WorkBeads™ Protein A

WorkBeads Protein A is a new affinity resin designed for the purification of monoclonal and polyclonal antibodies.

- **Higher dynamic binding capacity for monoclonal antibodies; excellent recovery & high purity**
- **Stronger coupling chemistry; high pH stability and low leakage**
- **Reliable, reproducible and efficient**

Media description

WorkBeads Protein A affinity chromatography media is produced from agarose using a cross-linking method that results in a highly porous and physically stable agarose matrix. Agarose based matrices have successfully been used for many years in biotechnology research and in the industrial purification of pro-teins. Agarose is proven to be exceptionally compatible with natural biomolecules, e.g., proteins, DNA and carbohydrates. The material shows minimal nonspecific interaction due to the hydrophilic nature of agarose.

The recombinant protein A attached to the medium 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 pro-teins. 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 bead using a bromohydrin-based method that gives high chemical stability and low ligand leakage. The high capacity, chemical stability and a well-established agarose basematrix make WorkBeads Protein A ideal for purification of monoclonal antibodies as well as polyclonal antibodies. For convenient labscale purification of antibodies WorkBeads A is also available in the BabyBio A 1ml and BabyBio Protein A 5 ml columns.

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Bio-Works

Medicago

Applications

The purification of a monoclonal IgG using WorkBead™ Protein A is exemplified in fig. 1.

Column:	BabyBio A 1 ml
Sample:	10 mL Clarified supernatant from CHO cells diluted 1:11 in PBS
Buffer A:	20 mM Na-phospate, 150 mM NaCl, pH 7.4
Buffer B:	100 mM Gly-HCl, pH 2.7
Flow:	1 mL/min

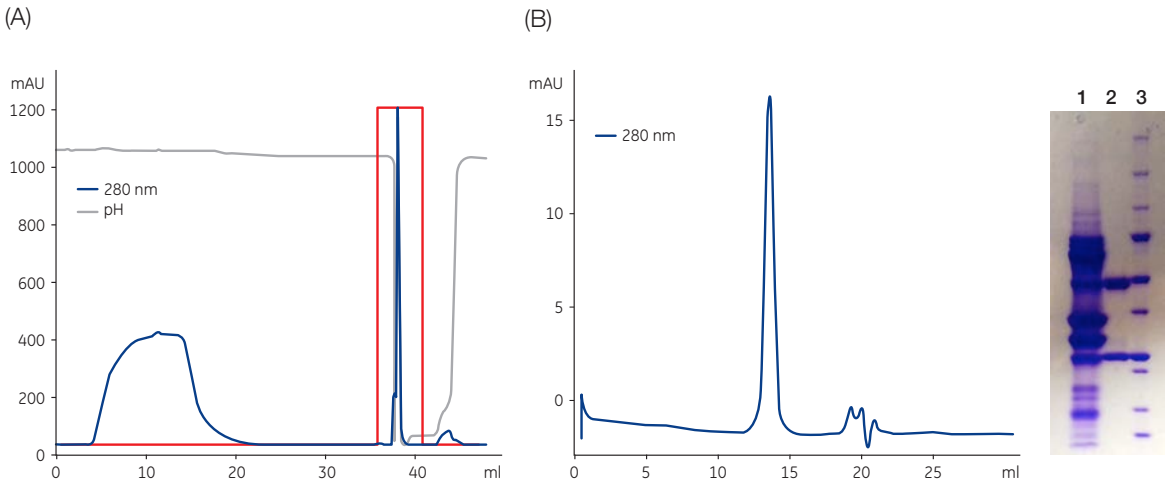

Figure 1. (A) Purification of a monoclonal IgG from CHO cell supernatant. (B) Analysis of the purified MAb by SEC and SDS-PAGE (1. Sample, 2. Purified MAb, 3. M_r markers; 250, 150, 100, 75, 50, 37, 25, 21, 6.9 kD)

High binding capacity

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

WorkBeads™ Protein A has a dynamic binding capacity of typically more than 40 mg IgG/ml media under standard binding conditions (PBS, pH 7.4 and 3 minutes residence time) see Fig 2. No further increase is seen in dynamic binding capacity at 4 or 6 minutes residence time, which indicates that most capacity is utilized at 3 minutes residence time and that total capacity is close to 45 mg IgG/ml.

