Bio-Works

INSTRUCTION

GoBio Prep charged NTA & IDA

GoBio Prep 16x100 Ni-NTA, GoBio Prep 26x100 Ni-NTA, GoBio Prep 16x100 Co-NTA, GoBio Prep 26x100 Co-NTA, GoBio Prep 16x100 Cu-NTA, GoBio Prep 26x100 Cu-NTA, GoBio Prep 16x100 Zn-NTA and GoBio Prep 26x100 Zn-NTA

GoBio Prep 16x100 Ni-IDA, GoBio Prep 26x100 Ni-IDA, GoBio Prep 16x100 Co-IDA, GoBio Prep 26x100 Co-IDA, GoBio Prep 16x100 Cu-IDA, GoBio Prep 26x100 Cu-IDA, GoBio Prep 16x100 Zn-IDA and GoBio Prep 26x100 Zn-IDA

GoBio™ Prep 16x100 NTA and GoBio 26x100 NTA are columns prepacked with WorkBeads™ 40 NTA precharged with Ni^{2+}, Co^{2+}, Cu^{2+} or Zn^{2+}. GoBio Prep 16x100 IDA and GoBio 26x100 IDA are columns prepacked with WorkBeads 40 IDA precharged with Ni^{2+}, Co^{2+}, Cu^{2+} or Zn^{2+}. These prepacked columns allows quick and easy purification of His-tagged proteins and other proteins with affinity for metal ions.

- Prepacked, ready-to-use columns for fast and reproducible purifications of hHs-tagged proteins
- High binding capacity and different selectivities.using different metal ions
- Easy scale-up

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.

GoBio prepacked column family is developed for convenient, reproducible, and fast results and can be used from small scale purification through process development to 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-IDA 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

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 immobilized nitrilotriacetic acid (NTA) or iminodiacetic acid (IDA) chelating ligands as illustrated in Figure 1.

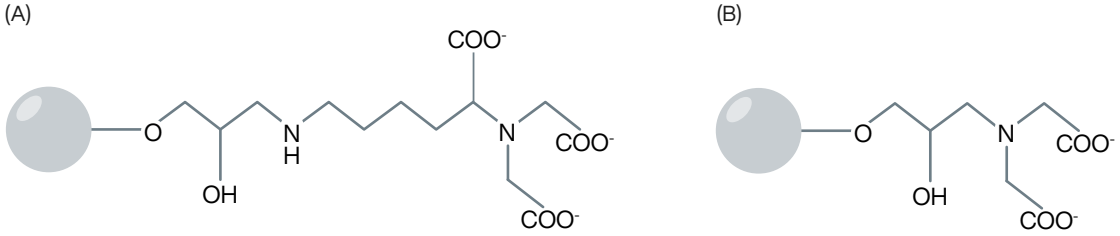

Figure 1. Structure of the chelating ligands used in WorkBeads 40 NTA (A) and WorkBeads 40 IDA (B) resins.

IMAC is commonly 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, screening the sample 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 offers two different Screening kits with precharged WorkBeads IMAC resins prepacked in GoBio Mini 1 mL and 5 mL columns.

Imidazole is recommended for elution. This is 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 agent in to prevent non-specific binding of endogenous proteins that may bind via histidine clusters for example. This is easily done by using the recommended binding buffer.

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

Note: If eucaryotic cells are used for expression and/or reducing agents like DTT and chelating agents like EDTA need to be included during purification use instead WorkBeads NiMAC, also available in several different prepacked GoBio column formats, read more on www.bio-works.com

GoBio Prep column characteristics

Make sure when using GoBio Prep columns that the connectors are tightened to prevent leakage. The pressure over the packed bed varies depending on different parameters such as the resin characteristics, sample/buffer viscosities and the tubings used. Make sure that the flow through the column is according to the arrow on the column.

These columns should not be opened and refilled.

Note: GoBio Prep column hardware is compatible with most aqueous chemicals, but NOT with concentrated alcohol. Maximal alcohol concentration is 20%.

Table 1. GoBio Prep 16x100 and GoBio Prep 26x100 columns characteristics.

