BabyBio A

BabyBio™ 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.

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

Resin description

BabyBio A columns are prepacked with WorkBeads Protein A resin. WorkBeads are agarose-based chromatographic resins manufactured by a proprietary method that results in porous beads with a tight size distribution and very high mechanical stability. Agarose based matrices have been successfully used for decades in biotechnology research from laboratory to production scale, due to their exceptional compatibility with biomolecules including proteins, peptides, nucleic acids and carbohydrates. WorkBeads resins are designed for separations requiring optimal capacity and purity.

The recombinant protein A attached to the resin is developed by Medicago and produced in *E. coli* under conditions free of components of animal origin and purified to high purity before coupling.

The protein A is engineered to facilitate an oriented coupling to the matrix. This allows high binding capacities for target proteins. The specificity of the recombinant protein A for the F_c region of IgG provides excellent purification. Each batch of protein A is tested according to stringent requirements.

The protein A ligand is coupled to the resin using a bromohydrin based method that gives high chemical stability and low ligand leakage. The high capacity, chemical stability and a well established agarose matrix make WorkBeads Protein A ideal for purification of both monoclonal antibodies and polyclonal antibodies.

Column description

The column is made from biocompatible polypropylene which does not significantly interact with biomolecules. The top and bottom filters are made from low protein binding polyethylene. The ready to use BabyBio columns are delivered with a plug in the inlet, a cut-off outlet and a cap for storage. The columns can be connected to a syringe, pump or chromatography system using finger tight fittings (coned 10–32) for 1/16” o.d. tubing (standard HPLC PEEK tubing).

The main characteristics of BabyBio A columns are shown in Table 1. For more details, please see instructions IN 45 605 010.

Table 1. Main characteristics of the BabyBio A 1 ml and BabyBio A 5 ml columns.

	BabyBio A
Target substance	Antibodies (IgG), bound via the F_c-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 x 28 mm (1 ml) 13 x 38 mm (5 ml)
Recommended flow rate	
BabyBio A 1 ml	0.5 - 1 ml/min (75 - 150 cm/h)
BabyBio A 5 ml	1 - 4 ml/min (45 - 180 cm/h)
Maximum flow rate[3]	
BabyBio A 1 ml	4 ml/min (620 cm/h)
BabyBio A 5 ml	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 BabyBio columns is slightly lower due to their short length.
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).

Applications

BabyBio A columns can successfully be used for purification of mono- and polyclonal antibodies using affinity chromatography technique. The columns are convenient tools in small scale purifications as well as in bioprocess development. For lab-scale purification of IgG, a set of standard conditions can often be used without further optimization.

Principle

Affinity chromatography is a useful technique for the separation of proteins by means of reversible interaction between the target protein and the ligand of 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. Very high purity is often achieved in a single step. Large sample volumes can be handled and samples are applied under conditions that favor specific binding to the ligand. Elution is often performed 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 ligand.

For more detailed description of affinity chromatography technique, please see instruction, IN 45 605 010.

Purification of monoclonal antibodies

The purification of a monoclonal IgG using BabyBio A is exemplified in Figure 1 and Figure 2.

Column:	BabyBio A 1 ml
Sample:	10 ml clarified supernatant from CHO cells diluted 1:11 in PBS, pH 7.4
Binding buffer:	20 mM Na-phosphate, 150 mM NaCl, pH 7.4
Elution buffer:	100 mM glycin-HCl, pH 2.7
Flow:	1 ml/min

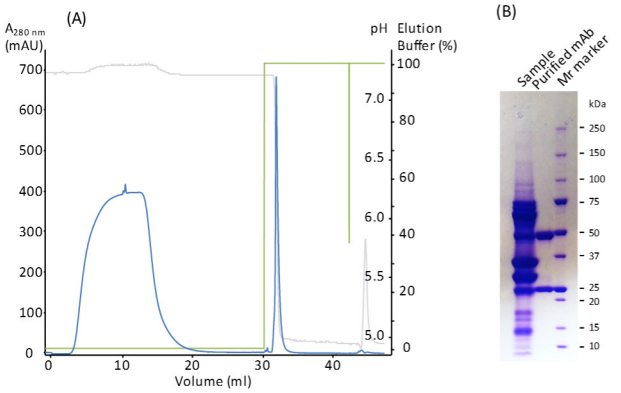

Figure 1. Purification of a monoclonal IgG from CHO cell supernatant using BabyBio A 1 ml column (A). The blue line corresponds to the absorbance at 280 nm, the green line to the concentration of elution buffer and the grey line to the pH. Analysis of the purified mAb by SDS-PAGE, reduced conditions (B).

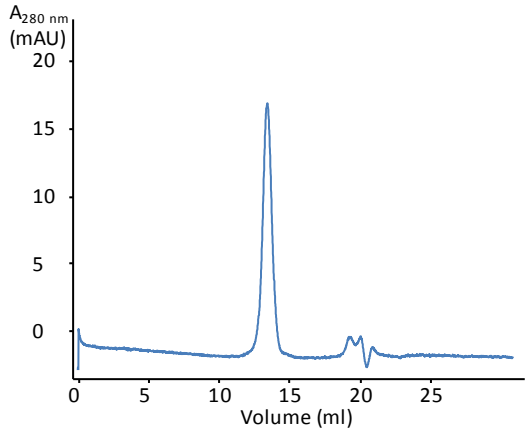

Figure 2. Size exclusion analysis of the purified mAb.

