Bio-Works

INSTRUCTION

GoBio Screen 7x100 NiMAC

GoBio™ Screen 7x100 NiMAC are prepacked columns for fast and easy optimization of methods and parameters, such as selectivity, binding and elution conditions, as well as for small-scale purifications. These columns are prepacked with WorkBeads™ NiMAC resin which allows quick and easy purification of His-tagged proteins and other proteins with an affinity for nickel ions. WorkBeads NiMAC resin is precharged with very strongly bound nickel ions resulting in very high resistance to reducing agents such as DTT and chelating substances such as EDTA. The high stability allows purification of proteins from sources such as eukaryotic cell extracts, that normally would cause significant nickel ion stripping from the resin. This reduces the need for sample pre-treatment. The resin provides high purity and binding capacity and the possibility to use high flow rates for minimized process time.

- Prepacked, ready-to-use columns for fast and reproducible optimization of methods and parameters
- Highly resistant to up to 20 mM DTT and 20 mM EDTA
- 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.

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 Sheet (SDS) for WorkBeads NiMAC and the safety instructions for any equipment to be used.

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.

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 NiMAC columns are recommended for His-tagged protein purification when minimal Ni^{2+} leakage is necessary and also when higher concentrations of DTT (20 mM) and EDTA (20 mM) have to be included in the sample/buffers. For optimization of for example purity, a screening is recommended using the eight available different pre-charged WorkBeads IMAC resins are recommended to find the optimal combination of ligand and metal ion, see “Related products”. Bio-Works also offer two different Screening kits with pre-charged WorkBeads IMAC resins prepacked in 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 column should be equilibrated with a binding buffer containing a low concentration of the competing ligand to prevent non-specific binding of host cell proteins that may bind via, e.g., histidine clusters on their surface.

Elution with a continuously decreasing pH gradient is an alternative to imidazole and after optimization a pH step gradient may be more appropriate for scale-up. At pH 3 - 5, the histidine residues (pK_a approx. 6) are protonated which leads to the loss of affinity for the metal ion and thus to the release of the protein. It is important to consider the target protein stability at low pH.

GoBio Screen 7x100 column characteristics

When using GoBio Screen 7x100 columns make sure that the connectors are tightened to prevent leakage. The pressure over the packed bed varies depending on 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: The GoBio Screen 7x100 column hardware is compatible with most aqueous chemicals, but NOT with concentrated alcohol. Maximum alcohol concentration is 20%.

Table 1. GoBio Screen 7x100 column characteristics.

Column characteristics	
Column hardware	Acrylic
Top and bottom plugs	Polypropylene
Top and bottom filters	Polyamide
Connections	1/16” female thread in both ends
Column volume	3.8 mL
Column dimension	7 × 100 mm
Maximum column hardware pressure[1]	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

WorkBeads NiMAC is an agarose-based chromatographic resin immobilized with chelating groups that bind nickel ions very tightly. The Ni^{2+}-charged resin binds His-tagged proteins but has unusually low affinity for other host proteins that tend to bind other resins made for Immobilized Metal Ion Affinity Chromato¬graphy (IMAC).

The very tightly bound nickel ions result in extremely low nickel ion leakage. This property makes WorkBeads NiMAC excellent for purification of His-tagged proteins from large feed volumes containing chelating substances. Typical sources of this problem are extracts from eukaryotic cells, e.g., insect cells, that commonly contain reducing agents added during the extraction. WorkBeads NiMAC resin cannot be stripped of the Ni^{2+} ion and recharged.

The characteristics of GoBio Screen 7x100 NiMAC are listed in section “Product description”.

General process development

The GoBio Screen 7x100 column format is very useful for screening of parameters and method optimization, as well as for robustness testing when developing a new purification protocol and process. The bed height of 100 mm in combination with the narrow column diameter of 7 mm gives a column volume of only 3.8 mL which minimizes both sample and buffer consumptions when performing scalable experiments at relevant process flow rates.

Below can be seen the typical steps during general process development. Remember to, right from the start, take into consideration process cost, resin cleaning possibilities and environmental constraints.

1. Initial experiments in which Design of Experiments (DoE) is an effective tool.
2. Screening of parameters and optimization.
3. Find optimal conditions by evaluation of data.
4. Test of robustness.
5. Scale-up.

In process development an important goal is to determine the robustness criteria for the process. The robustness test evaluates factors that may cause variability in, for example, yield and purity.

Purification planning

Unpacking and connecting GoBio Screen 7x100 column to a chromatography system

*Each packed column is sealed with a pressure syringe in the **bottom** end 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 Screen 7x100 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 Ni^{2+}-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 there is a risk that 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. Do an initial wash with 3 - 5 CV 0.5 M NaOH for 15 minutes followed by 5 - 10 CV deionized water.
3. Equilibrate with 5 - 10 CV binding buffer.
4. Apply the sample.
5. Wash with 5 - 20 CV binding buffer until the UV trace of the effluent returns to near baseline.
6. 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

7. Wash with 5 CV elution buffer including for example 1 M imidazole to remove any remaining impurities.
8. If required perform a cleaning-in-place (CIP), see page 8.
9. For storage wash the column with at least 5 CV 20% ethanol.

   Use a reduced flow rate, 50% of the maximum flow rate when equilibrating with the storage solution.
10. 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

A multitude of additives can be used in IMAC, including various buffer substances, including DTT, EDTA, 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.