Column characteristics	
Column hardware	Acrylic
Top and bottom plugs	Polypropylene
Top and bottom filters	Polyamid
Connections	1/16” female thread in both ends
Column volumes	20 mL (GoBio Prep 16x100) 53 mL (GoBio Prep 26x100)
Column dimensions	16 × 100 mm (GoBio Prep 16x100) 26 × 100 mm (GoBio Prep 26x100)
Maximal column hardware pressure1	5 bar, 0.5 MPa, 70 psi

[1] 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

Resins characteristics

The WorkBeads IMAC precharged 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.

The characteristics of GoBio Prep 16x100 NTA/IDA charged with $Ni2^{+}$, Co^{2+}, Cu^{2+} or Zn^{2+} ions and GoBio Prep 26x100 NTA/IDA charged with Ni^{2+}, Co^{2+}, Cu^{2+} or Zn^{2+} ions are listed in section “Product Description”.

Unpacking and connecting GoBio Prep 16x100 and GoBio Prep 26x100 columns to a chromatography system

*Each packed column is sealed with a pressure syringe on the **bottom** of the column. It is then placed in a sealed plastic bag.*

1. Cut the plastic bag and remove the column with care.
2. Follow the flow direction (indicated by an arrow on the column label) to clamp the column onto the chromatography system or to a vertical stand.
3. Prepare the chromatography system for connecting the column. The GoBio Prep 16x100 and GoBio Prep 26x100 columns are compatible with 1/16” male connectors with narrow heads. The length of the connector thread must be at least 7 mm to avoid leakage.

   Note: It is recommended to use the two red connectors attached to the transport syringe when connecting the column to a chromatography system. One red connector should be used in each end of the column.
4. Gently unhook the springs from the shaft top of the transport syringe using even force.
5. Remove the syringe and keep it for further use during storage.
6. Unscrew the top plug, some liquid may come out. Connect the column to the chromatography system using one of the red connectors “drop-to-drop” avoiding introducing air into the packed column.
7. Connect the bottom of the column to the chromatography system using the second red connector.

Buffer preparation

The buffer species and buffer concentration are important for robust and reproducible methods. Choose a suitable pH and buffer for the binding of the target protein.

The binding conditions should be optimized to achieve binding of the target molecule, while minimizing the binding of impurities. See examples of buffers to use for samples with unknown charge properties in Table 2.

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

Table 2. Recommended buffers for purification of His-tagged proteins.

Buffer	Composition
Binding buffer[1]	50 mM sodium phosphate buffer, 300 mM NaCl, 10 mM imidazole, pH 8.0
Washing buffer[1]	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

[1] The imidazole concentration may have to be optimized. A too high concentration may elute the target during washing. An imidazole concentration just below where the target proteins is 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.

Purification

When using affinity chromatography it is recommended to do an initial wash with a Cleaning-in-Place (CIP) solution before the first purification to wash out any loosely bound ligands and/or metal ions to stabilize the binding capacity over time.

Note: Do not exceed the maximum recommended flow rate and back pressure for the column, see Product description".

1. Wash out the storage solution with 1 - 2 column volumes (CV) deionized/distilled water if the binding buffer salts may precipitate upon exposure to ethanol. Use a reduced flow rate, 50% of the maximum flow rate when washing out the storage solution.

   This step can be omitted if precipitation is not likely to be a problem.
2. Equilibrate with 5 - 10 CV binding buffer.
3. Apply the sample.
4. Wash with 5 - 20 CV binding buffer until the UV trace of the effluent returns to near baseline.
5. Elute with elution buffer using either a linear gradient, for example, from 10 mM to 300 mM imidazole in 10 - 20 CV or step elution, 5 - 10 CV with binding buffer including the preferred imidazole concentration.

   When gradient elution is used most host cell protein impurities will elute earlier than the His-tagged protein. This reduces the need for a stringent washing solution
6. Wash with 5 CV elution buffer including for example 1 M imidazole to remove any remaining impurities.
7. If required perform a cleaning-in-place (CIP), see page 8.
8. For storage wash the column with at least 5 CV 20% ethanol.

   Use a reduced flow rate, 50% of the maximum flow rate when equilibration with the storage solution.
9. Make sure that the stop plugs are tight to prevent leakage.

   For prolonged storage, connect the included syringe filled with storage solution to the bottom end of the column.

Purification additives

GoBio Prep precharged 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.