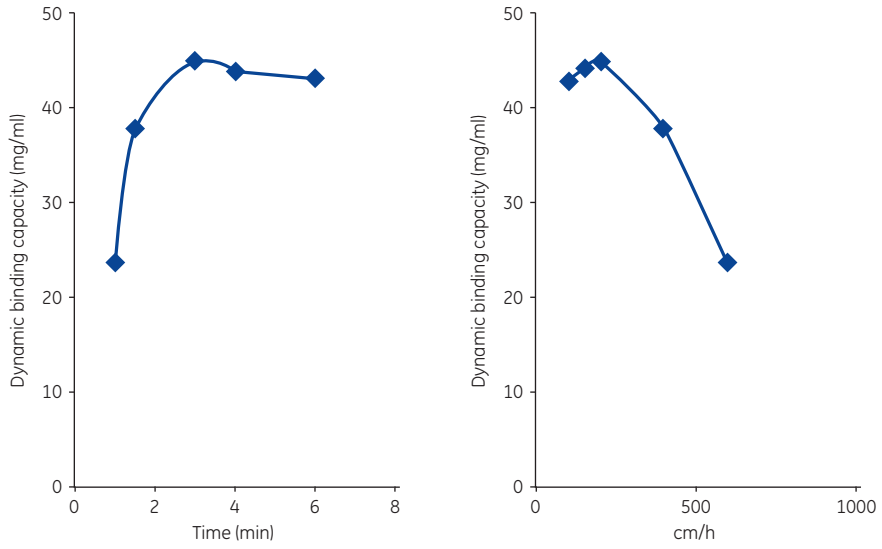

Figure 2. Dependency of the dynamic binding capacity on residence time. Frontal analysis using 1 mg/ml human normal serum IgG was performed in a 6.6 x 100 mm glass column (from Diba, Cambridge, UK) in the presence of PBS, pH 7.4.

Low ligand leakage

The multipoint attachment of protein A to the medium reduced the risk of releasing the ligand. The protein A leakage is therefore low and are similar or less to corresponding competing media in the market.

Purification in lab scale

In the research lab small amounts of antibodies can be purified on BabyBio A 1 ml and BabyBio A 5 ml that are packed with WorkBeads Protein A. More than 30 mg IgG can be purified using the 1-ml column, more than 150 mg IgG in the 5-ml column. See the Date sheet for these products.

Process development

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 comes from and which subclass it is. 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 value than required for desorption. For biopharmaceutical production using WorkBeads Protein A a polishing purification step based on, *e.g.*, ion exchange chromatography, could be added to the process in order to remove traces of leaked of 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

For repeated use it is recommended to perform cleaning-in-place (CIP) of WorkBeads Protein A by sequentially incubate the column or media 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 nonionic detergent followed by 20% ethanol can be used.

Alkaline stability

The stability of the dynamic binding capacity (QB10) a BabyBio A 1 mL column determined by frontal analysis using 1 mg/ml IgG in the presence of PBS, pH 7.4 was analyzed after various number of cleaning-in-place cycles each with 100 mM 1-thioglycerol, pH 8.5 (15 minutes incubation) followed by 15 mM NaOH for 15 minutes (open circles) or by 100 mM NaOH.

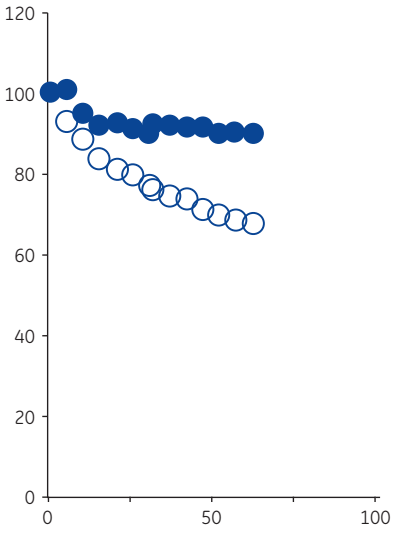

Figure 3. Alkaline stability of WorkBeads Protein A.

Media characteristics

Medium	WorkBeads Protein A
Target substance	Antibodies (IgG), bound via the Fc-region;
Matrix	Rigid, highly cross-linked agarose
Ligand	Recombinant protein A expressed in *E. coli* using animal-free medium
Coupling chemistry	Bromohydrin
Dynamic binding capacity (DBC)[1]	>40 mg human IgG/ml medium
Recommended flow rate[2]	250 cm/h
Max flow rate[3]	500 cm/h
Chemical stability	Compatible with all standard aqueous buffers used for protein purification, and 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.
Recommended working range pH Stability	3–10 short term 2–12 cleaning
Storage	+2°C to +8°C in 20% ethanol

[1] DBC was determined at 10% breakthrough ($Q_{B,10}$) by frontal analysis with 1 mg/ml human serum IgG in PBS, pH 7.4 at 240 cm/h in a column with a WorkBeads Protein A bed of 6 x 100 mm (=2.5 minutes residence time).

[2] At 20 °C using aqueous buffers.

[3] At 20 °C using aqueous buffers in 10 x 300 mm column bed. Decrease the max flow if the liquid has a higher viscosity. Higher viscosities can be caused by low temperature (use max flow/2 at 4 °C), or by additives (e.g, use max flow/2 for 20% ethanol). For large columns a lower max flow should be applied.

Ordering information

Product name	Pack size	Article number
WorkBeads Protein A	Bulk Media – 1.5 ml	40605001
	Bulk Media – 5 ml	40605002
	Bulk Media – 10 ml	40605003
	Bulk Media – 100 ml	40605004
	Bulk Media – > 1L inquiry	40605005

To purchase this separation media contact your local distributor.
You may also contact Bio-Works or Medicago directly at:

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For more information, please visit our website at:

www.bio-works.net
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