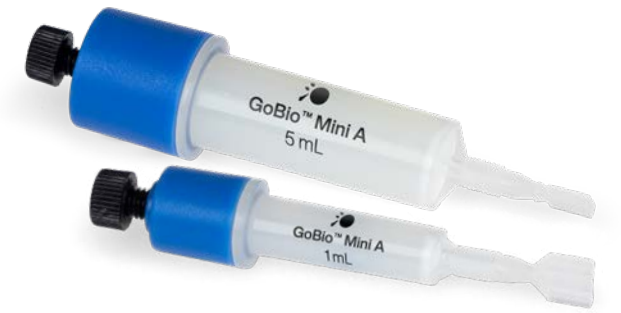
Bio-Works

INSTRUCTION

GoBio Mini A

GoBio™ Mini A columns are ready-to-use affinity chromatography columns for easy and convenient purification of monoclonal and polyclonal antibodies from cell culture supernatant, serum, ascites fluid or other sources. The columns are prepacked with WorkBeads™ Protein A resin and are available in two columns sizes, 1 mL and 5 mL.



- Swift purification of polyclonal and monoclonal antibodies
- High binding capacity and purity
- Simple and easy method giving reproducible results

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 Protein A 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

Short protocol

This general short protocol is for usage of GoBio Mini A columns. Detailed instructions and recommendations for optimization are given later in this instruction. Recommended buffers are listed in Table 2.

1. Connect the column to the chromatography system, syringe or pump.
2. Equilibrate the column using 10 column volumes (CV) binding buffer.
3. Apply a clarified sample under neutral conditions.
4. Wash using 10 – 20 CV binding buffer.
5. Elute the target protein with 5 CV elution buffer. Add 100 µL 1 M Tris-HCl, pH 9 per 1 mL collected fraction, in the fractionation tube.
6. Re-equilibrate with 10 CV binding buffer.
7. Equilibrate with 10 CV 20% ethanol for storage. Close the column using the included cap and plug.

Optimization may be needed for optimal purification results. See further details later in this instruction.

Principle

Affinity chromatography is a useful technique for the separation of proteins by means of the reversible interaction between the target protein and the ligand immobilized on the resin. The interaction can be biospecific, for example antibodies binding to protein A, or non-biospecific, for example histidine-tagged proteins binding to metal ions.

This chromatography technique provides high selectivity, high resolution and high capacity. High purity can often be achieved in a single step. Large sample volumes can be used and samples applied under conditions that favor specific binding to the ligand. Elution is often performed under gentle conditions that help to preserve bioactivity. The target protein is eluted in a purified and concentrated form by the modification of pH, ionic strength, or by introducing a competitive agent.

Instructions

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

1. Prepare the sample

After cell disruption or extraction, clarify the sample by centrifugation at 10 000 – 20 000 × g for 15 – 30 minutes. It is generally recommended also to pass the sample through a 0.22 – 0.45 µm filter (e.g. a syringe filter) to avoid inadvertently applying any remaining particles onto the column. If the sample contains only small amounts of particles, it may be enough only to carry out filtration. Application of a poorly sample may reduce the performance and lifetime of the column. The sample should be applied under conditions similar with those of the binding buffer.

2. Connect the column

Cut off or twist off the end at the outlet of the column, see Figure 1. Note: It is of high importance to cut off the tip at the very end of the cone, preferable using a scalpel. Incorrect removal of the end piece will affect the performance of the column.

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

Figure 1. Removal of the cut-off end at the column outlet should be done by cutting or by twisting (A) not bending (B).

Table 1. Recommended buffers for purification. Other buffers can possible be used.

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

3. Remove the storage solution

The column contains 20% ethanol on delivery. This storage solution should be washed out before use. Wash the column with 5 CV deionized water or buffer. Avoid flow rates higher than 2 mL/min for GoBio Mini 1 mL columns or 7 mL/min for GoBio Mini 5 mL columns before the storage solution has been removed to avoid overpressure due to high viscosity of the 20% ethanol solution.

4. Equilibrate the column

Equilibrate the column with 10 CV binding buffer.

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

Table 2. Recommended buffers for purification. Other buffers can possible be used.

Buffer	Composition
Binding buffer	20 mM sodium phosphate buffer, 150 mM NaCl, pH 7.4 (PBS)
Elution buffer	100 mM sodium citrate, pH 3.0

5. Apply the sample

Apply the sample at 0.3 – 0.6 mL/min (3 – 2 minutes residence time) for the GoBio Mini 1 mL or 1.7 – 2.5 mL/min (3 – 2 minutes residence time) for the GoBio Mini 5 mL columns. A too high flow rate may reduce the yield.

