WorkBeads Protein A
BabyBio A

WorkBeads™ Protein A resin is designed for purification of monoclonal- and polyclonal antibodies using affinity chromatography technique. For small scale purification and initial screening in process development we recommend BabyBio™ A 1 ml and 5 ml columns prepacked with WorkBeads Protein A resin. WorkBeads Protein A resin can also be used for applications in other formats, such as test tube batch adsorption, spin columns, gravity columns or multi-well filter plates. The resin can be used for immunoprecipitation experiments.

- High dynamic binding capacity for monoclonal and polyclonal antibodies, with excellent recovery and purity
- Strong coupling chemistry results in low leakage
- Reliable, reproducible and efficient

Resin description

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 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. 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 (mAb) and polyclonal antibodies.

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 monoclonal antibodies (mAb) as well as polyclonal antibodies. For convenient small-scale purifications of antibodies WorkBeads Protein A is available in BabyBio A 1 ml and BabyBio A 5 ml columns.

The main characteristics of WorkBeads Protein A and BabyBio A are shown in Tables 1 and 2. For more details, please see instructions, IN 40 605 010 (bulk) and IN 45 605 010 (BabyBio).

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).

Table 1. Main characteristics of WorkBeads Protein A resin.

	WorkBeads Protein A
Target substance	Antibodies (IgG), bound via the F_c-region
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
Maximum flow rate[3,4]	300 cm/h
Chemical stability	Compatible with all standard aqueous buffers used for protein purification, 10 mM HCl (pH 2), 10 mM NaOH (pH 12), 0.1 M sodium citrate-HCl (pH 3), 6 M guanidine-HCl, 20% ethanol Should not be stored at low pH for prolonged time
pH stability	3 - 10 (short term) 2 - 12 (cleaning)
Storage	2 to 8 °C in 20 % ethanol

Footnotes description, see below Table 2.

Table 2. 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[5]	
BabyBio A 1 ml	0.3 - 1 ml/min (47 - 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. 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 (240 cm/h) in a column packed with WorkBeads Protein A resin. Column bed 6.6 x 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. Maximum flow rate at 20 °C using aqueous buffers. Decrease the maximum flow rate if the liquid has a higher viscosity. Higher viscosities can be caused by low temperature (use half of the maximum flow rate when operating at 4 °C), or by additives (e.g., use half of the maximum flow rate for 20% ethanol).
4. **Note!** Optimal flow rate during binding is depending on the sample.
5. Recommended flow rates include the flow rates in all steps; cleaning, equilibration, applying sample, washing, elution etc.

Applications

WorkBeads Protein A resin is designed for purification of monoclonal and polyclonal antibodies using affinity chromatography technique.

Principle

Affinity chromatography is a useful technique for the separation of proteins by the reversible interaction between the target protein and the ligand of. 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. Large sample volumes can be handled and samples applied under conditions that favour 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 instructions, IN 40 605 010 or IN 45 605 010.

High binding capacity

The protein A is engineered to allow oriented coupling to the resin via multipoint attachment. This allows high utilization of the immobilized protein A resulting in high IgG binding capacity.

WorkBeads Protein A has a dynamic binding capacity of typically more than 40 mg IgG/ml resin under standard binding conditions (PBS, pH 7.4 and 3 minutes residence time), exemplified in Figure 1. No further increase is seen in dynamic binding capacity at 4 or 6 minutes' residence time, which indicates that most binding capacity is utilized at 3 minutes residence time and that total capacity is close to 45 mg IgG/ml.

Purification of monoclonal antibodies

The purification of a monoclonal IgG using BabyBio A 1 ml column is exemplified in Figure 2 and Figure 3.

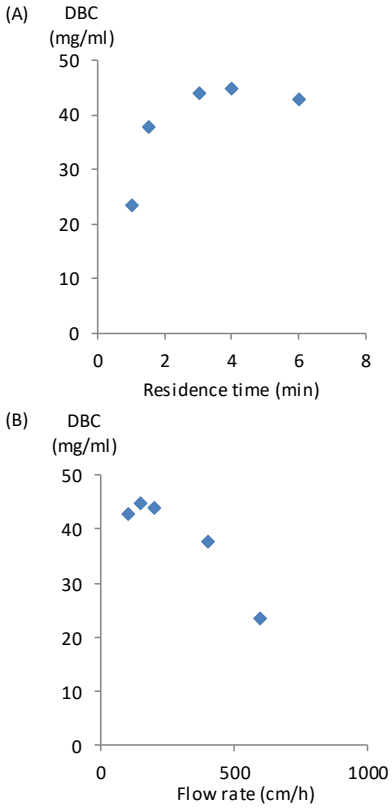

Figure 1. Dependency of dynamic binding capacity on residence time. Frontal analysis using 1 mg/ml human polyclonal IgG in PBS, pH 7.4 was performed in a 6.6 x 100 mm glass column (from Diba, Cambridge, UK). (A) DBC on WorkBeads Protein A versus residence time. (B) DBC on WorkBeads Protein A versus flow rate.