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 7.0 - 8.5. A lower pH protonates the histidine residues (pKa 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 this treatment. In some case 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 often gives increased purity compared to step elution, but step elution may be desired to obtain the highest possible concentration of the target protein and is most common in large-scale purifications. The imidazole concentration can be optimized for step elution by carry out an initial linear gradient test run to find required concentration for elution, see Figure 2.

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

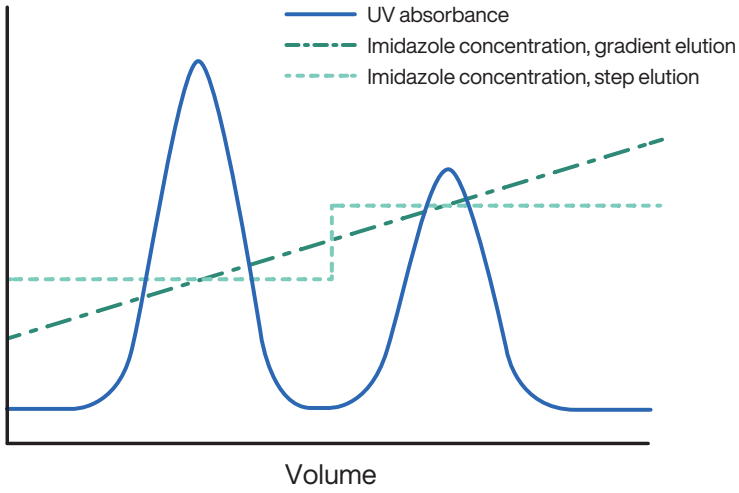

Figure 2. Optimization of step elution with salt. A test run with linear gradient elution gives information about suitable salt concentrations to be used in step elution.

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

Scale-up

After laboratory scale purifications using GoBio Prep 16x100 or GoBio Prep 26x100 columns the column volume can easily be scaled-up by using larger prepacked columns such as, GoBio Prod prepacked columns. GoBio Prod prepacked column family has column sizes starting from 1 L. Bulk packages of all different WorkBeads resins can also be packed into other column formats of choice.

Large-scale purification is often carried out in columns with bed heights of 200 - 300 mm and a diameter depending on the needed column volume.

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., 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:

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

Flow

The concepts of volumetric flow, linear flow rate and residence time are important when scaling-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 work 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 that was 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 3 for examples. If the column bed heights are kept constant during scale-up the linear flow rate should be kept constant (as well as the residence time).

Table 3. 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

His-tagged protein purification on WorkBeads IMAC resins 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 used to remove remaining proteins and/or impurities from the sample. In research-scale purification, size exclusion chromatography (SEC/gel filtration) 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. These resins are also available as ready-to-use prepacked GoBio columns with several different column sizes.

Desalting and buffer exchange

Buffer exchange or desalting of a sample can be used before analysis and/or after purification by 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 for larger sample volumes or when samples need to be processed rapidly to avoid degradation. For even larger sample volumes prepacked GoBio Prod Dsalt columns starting from 1 L are recommended or diafiltration can be used.

To find out more about Bio-Works' chromatography products visit www.bio-works.com

Maintenance

Cleaning-in-Place (CIP)

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:

Stripping and recharging with metal ions

1. 2-3 CV deionized water
2. 2-5 CV 50 mM Na_2EDTA, pH 8.0
3. 3-4 CV 100 mM NaOH
4. 5-10 CV deionized water
5. 2 CV 50 mM metal salt solution in deionized water
6. 5-10 CV deionized water
7. 3-5 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.

Storage

Store at 2 to 25°C in 20% ethanol.

For prolonged storage, connect the included transport syringe filled with storage solution to the bottom end of the column.

