WorkBeads Protein 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
- Stronger coupling chemistry; high pH stability and 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 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 monoclonal antibodies 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 resin are shown in Table 1. For more details, please see instructions, IN 40 605 010.

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
Recommended flow rate[3]	250 cm/h
Max flow rate[4]	500 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

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.
3. Recommended flow rate at 20 °C using aqueous buffers.
4. Max flow rate at 20 °C using aqueous buffers in 10 x 300 mm column bed. 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). For large columns a lower max flow should be applied.

Applications

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

For more detailed description of affinity chromatography technique please see instruction, IN 40 605 010.

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.

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.

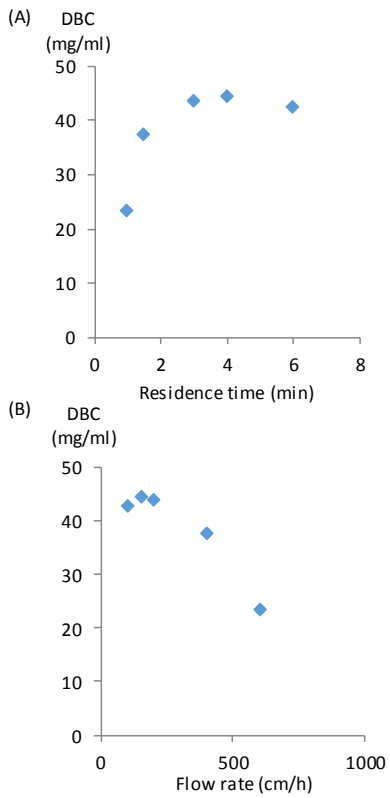

Figure 1. Dependency of dynamic binding capacity on residence time. Frontal analysis using 1 mg/ml human polyclonal IgG in PBS, pH 7.4 was performed in a 6.6 x 100 mm glass column (from Diba, Cambridge, UK). (A) DBC on WorkBeads Protein A versus residence time. (B) DBC on WorkBeads Protein A versus flow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

Purification of monoclonal antibodies

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

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

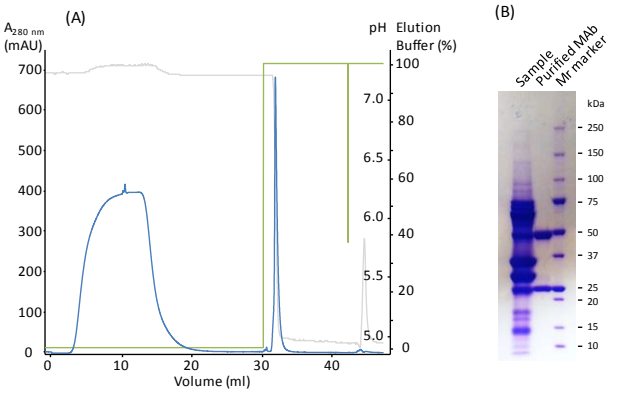

Figure 2. Purification of a monoclonal IgG from CHO cell supernatant using BabyBio A 1 ml column (A). The blue line corresponds to the absorbance at 280 nm, the green line to the concentration of elution buffer and the grey line to the pH. Analysis of the purified MAb by SDS-PAGE, reduced conditions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

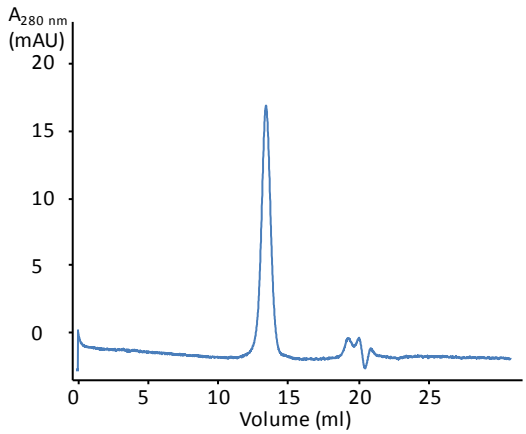

Figure 3. Size exclusion analysis of the purified Mab.

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.

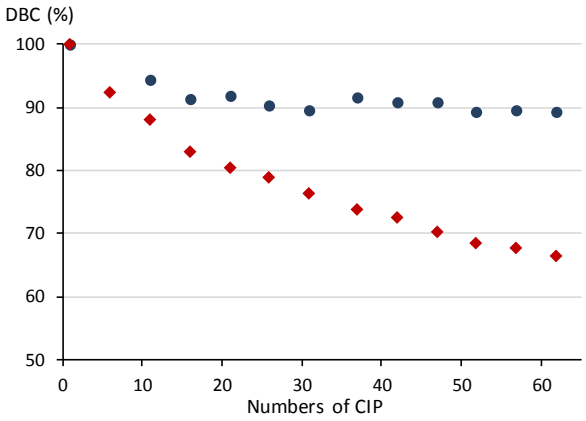

Figure 4. Alkaline stability of WorkBeads Protein A determined by frontal analysis using 1 mg/ml IgG in PBS, pH 7.4. CIP cycle: 100 mM 1-thioglycerol, pH 8.5, 15 minutes incubation; followed by 15 mM NaOH (blue circles) or 100 mM NaOH (red diamonds), for 15 minutes.

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 research antibodies are conveniently purified on BabyBio A 1 ml and BabyBio A 5 ml prepacked columns that are packed with WorkBeads Protein A. More than 30 mg IgG can be purified using the 1 ml column and more than 150 mg IgG on the 5 ml column. For more information, please, see Data sheet DS 45 605 010.

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 pretreatment 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 A 1 ml	1 x 1 ml	45 605 101
BabyBio A 5 ml	1 x 5 ml	45 605 105
BabyBio Dsalt 5 ml	1 x 5 ml	45 360 105
BabyBio S 1 ml	1 x 1 ml	45 200 105
BabyBio Q 1 ml	1 x 1 ml	45 100 105
BabyBio DEAE 1 ml	1 x 1 ml	45 150 105
OptioBio 40S 10x100	1 x 7.9 ml	55 420 011
OptioBio 40Q 10x100	1 x 7.9 ml	55 410 011
Bulk resins		
WorkBeads 40/1000 SEC	25 ml	40 300 001
WorkBeads 40S	25 ml	40 200 001
WorkBeads 40Q	25 ml	40 100 001
WorkBeads 40 DEAE	25 ml	40 150 001

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
WorkBeads Protein A	1.5 ml	40 605 001
	5 ml	40 605 002
	10 ml	40 605 003
	100 ml	40 605 004
	1 L	40 605 005

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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