

WorkBeads affimAb

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 base matrix in combination with an optimized alkaline-stable protein A ligand. This results in high dynamic binding capacity also at short residence times, and stable capacity over multiple purification cycles with cleaning-in-place with 0.5 M NaOH.

Prepacked BabyBio™ affimAb 1 ml and 5 ml columns are available for small scale purifications and condition screening in process development. WorkBeads affimAb resin can also be used for purifications in other formats, such as batch and centrifugation purifications.

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



Short protocol

This short protocol is for column packing of WorkBeads affimAb and antibody purification. Detailed instructions and recommendations for optimization are given later in this document. Recommended buffers are listed in Table 1.

1. Make a slurry of the desired resin concentration.
2. Pour the slurry into the column.
3. Pack the resin with an appropriate flow rate.
4. Apply an axial compression of less than 2%.
5. Equilibrate the column with binding buffer.
6. Apply sample.
7. Elute the target protein with elution buffer.
8. Wash the column with deionized water.
9. Equilibrate the column with 20% ethanol for storage.

Principle

Affinity chromatography is a useful technique for the separation of proteins by exploiting the reversible interaction between the target protein and the immobilized ligand. The interaction can be bio-specific, for example antibodies binding to protein A, or not bio-specific, 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. Large sample volumes can be processed, and samples applied under conditions that favour specific binding to the ligand. Elution is often carried out under gentle conditions which helps to preserve bioactivity. The target protein is eluted, in a purified and concentrated form, by modification of pH, ionic strength, or by introducing a competitive agent.

Column packing

WorkBeads resins are cross-linked using a proprietary method that results in a very rigid resin, which tolerate pressures of several bars and consequently can run at high flow rates. Follow both this general advice when packing a column and the column manufacturer's specific instructions. Preferably, use a column with an adjustable adaptor. In some instances, a packing reservoir or column extension may be needed.

Note: Always make sure that the maximum pressure of the column hardware is not exceeded. Backpressure caused by the chromatography system components connected downstream of the column may reduce the maximum flow that can be used. Wear eye protection.

1. Wash the resin

The resin is provided in 20% ethanol. To avoid undue backpressure when packing, wash the desired amount of resin with several column volumes of deionized water before packing.

2. Make a slurry

Add deionized water to the washed resin to obtain a 40% to 60% slurry concentration. Make approximately 15% extra slurry to compensate for the compression during packing. The amount of slurry to be prepared can be calculated according to:

$$\text{Slurry volume} = \frac{\text{bed volume} \times 100}{\% \text{ slurry}} \times 1.15$$

3. Pour the slurry into the column

Pour the slurry slowly down the side of the column to avoid formation of air bubbles. Preferably, use a packing reservoir or an extra column tube to extend the column volume to accommodate the entire slurry volume during packing. If no packing adaptor is available packing can be done by stepwise additions and packing. Although not recommended this will give acceptable results for most applications.

4. Pack the bed

Pack the resin with a downward flow higher than the intended operational flow. We recommend 300 cm/h for columns up to 25 mm i.d. and with 200 mm bed height. Make sure the packing flow rate does not exceed the maximum pressure of the column hardware or the resin. The operational flow should not be more than 75% of the packing flow rate.

5. Close the column

When the bed height is constant mark the bed height on the column. Stop the flow and remove the packing reservoir or extra tube. Note that the bed height will increase temporarily when the flow is stopped. If needed, adjust the bed height by removing excess resin. Be careful not to remove too much resin. Gently fill the column with packing solution to its rim without disrupting the packed bed. Insert the adjustable adapter on top of the packed bed. Apply a small axial compression of less than 2% of the final bed height by lowering the adapter into the packed bed.

6. Apply a flow

Apply a flow of up to 225 cm/h (taking account of section 4) and check for any gap formation above the surface of the resin bed. If a gap is observed, stop the flow and adjust the adaptor to eliminate the gap.

Purification

The following brief instruction gives general conditions for purification using a column packed with WorkBeads affmAb. Recommended buffers are listed in Table 1 and recommendations for optimization are mentioned below.

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

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

Sample preparation

Clarify the sample by centrifugation at 10 000 - 20 000 × *g* for 15 - 30 minutes. It is recommended to also pass the sample through a 0.22 - 0.45 µm filter to remove any remaining particles. If the sample contains only small amounts of particles, it may be enough to only carry out filtration. Make sure that the sample has a pH between 5 and 8. Preferably, the sample should have the same pH and ionic strength as the binding buffer.