6. Wash

After sample application, remove unbound impurities by washing the column with 20 – 30 CV of washing buffer or until desired A_{280} nm absorbance of the wash fractions (e.g., 0.01 – 0.02) is obtained.

7. Elute

The target protein is eluted by applying a low pH buffer. This can be done in different ways, for example:

Alternative 1: Desorb the target protein with 5 CV elution buffer.

Alternative 2: For high purity, gradient elution is recommended. For example, a gradient from 100 mM Na-citrate, pH 6.0 to 100 mM Na-citrate, pH 3.0 over 20 CV can be applied.

8. Re-equilibrate

Re-equilibrate the column with 10 CV binding buffer.

9. Column storage

Wash the column with 5 CV deionized water to remove the buffer and get the pH back to neutral.

Equilibrate the column with 10 CV 20% ethanol for storage. Close the column using the cap and plug (included).

Scale-up

GoBio Mini columns are easily connected without accessories. Up to five columns may be connected in series (column stacking). The pressure drop across each column bed will be the same as for a single column, but the upstream columns will be subjected to a higher internal pressure from the added pressure drops from downstream columns. It may therefore be necessary to decrease the flow rate accordingly to avoid exceeding the maximum pressure limit onto the first column. If possible, the maximum pressure of the chromatography system should be set according to Table 3. Remember always to take the system fluidics contribution to the pressure into account.

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

No. of columns in series	Max pressure GoBio Mini 1 mL (bar)	Max pressure GoBio Mini 5 mL (bar)
1	3.0	3.0
2	6.0	6.0
3	9.0	9.0
4	12	10[1]
5	15	10[1]

[1] The maximum pressure is defined by the column hardware maximum pressure

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

For columns larger than 20 mL, it is recommended to pack a single column using bulk resin as the limitations of column stacking will then impact chromatographic performance.

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Optimization

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

Optimization of coupling conditions

Human IgG and IgG from several other species bind to GoBio Mini A under neutral pH at moderate salt concentrations. Apart from the recommended binding buffer in Table 2, other buffers can be used. For example, 50 mM sodium phosphate, pH 7.4 or 50 mM sodium borate, pH 9. However, IgG with weaker affinity (e.g., mouse IgG_1) may need a binding buffer with a combination of high pH and ionic strength to be able to bind. For example, 50 mM sodium borate, 4 M NaCl, pH 9.

Optimization of elution

Run a test pH gradient elution with a small amount of sample to determine at what pH the target antibody is eluting. For example, a gradient from 100 mM sodium citrate, pH 6.0 to 100 mM sodium citrate, pH 3.0 over 10 – 20 CV can be tested. Elution will occur when the pH is low enough, while avoiding very low pH. The pH measured at the tail of the peak should be selected for elution. Prepare a 100 mM sodium citrate buffer with the selected elution pH as elution buffer. Apart from the elution buffer mentioned in Table 2, for example 100 mM glycine-HCl, pH 2.7 can also be used as elution buffer.

Desalting and buffer exchange

IgG can be sensitive to low pH. In order to avoid denaturation once the purification is completed, the pH can be neutralized by adding 100 µL of 1 M Tris-HCl, pH 9, per mL, to each tube before starting the collection of fractions. Immediately after fractionation, collect the target protein and perform buffer exchange using GoBio Mini Dsalt or GoBio Prep columns equilibrated with a neutral buffer, see “Related products.”

Additional purification

Antibody purification on GoBio Mini A columns gives high purity in a single step. For even higher purity requirements, it may be necessary to add a second purification step. The additional purification step is used to remove traces of leaked protein A ligand, antibody aggregates and remaining impurities from the sample. In fact, an added polishing step may allow the omission of optimization of the first purification step. WorkBeads 40/100 SEC, WorkBeads 40/1000 SEC and WorkBeads 40/10 000 SEC resins separates proteins of different size. WorkBeads 40S, WorkBeads 40Q, WorkBeads 40 DEAE and WorkBeads 40 TREN resins are used for ion exchange chromatographic purification. Note that all these resins also are available in different column formats in the GoBio prepacked column family.

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The polishing purification step may be based on several chromatographic techniques:

Size exclusion chromatography

Size exclusion chromatography (SEC) can be used for the separation of monomeric antibodies from dimeric antibodies, antibody aggregates as well as complexes of leaked protein A and antibody. SEC technique separates proteins and other biomolecules according to size hence the monomeric antibodies will elute after antibody dimer, aggregates and complexes of leaked protein A and the antibody. This technique is simple to run. It is carried out under neutral conditions, and is recommended for high purity demands in lab scale purification, (e.g. using WorkBeads 40/100 SEC). Optimization is often not required for significant purification but may sometimes be worthwhile. The technique is not recommended for bioprocess scale applications due to dilution effects, low capacity and that it is time consuming.