Column:	BabyBio A 1 ml
Sample:	10 ml Clarified supernatant from CHO cells diluted 1:11 in PBS
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

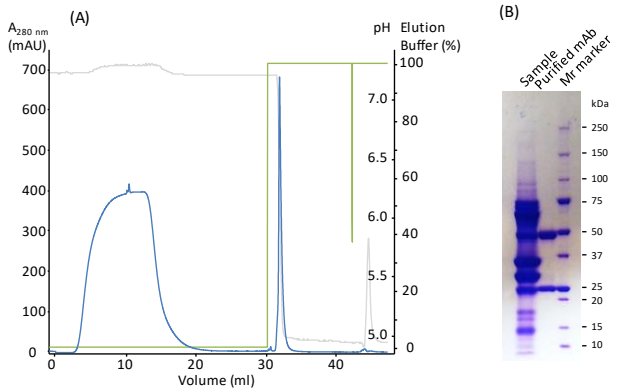

Figure 2. Purification of a monoclonal IgG from CHO cell supernatant using BabyBio A 1 ml column (A). The blue line corresponds to the absorbance at 280 nm, the green line to the concentration of elution buffer and the grey line to the pH. Analysis of the purified mAb by SDS-PAGE, reduced conditions (B).

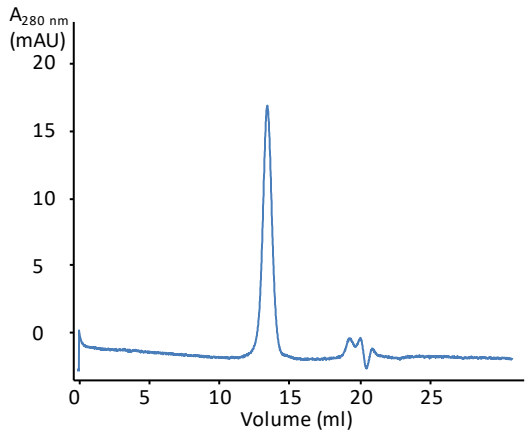

Figure 3. Size exclusion analysis of the purified mAb.

Alkaline stability

The alkaline stability of WorkBeads Protein A has been tested running DBC at $QB_{10\%}$ and frontal analysis. A 6.6 x 50 mm glass column was used and a solution of 1 mg/ml IgG in the presence of PBS, pH 7.4. The DBC was analysed after various number of Cleaning-in-place (CIP) cycles each with 100 mM 1-thioglycerol, pH 8.5 (15 minutes' incubation) followed by 15 mM NaOH or 100 mM NaOH for 15 minutes, exemplified in Figure 4.

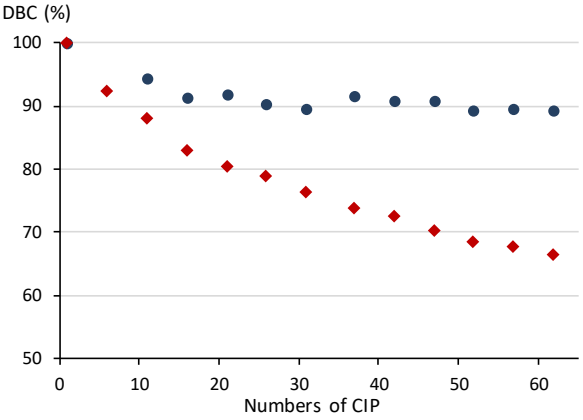

Figure 4. Alkaline stability of WorkBeads Protein A determined by frontal analysis using 1 mg/ml IgG in PBS, pH 7.4. CIP cycle: 100 mM 1-thioglycerol, pH 8.5, 15 minutes incubation; followed by 15 mM NaOH (blue circles) or 100 mM NaOH (red diamonds), for 15 minutes.

Low ligand leakage

The multipoint attachment of protein A to the resin reduces the risk of releasing the ligand. The protein A leakage is therefore low and similar or less to similar resins on the market.

Purification in lab scale

For small scale purifications antibodies are conveniently purified on BabyBio A 1 ml and BabyBio A 5 ml prepacked columns. More than 30 mg IgG can be purified using the 1 ml column and more than 150 mg IgG on the 5 ml column.

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. 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.

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 WorkBeads Protein A can be carried out by sequentially incubating the column or resin with 100 mM 1-thioglycerol, pH 8.5 for 15 minutes followed by 15 mM NaOH for 15 minutes. For CIP in lab-scale 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.

Storage

Store at 2 to 8 °C in 20% ethanol

Related products

Product name	Pack size[1]	Article number
Prepacked columns		
BabyBio TREN 1 ml	1 ml x 5	45 655 213
BabyBio TREN 5 ml	5 ml x 5	45 655 217
BabyBio Dsalt 5 ml	5 ml x 5	45 360 107
BabyBio IEX Screening Kit[2]	1 ml x 4	45 900 001
BabyBio S 5 ml	5 ml x 5	45 200 107
BabyBio Q	5 ml x 5	45 100 107
Bulk resins		
WorkBeads 40 TREN	25 ml	40 603 001
WorkBeads Dsalt	300 ml	40 360 003
WorkBeads 40S	25 ml	40 200 001
WorkBeads 40Q	25 ml	40 100 001
WorkBeads 40/1000 SEC	25 ml	40 300 001
	300 ml	40 300 003

1. All different pack sizes are available on www.bio-works.com
2. BabyBio IEX Screening Kit includes one of each: BabyBio S 1 ml, BabyBio Q 1 ml, BabyBio DEAE 1 ml and BabyBio TREN 1 ml.

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
WorkBeads Protein A	10 ml	40 605 003
	100 ml	40 605 004
	1 L	40 605 105

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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