BabyBio affimAb

BabyBio™ 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 also 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, recovery and reproducibility
- Negligible protein A leakage

Short protocol

This general short protocol is for usage of BabyBio 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, high resolution and high capacity. High purity can often be achieved in a single step. Large sample volumes can be handled and samples applied under conditions that favor specific binding to the ligand. 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, centrifugation may be omitted and it is enough only to carry out filtration. Application of a not clarified sample may reduce the performance and lifetime of the column. The sample should be applied under conditions similar to 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 (BabyBio 1 ml column) or 5 ml/min (BabyBio 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 connectors for coupling BabyBio columns to the equipment of choice.

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 BabyBio 1 ml columns or 7 ml/min for BabyBio 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	PBS; 20 mM Na-phosphate buffer, 150 mM NaCl, pH 7.4
Elution buffer	100 mM Na-citrate, pH 3.0 or 100 mM glycine-HCl, pH 2.7

5. Apply the sample

Apply the sample at 0.5 - 1 ml/min for the BabyBio 1 ml or 2 - 5 ml/min for the BabyBio 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. In order 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 BabyBio Dsalt column equilibrated with a neutral buffer, see *Related products*.

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

BabyBio columns are easily connected together 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 in order 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 BabyBio columns connected in series. Notice that the maximum pressure over each column is always 3 bar.

Number of columns in series	Max pressure BabyBio 1 ml (bar)	Max pressure BabyBio 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 as the limitations of column stacking will impact the chromatographic performance. To find out more about Bio-Works bulk chromatography resins, please see ordering information or 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 BabyBio 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 Na-phosphate, pH 7.4 or 50 mM Na-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 Na-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. 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 a BabyBio Dsalt column equilibrated with a neutral buffer. Complete buffer exchange can be obtained in some few minutes.

Additional purification

Antibody purification on BabyBio 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 40/1000 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. These ion exchange resins are also available as ready-to-use BabyBio Q and BabyBio S columns (see *Related products*).

To find out more about Bio-Works chromatography resins for additional purification, please 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 40/1000 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 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 will bind to WorkBeads 40 TREN.

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 BabyBio 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 (BabyBio affimAb 1 ml) or 4 ml/min (BabyBio 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.

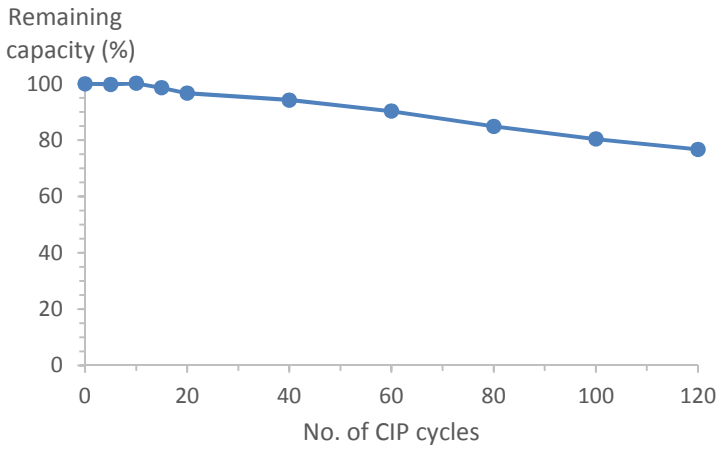

Figure 2. DBC for polyclonal human IgG on WorkBeads affimAb determined by frontal analysis at 2.5 minutes residence time after 120 CIP cycles with 0.5 M NaOH at 15 minutes contact time.

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.

Additional information

Intended use

BabyBio affimAb is intended for research and for process development.

Safety

Please read the associated Safety Data Sheet (SDS) for BabyBio columns, and the safety instructions for any equipment to be used with BabyBio. Note that the maximum backpressure of BabyBio affimAb columns is 0.3 MPa, 3 bar, 43 psi.

Product information

	BabyBio affimAb
Target substance	Antibodies (IgG), bound via the F_c-region
Resin	WorkBeads affimAb
Matrix	Rigid, highly cross-linked agarose
Average particle size[1] (D_{v50})	50 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 x 28 mm (1 ml) 13 x 38 mm (5 ml)
Recommended flow rate	
BabyBio affimAb 1 ml	0.5 - 1 ml/min (75 - 150 cm/h)
BabyBio affimAb 5 ml	1 - 4 ml/min (45 - 180 cm/h)
Maximum flow rates[3]	
BabyBio affimAb 1 ml	4 ml/min (620 cm/h)
BabyBio affimAb 5 ml	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 - 10
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 x 100 mm.
3. 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).

Related products

Product name	Pack size[1]	Article number
Prepacked columns		
BabyBio Dsalt 5 ml	5 ml x 5	45 360 107
BabyBio S 5 ml	5 ml x 5	45 200 107
BabyBio Q 5 ml	5 ml x 5	45 100 107
BabyBio TREN 5 ml	5 ml x 5	45 655 217
Bulk resins		
WorkBeads affimAb	25 ml	40 800 001
WorkBeads affimAb	200 ml	40 800 002
WorkBeads affimAb	1 L	40 800 010
WorkBeads 40S	25 ml	40 200 001
WorkBeads 40Q	25 ml	40 100 001
WorkBeads 40 TREN	25 ml	40 603 001
WorkBeads 40/1000 SEC	25 ml	40 300 001
WorkBeads 40/1000 SEC	300 ml	40 300 003
WorkBeads 40/1000 SEC	1 L	40 300 010

1. Other pack sizes can be found in the complete product list on www.bio-works.com

Ordering information

Product name	Pack size	Article number
BabyBio affimAb 1 ml	1 ml x 1	45 800 101
	1 ml x 2	45 800 102
	1 ml x 5	45 800 103
	1 ml x 10	45 800 104
BabyBio affimAb 5 ml	5 ml x 1	45 800 105
	5 ml x 2	45 800 106
	5 ml x 5	45 800 107
	5 ml x 10	45 800 108

Orders: E-mail us, sales@bio-works.com or contact your local distributor.

For more information about local distributors and products please visit www.bio-works.com or contact us at info@bio-works.com

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