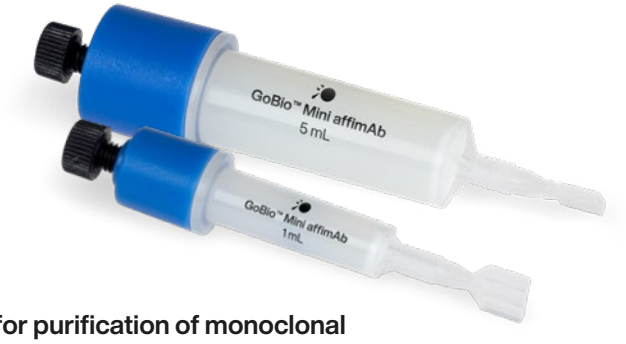
Bio-Works

INSTRUCTION

GoBio Mini affimAb



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

WorkBeads affimAb resin is an alkaline-stable resin designed for purification of monoclonal and polyclonal antibodies in laboratory to process scale. This resin has a superior basematrix in combination with an optimized alkaline-stable protein A ligand. This results in high dynamic binding capacity even at short residence times, and stabile capacity over multiple purification cycles with cleaning-in-place using 0.5 M NaOH.

- Top performance dynamic binding capacity also at short residence times
- Outstanding alkaline stability with 0.5 M NaOH, extends the number of purification cycles
- Excellent purity, reproducibility and recovery at high sample loads
- Negligible protein A leakage

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 affimAb 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 affimAb 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, resolution, and capacity. High purity is often achieved in a single step. Feeds with low target expression can be purified using WorkBeads affimAb, provided that the loading exceeds 50% of the resin's dynamic binding capacity (DBC) to ensure optimal recovery. Elution is performed at a lower pH, yielding a purified and concentrated antibody. The pH should then be neutralized to avoid affecting the antibody's bioactivity.

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 sample that has not been properly clarified 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, preferably using a scalpel. The function of the cone is to give a tight seal when the column is connected. Incorrect removal of the end piece will affect the performance of the column.

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

Figure 2. Removal of the cut-off end at the column outlet should be done by cutting or by twisting (A) not bending (B).

Table 1. Recommended connectors for coupling GoBio Mini columns to the equipment of choice.

Equipment	Accessories for connection
Syringe	Female luer/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.

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 or 100 mM glycine-HCl, pH 2.7

5. Apply the sample

Apply the sample at 0.2 – 0.5 mL/min (6 – 2.2 minutes residence time) for the GoBio Mini 1 mL or 0.9 – 2.3 mL/min (6 – 2.2 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 washing buffer or until desired $A_{280\,nm}$ absorbance of the wash fractions (e.g., 0.01 – 0.02) is obtained.

7. Elute

The antibodies are eluted by applying a low pH buffer. The standard is to elute with 5 CV elution buffer.

Note: Antibodies can be sensitive to low pH. To avoid denaturation after elution with low pH, the pH can be neutralized by adding 100 µL of 1 M Tris-HCl, pH 9 per mL collected fraction to each fractionation tube before starting the purification or immediately after completed elution.

Immediately after fractionation, collect the target protein and perform buffer exchange using a GoBio Mini Dsalt column equilibrated with a neutral buffer, see "Related products", or perform an anion exchange step as a polishing step.

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 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 or chromatography system components. 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 antibody to be purified. A general recommendation is to load approx. 80% of the column binding capacity. 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 or use one of the larger columns in the GoBio prepacked column family, as the limitations of column stacking will impact the chromatographic performance, see "Related products".

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

Optimization

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

Optimization of binding

Human IgG and IgG from several other species bind to GoBio Mini affimAb 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 IgG1) 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, 3 M NaCl, pH 9.

Optimization of elution

Run a test pH gradient elution with the sample to determine at what pH the target antibody is eluting. For example, a gradient from 100 mM Na-citrate, pH 6.0 to 100 mM Na-citrate, pH 3.0 over 10 – 20 CV. 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 Na-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. 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 a GoBio Mini Dsalt column equilibrated with a neutral buffer. Complete buffer exchange can be obtained in few minutes.

Additional purification

Antibody purification on GoBio Mini affimAb 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 resins separates proteins of different size and is a useful resin for these applications. WorkBeads 40S and WorkBeads 40Q resins are used for ion exchange chromatographic purification. All these resins are also available as prepacked GoBio columns, see “Related products”.

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

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 separates proteins and other biomolecules according to size, hence the monomeric antibodies will elute after antibody dimers, aggregates and complexes of leaked protein A and 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, also available in prepacked) columns). 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 processes. 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 and will elute in the flow through. In addition, the use of this technique as a polishing step usually requires optimization for optimal antibody purification.