Standard purification

1. Equilibrate the column using 10 column volumes (CV) binding buffer.
2. Apply a clarified sample under neutral conditions.
3. Wash using 10-20 CV binding buffer.
4. Elute with 5 CV elution buffer. Include 100 µl 1 M Tris-HCl, pH 9 per 1 ml collected fraction, to prevent degradation of eluted target protein.
5. Re-equilibrate with 10 CV binding buffer.
6. Equilibrate with 10 CV 20% ethanol for storage.

Before starting a purification run, it is recommended to make a blank run (with no sample applied) to remove any loosely bound ligands or impurities on the resin. Do this also for a newly packed resin. Although the above standard conditions usually give excellent results it may be worthwhile to optimize the purification protocol for highest purity of the target protein, see *Optimization*.

Optimization

Selection of column size

The column size should be selected based on estimated amount of target protein in each run, and the dynamic binding capacity (DBC) of the resin. DBC is the capacity obtained under the chosen run conditions and is usually lower than the static binding capacity (total binding capacity). Figure 1 shows an example of DBC at different flow rates (or residence times). At a low flow rate, the capacity is higher. At increasing flow rates the binding capacity usually decreases.

To obtain the highest possible recovery of the target protein we recommend the loading of no more than 80% of the capacity of the packed column at the selected flow rate. Consider using a larger column or dividing the sample into repeated purification runs if needed. Collect the flow through material for subsequent analysis to determine whether the column was “over-saturated”. If desired the collected flow through material can be reapplied on the packed column after proper regeneration, in a new purification run.

Binding capacity

Antibody binding capacity depends on the flow rate used for binding and may differ between different antibody species and subclasses. For WorkBeads affimAb, the binding capacity is more than 40 mg human polyclonal IgG/ml resin at 2.5 minutes residence time in a 6.6 x 100 mm column, which corresponds to a linear flow rate of 240 cm/min. Normally the binding capacity decreases with increased flow rate, see Figure 1.

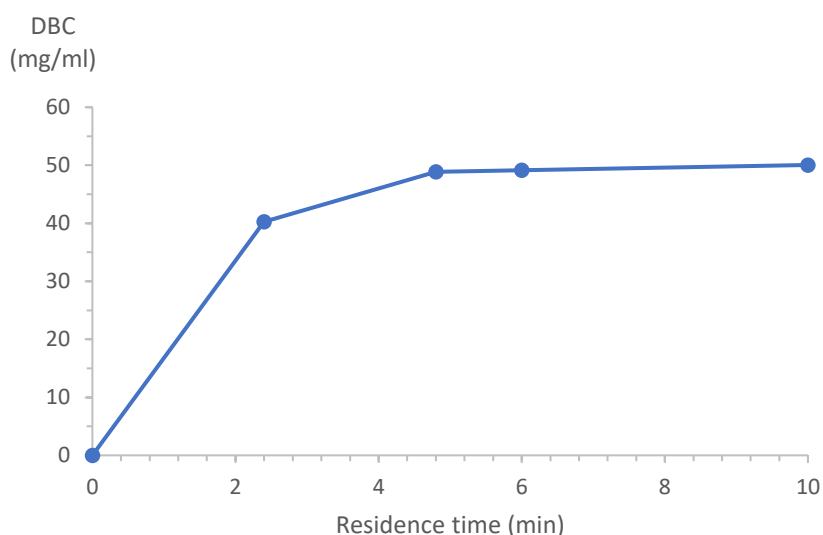


Figure 1. Dependency of dynamic binding capacity to residence time. Frontal analysis using 1 mg/ml human polyclonal IgG in PBS, pH 7.4 in a 6.6 x 100 mm glass column.

Optimization of binding

Human IgG and IgG from several other species bind to WorkBeads affimAb under neutral pH at moderate salt concentrations. Apart from the recommended binding buffer in Table 1, other buffers can be used. For example, 50 mM Na-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.

Extra wash step

To remove weakly adsorbed impurities, it may be useful to add an extra washing step after the standard wash. This can be done using a buffer with slightly increased ionic strength compared to the binding buffer or by a small decrease in pH that do not elute the target protein, see *Optimization of elution*.

