
GoBio Prod 130x200 Co-NTA <sup>3</sup>	2.7 L × 1	55 651 462
GoBio Prod 200x200 Co-NTA <sup>3</sup>	6 L × 1	55 651 472
GoBio Prod 240x200 Co-NTA <sup>3</sup>	9 L × 1	55 651 482
GoBio Prod 330x250 Co-NTA <sup>3</sup>	21.4 L × 1	55 651 493
GoBio Prod 80x200 Cu-NTA <sup>3</sup>	1 L × 1	55 651 342
GoBio Prod 130x200 Cu-NTA <sup>3</sup>	2.7 L × 1	55 651 362
GoBio Prod 200x200 Cu-NTA <sup>3</sup>	6 L × 1	55 651 372
GoBio Prod 240x200 Cu-NTA <sup>3</sup>	9 L × 1	55 651 382
GoBio Prod 330x250 Cu-NTA <sup>3</sup>	21.4 L × 1	55 651 393

Product name	Pack size <sup>1</sup>	Article number
GoBio Prod 80x200 Zn-NTA <sup>3</sup>	1 L × 1	55 651 542
GoBio Prod 130x200 Zn-NTA <sup>3</sup>	2.7 L × 1	55 651 562
GoBio Prod 200x200 Zn-NTA <sup>3</sup>	6 L × 1	55 651 572
GoBio Prod 240x200 Zn-NTA <sup>3</sup>	9 L × 1	55 651 582
GoBio Prod 330x250 Zn-NTA <sup>3</sup>	21.4 L × 1	55 651 593
GoBio Prod 80x200 Ni-IDA <sup>3</sup>	1 L × 1	55 650 042
GoBio Prod 130x200 Ni-IDA <sup>3</sup>	2.7 L × 1	55 650 062
GoBio Prod 200x200 Ni-IDA <sup>3</sup>	6 L × 1	55 650 072
GoBio Prod 240x200 Ni-IDA <sup>3</sup>	9 L × 1	55 650 082
GoBio Prod 330x250 Ni-IDA <sup>3</sup>	21.4 L × 1	55 650 093
GoBio Prod 80x200 Co-IDA <sup>3</sup>	1 L × 1	55 650 442
GoBio Prod 130x200 Co-IDA <sup>3</sup>	2.7 L × 1	55 650 462
GoBio Prod 200x200 Co-IDA <sup>3</sup>	6 L × 1	55 650 472
GoBio Prod 240x200 Co-IDA <sup>3</sup>	9 L × 1	55 650 482
GoBio Prod 330x250 Co-IDA <sup>3</sup>	21.4 L × 1	55 650 493
GoBio Prod 80x200 Cu-IDA <sup>3</sup>	1 L × 1	55 650 342
GoBio Prod 130x200 Cu-IDA <sup>3</sup>	2.7 L × 1	55 650 362
GoBio Prod 200x200 Cu-IDA <sup>3</sup>	6 L × 1	55 650 372
GoBio Prod 240x200 Cu-IDA <sup>3</sup>	9 L × 1	55 650 382
GoBio Prod 330x250 Cu-IDA <sup>3</sup>	21.4 L × 1	55 650 393
GoBio Prod 80x200 Zn-IDA <sup>3</sup>	1 L × 1	55 650 542
GoBio Prod 130x200 Zn-IDA <sup>3</sup>	2.7 L × 1	55 650 562
GoBio Prod 200x200 Zn-IDA <sup>3</sup>	6 L × 1	55 650 572
GoBio Prod 240x200 Zn-IDA <sup>3</sup>	9 L × 1	55 650 582
GoBio Prod 330x250 Zn-IDA <sup>3</sup>	21.4 L × 1	55 650 593
<b>Bulk resins</b>		
WorkBeads Dsalt	300 mL	40 360 003
	1 L	40 360 010

<sup>1</sup> All different pack sizes are available on [www.bio-works.com](http://www.bio-works.com)

<sup>2</sup> Includes one column each charged with Ni<sup>2+</sup>, Co<sup>2+</sup>, Cu<sup>2+</sup> or Zn<sup>2+</sup>.

<sup>3</sup> Packed on request.

## Ordering information

Product name	Pack size	Article number
WorkBeads 40 Ni-NTA	25 mL	40 651 001
	150 mL	40 651 003
	1 L	40 651 010
WorkBeads 40 Co-NTA	25 mL	40 651 401
	150 mL	40 651 403
	1 L	40 651 410
WorkBeads 40 Cu-NTA	25 mL	40 651 301
	150 mL	40 651 303
	1 L	40 651 310
WorkBeads 40 Zn-NTA	25 mL	40 651 501
	150 mL	40 651 503
	1 L	40 651 510
WorkBeads 40 Ni-IDA	25 mL	40 650 001
	150 mL	40 650 003
	1 L	40 650 010
WorkBeads 40 Co-IDA	25 mL	40 650 401
	150 mL	40 650 403
	1 L	40 650 410
WorkBeads 40 Cu-IDA	25 mL	40 650 301
	150 mL	40 650 303
	1 L	40 650 310
WorkBeads 40 Zn-IDA	25 mL	40 650 501
	150 mL	40 650 503
	1 L	40 650 510

Orders: [sales@bio-works.com](mailto:sales@bio-works.com) or contact your local distributor.

For more information about local distributor and products visit [www.bio-works.com](http://www.bio-works.com) or contact us at [info@bio-works.com](mailto:info@bio-works.com)

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IN 40 650 010 BB