WorkBeads 40 TREN is another type of anion exchanger that offers a unique separation by multimodal ion exchange chromatography. This resin is useful as a “guard” column before loading the crude antibody sample directly on the protein A resin to prevent fouling and increase the lifetime of the protein A resin. Several of the host cell proteins, for example chromatin, as well as viruses will bind to WorkBeads 40 TREN.

Desalting and buffer exchange

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

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 0.5 M NaOH as the most common method, see Figure 2. CIP of GoBio Mini affimAb 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 Na-citrate, pH 3.
2. Wash the column with 5 CV deionized water.
3. Clean by passing 5-10 CV 0.5 M NaOH at 1 mL/min (GoBio Mini affimAb 1 mL) or 4 mL/min (GoBio Mini affimAb 5 mL).
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.

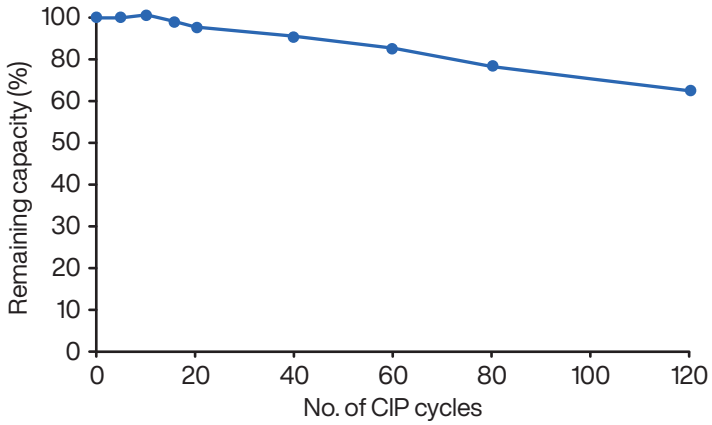


Figure 3. DBC for polyclonal human IgG on WorkBeads affimAb determined by frontal analysis at 2.5 minutes residence time after 120 CIP cycles using 0.5 M NaOH at 15 minutes contact time.

Storage

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

Store the column at 2 to 8 °C.

Product information

	GoBio Mini affimAb
Target substance	Antibodies (IgG), bound via the Fc-region
Resin	WorkBeads affimAb
Matrix	Rigid, highly cross-linked agarose
Average particle size (D_{v50})[1]	50 µm
Ligand	Recombinant protein A expressed in *E. coli* using animal free medium
Dynamic binding capacity (DBC)[2]	> 40 mg human IgG/mL resin
Sample load	> 50% of DBC[3]
Column volume	1 mL 5 mL
Column dimension	7 × 28 mm (1 mL) 13 × 38 mm (5 mL)
Recommended flow rates[4] GoBio Mini affimAb 1 mL GoBio Mini affimAb 5 mL	 0.2 – 1 mL/min (28 – 150 cm/h) 0.9 – 4 mL/min (38 – 180 cm/h)
Maximum flow rates[5] GoBio Mini affimAb 1 mL GoBio Mini affimAb 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 0.5 M NaOH and all standard aqueous buffers used for protein purification. 10 mM HCl (pH 2), 0.5 M NaOH (pH 12), 0.1 M sodium citrate buffer (pH 3), 6 M guanidine-HCl, 20% ethanol Should not be stored at low pH for prolonged time.
pH stability	3 – 12
Cleaning-in-place stability	Up to 0.5 M NaOH
Storage	2 to 8 °C in 20% ethanol

[1] The median particle size of the cumulative volume distribution.
[2] DBC 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 (245 cm/h, 2.5 minutes residence time) in a column packed with WorkBeads affimAb resin, column bed 6.6 × 100 mm.
[3] It is recommended to load 50-80% of resin's DBC to achieve maximum yield.
[4] Recommended flow rates include the flow rates in all steps; cleaning, equilibration, applying sample, washing, elution, etc.
[5] 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 flowrate 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 S 5 mL	5 mL × 5	45 200 107
GoBio Mini Q 5 mL	5 mL × 5	45 100 107
GoBio Mini TREN 5 mL	5 mL × 5	45 655 217
GoBio Mini Dsalt 5 mL	5 mL × 5	45 360 107
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 affimAb	25 mL 200 mL 1 L	40 800 001 40 800 002 40 800 010
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 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 affimAb 1 mL	1 mL × 1 1 mL × 5 1 mL × 10	45 800 101 45 800 103 45 800 104
GoBio Mini affimAb 5 mL	5 mL × 1 5 mL × 5 5 mL × 10	45 800 105 45 800 107 45 800 108

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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