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

Product description NTA based resins

	GoBio Prep 16x100 Ni-NTA **GoBio Prep 26x100 Ni-NTA**	**GoBio Prep 16x100 Co-NTA** **GoBio Prep 26x100 Co-NTA**	**GoBio Prep 16x100 Cu-NTA** **GoBio Prep 26x100 Cu-NTA**	**GoBio Prep 16x100 Zn-NTA** **GoBio Prep 26x100 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
agarose	45 µm	45 µm	45 µm	45 µm
Average particle size (D_{V50})[1]	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[2]	NA	NA	50 - 60 µmol Cu^{2+}/mL resin	NA
Dynamic binding capacity[3]	> 60 mg His_6-GFP/mL resin	NA	NA	NA
Column volumes	20 mL (16x100) 53 mL (26x100)	20 mL (16x100) 53 mL (26x100)	20 mL (16x100) 53 mL (26x100)	20 mL (16x100) 53 mL (26x100)
Column dimensions	16 × 100 mm 26 × 100 mm	16 × 100 mm 26 × 100 mm	16 × 100 mm 26 × 100 mm	16 × 100 mm 26 × 100 mm
Recommended flow rate[4]				
(16x100)	4 - 6 mL/min (120 - 180 cm/h)	4 - 6 mL/min (120 - 180 cm/h)	4 - 6 mL/min (120 - 180 cm/h)	4 - 6 mL/min (120 - 180 cm/h)
(26x100)	10 - 15 mL/min (115 - 170 cm/h)	10 - 15 mL/min (115 - 170 cm/h)	10 - 15 mL/min (115 - 170 cm/h)	10 - 15 mL/min (115 - 170 cm/h)
Maximum flow rate[5]				
(16x100)	8 mL/min (240 cm/h)	8 mL/min (240 cm/h)	8 mL/min (240 cm/h)	8 mL/min (240 cm/h)
(26x100)	20 mL/min (230 cm/h)	20 mL/min (230 cm/h)	20 mL/min (230 cm/h)	20 mL/min (230 cm/h)
Maximum back pressure[6]	5 bar, 0.5 MPa, 70 p	5 bar, 0.5 MPa, 70 p	5 bar, 0.5 MPa, 70 p	5 bar, 0.5 MPa, 70 p
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)[7] 2 - 12 (cleaning, stripped resin)	7 - 9 (working range)[7] 2 - 12 (cleaning, stripped resin)	7 - 9 (working range)[7] 2 - 12 (cleaning, stripped resin)	7 - 9 (working range)[7] 2 - 12 (cleaning, stripped resin)
Storage	2 to 25 °C in 20% ethanol	2 to 25 °C in 20% ethanol	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 Metal ion capacity is determined by frontal analysis at 50% breakthrough using cupper solution.
3 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 of impurities.
4 Optimal flow rate during binding is depending on the sample.
5 Maximum flow rate for 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 for 20% ethanol).
6 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.
7 This is the most common pH range for purification of His-tagged proteins.

Product description IDA based resins

	GoBio Prep 16x100 Ni-IDA **GoBio Prep 26x100 Ni-IDA**	**GoBio Prep 16x100 Co-IDA** **GoBio Prep 26x100 Co-IDA**	**GoBio Prep 16x100 Cu-IDA** **GoBio Prep 26x100 Cu-IDA**	**GoBio Prep 16x100 Zn-IDA** **GoBio Prep 26x100 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 (D_{V50})[1]	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[2]	NA	NA	50 - 60 µmol Cu^{2+}/mL resin	NA
Dynamic binding capacity[3]	> 60 mg His_6-GFP/mL resin	NA	NA	NA
Column volumes	20 mL (16x100) 53 mL (26x100)	20 mL (16x100) 53 mL (26x100)	20 mL (16x100) 53 mL (26x100)	20 mL (16x100) 53 mL (26x100)
Column dimensions	16 × 100 mm 26 × 100 mm	16 × 100 mm 26 × 100 mm	16 × 100 mm 26 × 100 mm	16 × 100 mm 26 × 100 mm
Recommended flow rate[4]				
(16x100)	4 - 6 mL/min (120 - 180 cm/h)	4 - 6 mL/min (120 - 180 cm/h)	4 - 6 mL/min (120 - 180 cm/h)	4 - 6 mL/min (120 - 180 cm/h)
(26x100)	10 - 15 mL/min (115 - 170 cm/h)	10 - 15 mL/min (115 - 170 cm/h)	10 - 15 mL/min (115 - 170 cm/h)	10 - 15 mL/min (115 - 170 cm/h)
Maximum flow rate[5]				
(16x100)	8 mL/min (240 cm/h)	8 mL/min (240 cm/h)	8 mL/min (240 cm/h)	8 mL/min (240 cm/h)
(26x100)	20 mL/min (230 cm/h)	20 mL/min (230 cm/h)	20 mL/min (230 cm/h)	20 mL/min (230 cm/h)
Maximum back pressure[6]	5 bar, 0.5 MPa, 70 psi	5 bar, 0.5 MPa, 70 psi	5 bar, 0.5 MPa, 70 psi	5 bar, 0.5 MPa, 70 psi
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)[7] 2 - 12 (cleaning, stripped resin)	7 - 9 (working range)[7] 2 - 12 (cleaning, stripped resin)	7 - 9 (working range)[7] 2 - 12 (cleaning, stripped resin)	7 - 9 (working range)[7] 2 - 12 (cleaning, stripped resin)
Storage	2 to 25 °C in 20% ethanol	2 to 25 °C in 20% ethanol	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 Metal ion capacity is determined by frontal analysis at 50% breakthrough using cupper solution.
3 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 of impurities.
4 Optimal flow rate during binding is depending on the sample.
5 Maximum flow rate for 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 for 20% ethanol).
6 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.
7 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
GoBio Mini NTA His-tag Screening kit 1 m[2]	1 mL × 4	45 700 101
GoBio Mini NTA His-tag Screening kit 5 mL[2]	5 mL × 4	45 700 102
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 IDA His-tag Screening kit 1 mL[2]	1 mL × 4	45 700 001
GoBio Mini IDA His-tag Screening kit 5 mL[2]	5 mL × 4	45 700 002
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 Dsalt 1 mL	1 mL × 5	45 360 103
GoBio Mini Dsalt 5 mL	5 mL × 5	45 360 107
GoBio Prep 16x100 Dsalt[3]	20 mL × 1	55 700 021
GoBio Prep 26x100 Dsalt	53 mL × 1	55 700 031