Optimization of elution

Apart from the recommended elution buffer in Table 1, other buffers can be used. For example, 100 mM glycine-HCl, pH 2.7. IgG 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. Alternatively, collect the target protein and perform buffer exchange using a BabyBio Dsalt column equilibrated with a neutral buffer or pack your own column using WorkBeads Dsalt, see *Related products*. In large-scale purifications or means of buffer exchange, e.g., diafiltration, may be more suitable.

The purity can sometimes be increased by applying a pH gradient for elution. This reduces the risk of elution of impurities adsorbed to the resin and denaturation of the target antibody.

For example, 100 mM sodium citrate, pH 6.0 to 100 mM sodium citrate, pH 3.0 over 20 CV can be applied. Desorption will occur when the pH is low enough, while avoiding too low pH. The pH measured at the tail of the peak can be selected for elution. Prepare a 100 mM sodium citrate buffer with the selected elution pH and use it in step elution in for scale-up runs.

Scale-up

After developing a chromatographic procedure in a small-scale column, e.g., 10 (i.d.) × 100 mm (bed height), WorkBeads resins can be packed into larger columns for scale-up. Large-scale purification is often carried out in columns with bed heights of 200 - 300 mm.

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 is important when doing scale-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 phase, it is common to use a small column, e.g., 10 × 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 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 2 for examples. If the column bed heights are constant during scale-up the linear flow rate should be also constant (as well as the residence time).

Table 2. Example of scale-up parameters.

Column dimension	Residence time (minutes)	Linear flow rate (cm/h)	Volumetric flow rate (ml/min)
10 × 100	4	150	1.96
25 × 200	4	300	24.5
50 × 200	4	300	98.2

Additional purification

Antibody purification on WorkBeads affimAb frequently gives high purity in a single step. For very high purity requirements, it may be necessary to add a second purification step. The additional purification step is used to remove traces of leached protein A ligand, host cell proteins and nucleic acids, aggregates of the target antibody, and other remaining impurities from the sample. In research-scale purification, size exclusion chromatography (gel filtration) is often a good polishing step since it removes impurities and potential aggregates of the target protein. Size exclusion chromatography can be done using WorkBeads 40/1000 SEC.

Ion exchange chromatography is suitable for both research scale purification and industrial purification. WorkBeads TREN, WorkBeads 40S, WorkBeads 40Q and WorkBeads 40 DEAE resins provide different selectivities for ion exchange chromatography. These resins are also available as ready-to-use 1 ml and 5 ml BabyBio TREN, BabyBio S, BabyBio Q and BabyBio DEAE columns, see *Related products*.

The polishing purification step can be based on several chromatographic techniques depending on the target molecule and the contaminants.

Size exclusion chromatography

Size exclusion chromatography (SEC) can be used for the separation of monomeric antibodies from dimeric antibodies and aggregates as well as complexes of leached 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 leached 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 this technique, although it may sometimes be worthwhile. SEC 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 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 leached 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. Also, the use of this technique as a polishing step, usually require optimization for optimal antibody purification.

Multimodal anion exchange chromatography

WorkBeads 40 TREN is another type of anion exchanger that offers a unique separation possibility with the immobilized TREN ligand. 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 as well as chromatin (large DNA-protein complexes) will bind to WorkBeads 40 TREN.

Find out more about Bio-Works chromatography resins for additional purification on www.bio-works.com

Desalting and buffer exchange

Protein samples collected after purification on protein A, using the conditions describe, will contain low pH and sometimes a relatively high concentration of salt which usually need to be removed. Buffer exchange or desalting of the sample can be done, using BabyBio Dsalt 1 ml or BabyBio Dsalt 5 ml. Larger columns are easlity packed with WorkBeads Dsalt (see *Related products*). Chromatographic desalting is especially useful when the sample needs to be processed rapidly to avoid degradation. For process-scale, diafiltration should be considered.

Maintenance of the resin

Unpacking and inspection

Unpack the shipment as soon as it arrives and inspect it for damage. Promptly report any damage or discrepancies to your local supplier.

Cleaning using NaOH

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 packed column, and the pre-treatment of the sample. The bound impurities may reduce the performance of the column over time. Regular cleaning (Cleaning-in-place, CIP) keeps the resin clean, reduces the rate of further contamination, and retains the capacity, resolution and flow properties of the column. Cleaning the resin using 0.5 M NaOH gives efficient cleaning.