Alkaline stability

The alkaline stability of WorkBeads Protein A resin has been tested by running DBC at $QB_{10\%}$ was determined by frontal analysis using. A 6.6 x 50 mm glass column was used and a solution of 1 mg/ml IgG in PBS, pH 7.4. The DBC was analysed after various number of Cleaning-in-place (CIP) cycles using 100 mM 1-thioglycerol, pH 8.5 (15 minutes incubation) followed by 15 mM NaOH or 100 mM NaOH for 15 minutes, see Figure 3.

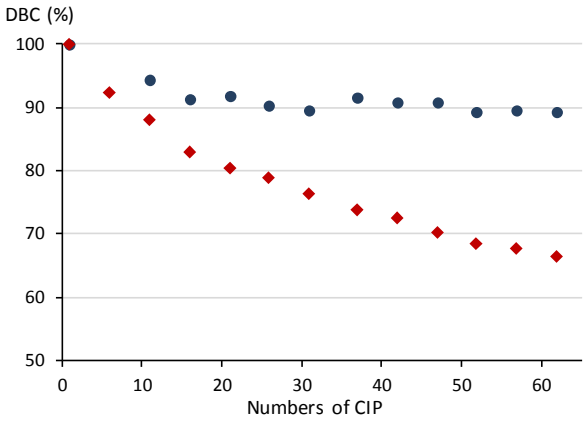

Figure 3. Alkaline stability of WorkBeads Protein A determined by frontal analysis using 1 mg/ml IgG in the presence of PBS at pH 7.4. CIP cycle: 100 mM 1-thioglycerol, pH 8.5, 15 minutes' incubation; followed by 15 mM NaOH (blue circles) or 100 mM NaOH (red diamonds), for 15 minutes.

Process optimization

The primary aim of method optimization is to find the suitable binding and elution conditions. The binding affinity for IgG to protein A varies depending on what species the IgG originates from and which subclass it belongs to. There may also be a difference between individual IgG species. Typical binding conditions are low salt concentration buffers at neutral pH. For efficient capture of weakly bound antibodies, it is often necessary to increase the pH and/or salt concentration in the binding buffer. This is for example common for mouse IgG. Elution is normally performed at reduced pH, down to pH 2.7 depending on species and subclass. To avoid denaturation of the IgG the elution should not be performed at lower pH than required for desorption. A quick and convenient buffer exchange of the eluted antibody to neutral pH can easily be done using BabyBio Dsalt columns. For biopharmaceutical production using WorkBeads Protein A, one or two polishing purification steps based on e.g., ion exchange chromatography could be added to the process in order to remove traces of leaked protein A and impurities from the feed. After optimizing the antibody purification at laboratory scale, the process can be scaled up by keeping the linear flow rate and sample to bed volume ratio constant, and increasing the column diameter.

Scale-up

Scale-up can conveniently be carried out from a 1 ml column to a 5 ml column. Column binding capacity can also be increased by coupling several columns in series. Note that the backpressure will increase. Further scale-up can be done by packing bulk WorkBeads Protein A resins in larger columns (see Related products). For more detailed description, please see instructions IN 45 605 010.

Cleaning-in-place

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 column, and the pre-treatment of the sample. The impurities may reduce the performance of the column over time. Regular cleaning (Cleaning-in-place, CIP) keep the resin clean, reduces the rate of further contamination, and prolongs the capacity, resolution and flow properties of the column.

CIP of BabyBio A can be carried out by sequentially incubating the column with 100 mM 1-thioglycerol, pH 8.5 for 15 minutes followed by 15 mM NaOH for 15 minutes. Alternatively, 6 M guanidinium hydrochloride or 6 M urea for 1 h or overnight can be used. Extended periods with low pH should be avoided. For removal of hydrophobically bound substances a solution of non-ionic detergent followed by 20% ethanol can be used.

Equipment

Prepacked BabyBio A ready-to-use columns can be used with most standard liquid chromatography equipment. Purification can also be carried out using a syringe connected to the column by a luer or a std HPLC connector.

Storage

Between uses equilibrate the column in 20% ethanol and close it securely using the included plug and cap. Store the column at 2 to 8°C.

Related products

Product name	Pack size[1]	Article number
Prepacked columns		
BabyBio Dsalt 5 ml	5 ml x 5	45 360 107
BabyBio S 1 ml	1 ml x 5	45 200 003
BabyBio Q 1 ml	1 ml x 5	45 100 003
BabyBio DEAE 1 ml	1 ml x 5	45 150 003
Bulk resins		
WorkBeads Protein A	1.5 ml	40 605 001
WorkBeads 40/1000 SEC	25 ml	40 300 001

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 A 1 ml	1 ml x 1	45 605 101
	1 ml x 2	45 605 102
	1 ml x 5	45 605 103
	1 ml x 10	45 605 104
BabyBio A 5 ml	5 ml x 1	45 605 105
	5 ml x 2	45 605 106
	5 ml x 5	45 605 107
	5 ml x 10	45 605 108

Orders: sales@bio-works.com or contact your local distributor.
For more information about local distributor and products please visit www.bio-works.com or contact us at info@bio-works.com

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