Product name	Pack size[1]	Article number
GoBio Screen 7x100 Ni-NTA[3]	3.8 mL × 1	55 651 001
GoBio Screen 7x100 Co-NTA[3]	3.8 mL × 1	55 651 401
GoBio Screen 7x100 Cu-NTA[3]	3.8 mL × 1	55 651 301
GoBio Screen 7x100 Zn-NTA[3]	3.8 mL × 1	55 651 401
GoBio Screen 7x100 Ni-IDA[3]	3.8 mL × 1	55 650 001
GoBio Screen 7x100 Co-IDA[3]	3.8 mL × 1	55 650 401
GoBio Screen 7x100 Cu-IDA[3]	3.8 mL × 1	55 650 301
GoBio Screen 7x100 Zn-IDA[3]	3.8 mL × 1	55 650 501
GoBio Prod 80x200 Ni-NTA[3]	1 L	55 651 041
GoBio Prod 130x200 Ni-NTA[3]	2.7 L	55 651 062
GoBio Prod 200x200 Ni-NTA[3]	6 L	55 651 072
GoBio Prod 240x200 Ni-NTA[3]	9 L	55 651 082
GoBio Prod 330x250 Ni-NTA[3]	21.4 L	55 651 093
GoBio Prod 80x200 Co-NTA[3]	1 L	55 651 442
GoBio Prod 130x200 Co-NTA[3]	2.7 L	55 651 462
GoBio Prod 200x200 Co-NTA[3]	6 L	55 651 472
GoBio Prod 240x200 Co-NTA[3]	9 L	55 651 482
GoBio Prod 330x250 Co-NTA[3]	21.4 L	55 651 493
GoBio Prod 80x200 Cu-NTA[3]	1 L	55 651 342
GoBio Prod 130x200 Cu-NTA[3]	2.7 L	55 651 362
GoBio Prod 200x200 Cu-NTA[3]	6 L	55 651 372
GoBio Prod 240x200 Cu-NTA[3]	9 L	55 651 382
GoBio Prod 330x250 Cu-NTA[3]	21.4 L	55 651 393
GoBio Prod 80x200 Zn-NTA[3]	1 L	55 651 542
GoBio Prod 130x200 Zn-NTA[3]	2.7 L	55 651 562
GoBio Prod 200x200 Zn-NTA[3]	6 L	55 651 572
GoBio Prod 240x200 Zn-NTA[3]	9 L	55 651 582
GoBio Prod 330x250 Zn-NTA[3]	21.4 L	55 655 193
GoBio Prod 80x200 Ni-IDA[3]	1 L	55 650 042
GoBio Prod 130x200 Ni-IDA[3]	2.7 L	55 650 062
GoBio Prod 200x200 Ni-IDA[3]	6 L	55 650 072
GoBio Prod 240x200 Ni-IDA[3]	9 L	55 650 082
GoBio Prod 330x250 Ni-IDA[3]	21.4 L	55 650 093
GoBio Prod 80x200 Co-IDA[3]	1 L	55 650 442
GoBio Prod 130x200 Co-IDA[3]	2.7 L	55 650 462
GoBio Prod 200x200 Co-IDA[3]	6 L	55 650 472
GoBio Prod 240x200 Co-IDA[3]	9 L	55 650 482
GoBio Prod 330x250 Co-IDA[3]	21.4 L	55 650 493