Cation exchange chromatography

Cation exchange chromatography is commonly used as a polishing step in antibody purification strategies. Many antibodies are weakly basic at neutral pH and will hence bind to a cation exchange chromatography resin (e.g., WorkBeads 40S). Conversely, protein A does not bind to a cation exchange resin under the same conditions. Dissociation between antibodies and potential leakage of protein A can therefore be carried out by cation exchange chromatography technique under neutral pH. This technique usually requires optimization for each specific antibody to be purified.

Anion exchange chromatography

Anion exchange chromatography technique is often used in a negative chromatography mode, during the polishing antibody purification. Potential leakage of protein A, as well as complexes between protein A and the antibody, tend to bind to an anion exchange chromatography resin (e.g., WorkBeads 40Q) at neutral pH, whereas the antibody itself usually does not bind. The use of this technique as a polishing step usually requires optimization for optimal antibody purification.

Maintenance of the column

Cleaning using NaOH

Small amounts of impurities can be found in samples that tend to adsorb to the resin as the result of unspecific interactions. This may reduce the packed column performance. It is therefore common to make regular Cleaning-in-Place (CIP). Using NaOH is the most common method, although prolonged wash with alkaline conditions will reduce the functionality of the resin and must therefore be kept to a minimum (see Figure 2). CIP of the column can be carried out as followed:

1. Unless elution was carried out at very low pH there may be a need for regeneration by cleaning the column with, for example, 10 CV 100 mM glycine-HCl, pH 2.7 or 100 mM sodium citrate, pH 3.
2. Wash the column with 5 CV deionized water.
3. Clean by passing 15 CV 15 mM NaOH at 1 mL/min (GoBio Mini A 1 mL) or 4 mL/min (GoBio Mini A 5 mL); for harsher conditions try 50 mM NaOH. For increased efficiency, before the NaOH wash, include a passage of 15 CV 100 mM 1-thioglycerol, pH 8.5 to reduce any oxidized aggregates adsorbed to the column.
4. Wash with 10 CV neutral buffer. Make sure that neutral pH is restored in the column. Prolonged exposure to extreme pH may harm the column.
5. Wash with 10 CV deionized water.
6. Wash with 10 CV 20% ethanol before storage.

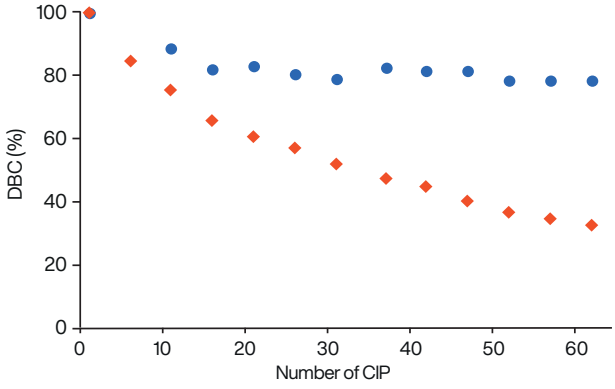

Figure 2. Alkaline stability of WorkBeads Protein A determined by frontal analysis using 1 mg/mL IgG in the presence of PBS, pH 7.4. CIP cycle: 100 mM 1-thioglycerol, pH 8.5, 15 minutes incubation; followed by 15 mM NaOH (circles) or 100 mM NaOH (diamonds) for 15 minutes.

Cleaning using strong denaturants

Guanidine hydrochloride and urea can be used for CIP in lab scale. For industrial scale CIP, these compounds are not recommended due to cost and negative environmental effects when used in large quantities.

1. Unless elution was done at very low pH there may be a need for regeneration by cleaning the column with, for example, 10 CV 100 mM glycine-HCl, pH 2.7 or 100 mM sodium citrate, pH 3.
2. Wash the column with 5 CV deionized water.
3. Clean by passing 10 CV 6 M urea or 6 M guanidine hydrochloride at 0.5 mL/min (GoBio Mini A 1 mL) or 2 mL/min (GoBio Mini A 5 mL). Alternatively, stop the flow and incubate overnight.
4. Wash with 10 CV deionized water.
5. Wash with 10 CV 20% ethanol before storage.

Storage

Equilibrate the column in 20% ethanol and close it securely using the included plug and cap.

Store the column at 2 to 8 °C.