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. Prolonged wash with alkaline conditions will reduce the functionality of the column and must therefore be kept to a minimum (see Figure 2).

Regeneration of WorkBeads affimAb

After purification using WorkBeads affimAb perform the following steps:

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. Cleaning-in-place by passing 5-10 CV 0.5 M NaOH over 15-30 minutes.
For increased efficiency, before the NaOH wash, include a passage of 10 CV 100 mM 1-thioglycerol, pH 8.5, over 15 minutes 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 resin.
5. Wash with 10 CV deionized water.
6. Wash with 10 CV 20% ethanol before storage.

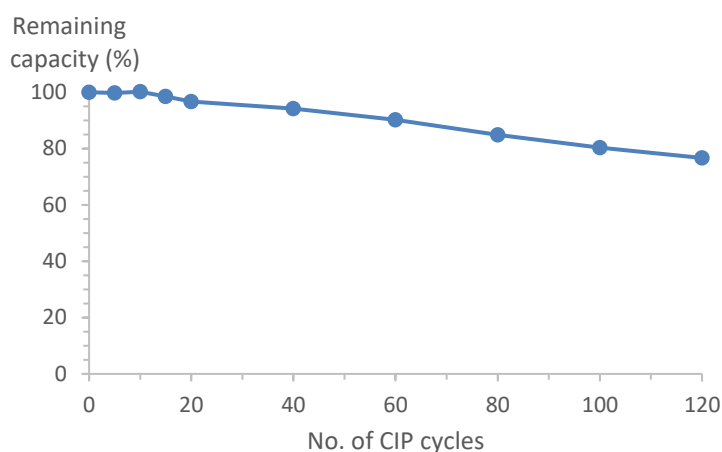


Figure 2. DBC for polyclonal human IgG on WorkBeads affimAb determined by frontal analysis at 2.5 minutes residence time after 120 CIP cycles with 0.5 M NaOH at 15 minutes contact time.

Storage

Store at 2 to 8°C in 20% ethanol.

Additional information

Product description

	WorkBeads affimAb
Target substance	Antibodies (IgG), bound via the F _c -region
Matrix	Rigid, highly cross-linked agarose
Average particle size ¹ (D _{V50})	50 µm
Ligand	Recombinant protein A expressed in <i>E. coli</i> using animal-free medium
Dynamic binding capacity ² (DBC)	> 40 mg human IgG/ml resin
Max recommended flow rate ³	300 cm/h
Chemical stability	Compatible with 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 (Q_{0.10%}) 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. Max recommended flow rate at 20 °C using aqueous buffers. 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).

Intended use

WorkBeads resins are developed and supported for both research and production-scale chromatography. WorkBeads resins are produced with validated methods and Regulatory Support Files (RSF) are available to assist the process validation and submissions to regulatory authorities.

Safety

Please read the associated Safety Data Sheets (SDS) for WorkBeads affimAb resin, and the safety instructions for any equipment to be used.

Related products

Product name	Pack size ¹	Article number
Prepacked columns		
BabyBio affimAb 1 ml	1 ml x 5	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
BabyBio S 5 ml	5 ml x 5	45 200 107
BabyBio Q 5 ml	5 ml x 5	45 100 107
babyBio DEAE 5 ml	5 ml x 5	45 150 107
BabyBio TREN 5 ml	5 ml x 5	45 655 217
BabyBio Dsalt 5 ml	5 ml x 5	45 360 107
Bulk resins		
WorkBeads 40S	25 ml	40 200 001
	200 ml	40 200 002
WorkBeads 40Q	25 ml	40 100 001
	200 ml	40 100 002
WorkBeads 40 DEAE	25 ml	40 150 001
	200 ml	40 150 002
WorkBeads 40 TREN	25 ml	40 603 001
	150 ml	40 603 003
WorkBeads Dsalt	300 ml	40 360 003

1. All different pack sizes are available on www.bio-works.com

Ordering information

Product name	Pack size	Article number
WorkBeads affimAb	25 ml	40 800 001
	200 ml	40 800 002
	1 L	40 800 010
	5 L	40 800 050
	10 L	40 800 060

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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