Product name	Pack size[1]	Article number
GoBio Prod 80x200 Cu-IDA[3]	1 L	55 650 342
GoBio Prod 130x200 Cu-IDA[3]	2.7 L	55 650 362
GoBio Prod 200x200 Cu-IDA[3]	6 L	55 650 372
GoBio Prod 240x200 Cu-IDA[3]	9 L	55 650 382
GoBio Prod 330x250 Cu-IDA[3]	21.4 L	55 650 393
GoBio Prod 80x200 Zn-IDA[3]	1 L	55 650 542
GoBio Prod 130x200 Zn-IDA[3]	2.7 L	55 650 562
GoBio Prod 200x200 Zn-IDA[3]	6 L	55 650 572
GoBio Prod 240x200 Zn-IDA[3]	9 L	55 650 582
GoBio Prod 330x250 Zn-IDA[3]	21.4 L	55 650 593
Bulk resins		
WorkBeads 40 Ni-NTA	25 mL 150 mL 1 L	40 651 001 40 651 003 40 651 010
WorkBeads 40 Co-NTA	25 mL 150 mL 1 L	40 651 401 40 651 403 40 651 410
WorkBeads 40 Cu-NTA	25 mL 150 mL 1 L	40 651 301 40 651 303 40 651 310
WorkBeads 40 Zn-NTA	25 mL 150 mL 1 L	40 651 501 40 651 503 40 651 510
WorkBeads 40 Ni-IDA	25 mL 150 mL 1 L	40 650 001 40 650 003 40 650 010
WorkBeads 40 Co-IDA	25 mL 150 mL 1 L	40 650 401 40 650 403 40 650 410
WorkBeads 40 Cu-IDA	25 mL 150 mL 1 L	40 650 301 40 650 303 40 650 310
WorkBeads 40 Zn-IDA	25 mL 150 mL 1 L	40 650 501 40 650 503 40 650 510
WorkBeads Dsalt	300 mL 1 L	40 360 003 40 360 010

1 All different pack sizes are available on www.bio-works.com
2 Includes one column each charged with Ni^{2+}, Co^{2+}, Cu^{2+} or Zn^{2+}
3 Packed on request.

Ordering information

Product name	Pack size	Article number
GoBio Prep 16x100 Ni-NTA[1]	20 mL × 1	55 651 021
GoBio Prep 26x100 Ni-NTA[1]	53 mL × 1	55 651 031
GoBio Prep 16x100 Co-NTA[1]	20 mL × 1	55 651 421
GoBio Prep 26x100 Co-NTA[1]	53 mL × 1	55 651 431
GoBio Prep 16x100 Cu-NTA[1]	20 mL × 1	55 651 321
GoBio Prep 26x100 Cu-NTA[1]	53 mL × 1	55 651 331
GoBio Prep 16x100 Zn-NTA[1]	20 mL × 1	55 651 521
GoBio Prep 26x100 Zn-NTA[1]	53 mL × 1	55 651 531
GoBio Prep 16x100 Ni-IDA[1]	20 mL × 1	55 650 021
GoBio Prep 26x100 Ni-IDA[1]	53 mL × 1	55 650 031
GoBio Prep 16x100 Co-IDA[1]	20 mL × 1	55 650 421
GoBio Prep 26x100 Co-IDA[1]	53 mL × 1	55 650 431
GoBio Prep 16x100 Cu-IDA[1]	20 mL × 1	55 650 321
GoBio Prep 26x100 Cu-IDA[1]	53 mL × 1	55 650 331
GoBio Prep 16x100 Zn-IDA[1]	20 mL × 1	55 650 521
GoBio Prep 26x100 Zn-IDA[1]	53 mL × 1	55 650 531

[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

Bio-Works, Virdings allé 18, 754 50 Uppsala, Sweden. For local office contact information, visit bio-works.com/contact.

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