Additional information

Additional information

	GoBio Mini A
Target substance	Antibodies (IgG), bound via the Fc-region
Resin	WorkBeads Protein A
Matrix	Rigid, highly cross-linked agarose
Average particle size[1] (D_{v50})	45 µm
Ligand	Recombinant protein A expressed in *E. coli* using animal free medium
Dynamic binding capacity[2] (DBC)	> 40 mg human IgG/mL resin
Column volume	1 mL 5 mL
Column dimension	7 × 28 mm (1 mL) 13 × 38 mm (5 mL)
Recommended flow rate[3] GoBio Mini A 1 mL GoBio Mini A 5 mL	 0.3 – 1 mL/min (47 – 150 cm/h) 1 – 4 mL/min (45 – 180 cm/h)
Maximum flow rate[4] GoBio Mini A 1 mL GoBio Mini A 5 mL	 4 mL/min (620 cm/h) 15 mL/min (670 cm/h)
Maximum back pressure	0.3 MPa, 3 bar, 43 psi
Chemical stability	Compatible with all standard aqueous buffers used for protein purification
pH stability	3 – 10 (short term) 2 – 12 (cleaning)
Storage	2 to 8°C in 20 % ethanol

[1] The median particle size of the cumulative volume distribution.
[2] Dynamic binding capacity was determined at 10% breakthrough ($QB_{10\%}$) by frontal analysis with 1 mg/mL human polyclonal IgG in PBS, pH 7.4 at 1.4 mL/min (240 cm/h) in a column with a WorkBeads Protein A bed height of 100 mm and 2.5 minutes residence time. Notice that the dynamic binding capacity at corresponding flow rate in GoBio Mini columns is slightly lower due to their short length.
[3] Recommended flow rates include the flow rates in all steps; cleaning, equilibration, applying sample, washing, elution etc.
[4] Decrease the max flow rate if the liquid has a higher viscosity. Higher viscosities can be caused by low temperature (use half of the max flow rate when operating at 4 °C), or by additives (e.g. use half of the max flow rate for 20% ethanol).

GoBio prepacked column family

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

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

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

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

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

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

Related products

Product name	Pack size[1]	Article number
Prepacked columns		
GoBio Mini Dsalt 5 mL	5 mL × 5	45 360 107
GoBio Mini S 1 mL	1 mL × 5	45 200 003
GoBio Mini S 5 mL	5 mL × 5	45 200 007
GoBio Mini Q 1 mL	1 mL × 5	45 100 003
GoBio Mini Q 5 mL	5 mL × 5	45 100 007
GoBio Mini TREN 1 mL	1 mL × 5	45 655 213
GoBio Mini TREN 5 mL	5 mL × 5	45 655 217
GoBio Prep 16x100 40S	20 mL × 1	55 420 021
GoBio Prep 16x100 40Q	20 mL × 1	55 410 021
GoBio Prep 16x100 40 TREN	20 mL × 1	55 463 021
GoBio Prep 16x100 Dsalt[2]	20 mL × 1	55 700 021
GoBio Prep 26x100 40S	53 mL × 1	55 420 031
GoBio Prep 26x100 40Q	53 mL × 1	55 410 031
GoBio Prep 26x100 40 TREN[2]	53 mL × 1	55 463 031
GoBio Prep 26x100 Dsalt	53 mL × 1	55 700 031
GoBio Prep 16x600 40/100 SEC	120 mL × 1	55 434 026
GoBio Prep 26x600 40/100 SEC	320 mL × 1	55 434 036
GoBio Prep 16x600 40/1000 SEC	120 mL × 1	55 430 026
GoBio Prep 26x600 40/1000 SEC	320 mL × 1	55 430 036
Bulk resins		
WorkBeads Protein A	10 mL 100 mL 1 L	40 605 003 40 605 004 40 605 005
WorkBeads 40S	25 mL 200 mL	40 200 001 40 200 002
WorkBeads 40Q	25 mL 200 mL	40 100 001 40 100 002
WorkBeads 40 DEAE	25 mL 200 mL	40 150 001 40 150 002
WorkBeads 40 TREN	25 mL 150 mL	40 603 001 40 603 003
WorkBeads Dsalt	300 mL	40 360 003

[1] Other pack sizes can be found in the complete product list on www.bio-works.com
[2] Packed on request.

Ordering information

Product name	Pack size	Article number
GoBio Mini A 1 mL	1 mL × 1 1 mL × 5 1 mL × 10	45 605 101 45 605 103 45 605 104
GoBio Mini A 1 mL	5 mL × 1 5 mL × 5 5 mL × 10	45 605 105 45 605 107 45 605 108

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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