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 (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 this treatment. In some case subsequent renaturation is desired.

Addition of reducing agents

It is possible to include up to 20 mM DTT or other similar reducing agents, and 20 mM EDTA or other chelators both in the sample and buffers without losing any binding capacity.

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 1.

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

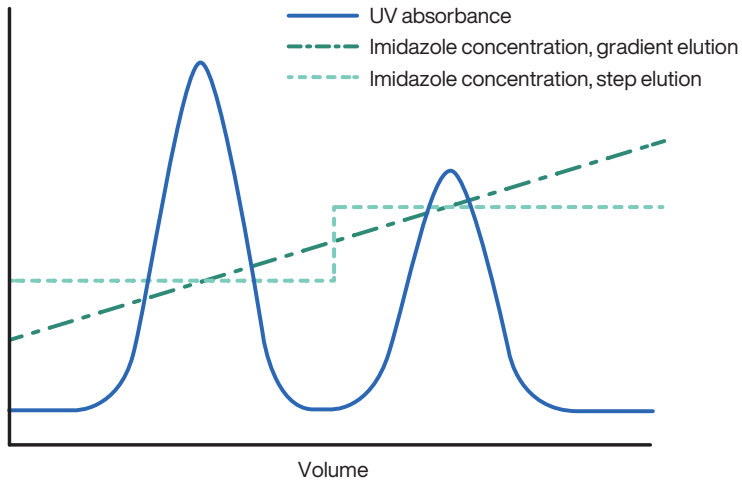

Figure 1. Optimization of step elution with salt. A test run with linear gradient elution gives information about suitable salt concentrations to be used in step elution. Note: Remember to take the system dead volume into account when comparing the gradient and the trace.

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 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 NiMAC resin 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 and WorkBeads 40 DEAE resins provide different selectivities for ion exchange chromatography.

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) prepacked columns depending on sample volumes. Go Bio 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.

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

Maintenance

Cleaning-in-Place (CIP)

When running complex feeds, small amounts of impurities tend to adsorb to the resin by unspecific interactions. Cleaning of resin with up to 0.5 M NaOH for 15 minutes followed by 5 - 10 CV deionized H_2O in repeated cycles are recommended if resin gets fouled.

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 descriptions

	GoBio Screen 7x100 NiMAC
Target substance	His-tagged proteins
Resin	WorkBeads NiMAC
Matrix	Highly cross-linked agarose
Average particle size1 (D_{V50})	45 µm
Pre-charged ions	Nickel (II) ions, Ni^{2+}
Static binding capacity	> 80 mg/mL resin
Dynamic binding capacity[2]	> 40 mg/mL resin
Metal ion capacity[3]	> 60 µmol Cu^{2+}/mL resin
Column volume	3.8 mL
Column dimension	7 × 100 mm
Recommended flow rate[4]	1.0 - 2.6 mL/min (155 - 405 cm/h)
Maximum flow rate[5]	5 mL/min (780 cm/h)
Maximum back pressure[6]	5 bar, 0.5 MPa, 70 psi
Chemical stability	Compatible with all standard aqueous buffers used for protein purification, and additives such as 20 mM Na_2-EDTA, 20 mM dithiothreitol (DTT), 20 mM TCEP, 20 mM β-mercaptoethanol, 8 M urea, 6 M guanidine-HCl, non-ionic detergents, 500 mM imidazole, 30% isopropanol, 0.5 M NaOH
pH stability	3 - 9 (working range)[7] 2 - 14 (cleaning-in-place)
Storage	2 to 25 °C in 20% ethanol

1 The median particle size of the cumulative volume distribution.
2 Binding capacity may vary depending on protein characteristics and on flow rate used. A lower flow rate usually increases the dynamic binding capacity.
3 Metal ion capacity is determined by frontal analysis at 50% breakthrough using copper solution.
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 pH range is the most used pH 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 NiMAC 1 mL	1 mL × 1 1 mL × 5 1 mL × 10	45 655 311 45 655 313 45 655 314
GoBio Mini NiMAC 5 mL	5 mL × 1 5 mL × 5 5 mL × 10	45 655 315 45 655 317 45 655 318
GoBio Prep 16x100 NiMAC[2]	20 mL × 1	55 653 021
GoBio Prep 26x100 NiMAC[2]	53 mL × 1	55 653 031
GoBio Prod 80x200 NiMAC[2]	1 L × 1	55 653 042
GoBio Prod 130x200 NiMAC[2]	2.7 L × 1	55 653 062
GoBio Prod 200x200 NiMAC[2]	6 L × 1	55 653 072
GoBio Prod 240x200 NiMAC[2]	9 L × 1	55 653 082
GoBio Prod 330x250 NiMAC[2]	21.4 L × 1	55 653 093
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[2]	20 mL × 1	55 700 021
GoBio Prep 26x100 Dsalt	53 mL × 1	55 700 031
Bulk resins		
WorkBeads NiMAC	25 mL 150 mL 1 L	40 653 001 40 653 003 40 653 010
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] Packed on request.

Ordering information

Product name	Pack size	Article number
GoBio Screen 7x100 NiMAC[1]	3.8 mL × 1	55 653 001

[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

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

IN 55 653 001 BC

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