BabyBio A™

BabyBio A ready to use mini columns available in 1 ml and 5 ml. Products allow quick, easy and convenient purification of monoclonal and polyclonal antibodies.

- **Swifter purification of polyclonal and monoclonal antibodies**
- **Higher binding capacity and purity in one step**
- **Simple and easy method giving reproducible results**

Media description

BabyBio A columns are packed with WorkBeads Protein A medium. The medium is based on agarose from a cross-linking method that results in a highly porous and physically stable agarose matrix. Agarose matrices have successfully been used for many years in biotechnology research and in the industrial purification of proteins. Agarose is proven to be exceptionally compatible with natural biomolecules like proteins, DNA, carbohydrates etc. The material shows minimal non-specific interaction due to the hydrophilic nature of agarose. Recombinant Protein A developed and produced by Mediacago is coupled to the matrix using a bromohydrin method. The coupling method gives high chemical stability and low ligand leakage.

The recombinant protein A is produced in *E. coli* and is engineered to facilitate an oriented coupling giving a higher binding capacity. The specificity of the recombinant protein A for the Fc region of IgG provides excellent purification. No animal derived ingredients are used in the fermentation process or the purification of the protein. Each batch of protein A is tested, using QC methods for IgG binding (>90%), amino acid analysis of content (>85%), as well as for bacterial burden (<100 CFU/g). The high capacity, chemical stability and a established agarose base matrix makes BabyBio A ideal for purification of monoclonal antibodies.

Bio-Works

Medicago

Column characteristics

The BabyBio column body is made from biocompatible polypropylene, which does not significantly interact with biomolecules. The top and bottom filters are made from polyethylene. These ready to use columns are delivered with plugs in the inlet and a snap-off end at the outlet. A cap for the outlet is included for closing the column during storage. The columns can be connected a syringe, pump or chromatography system using fingertight fittings (coned 10-32) for 1/16" o.d. tubing.

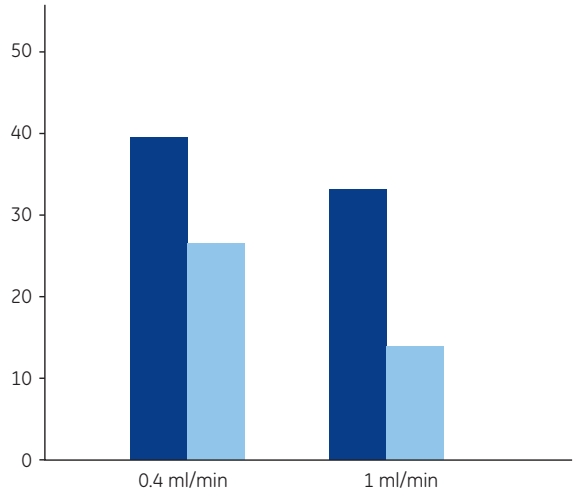

Figure 1. Dynamic binding capacity for human serum IgG on BabyBio A 1 mL (dark blue) and corresponding 1 ml column from competitor product (data from data sheet). Competitor values are obtained from corresponding data sheet and are based on experiments under similar conditions.

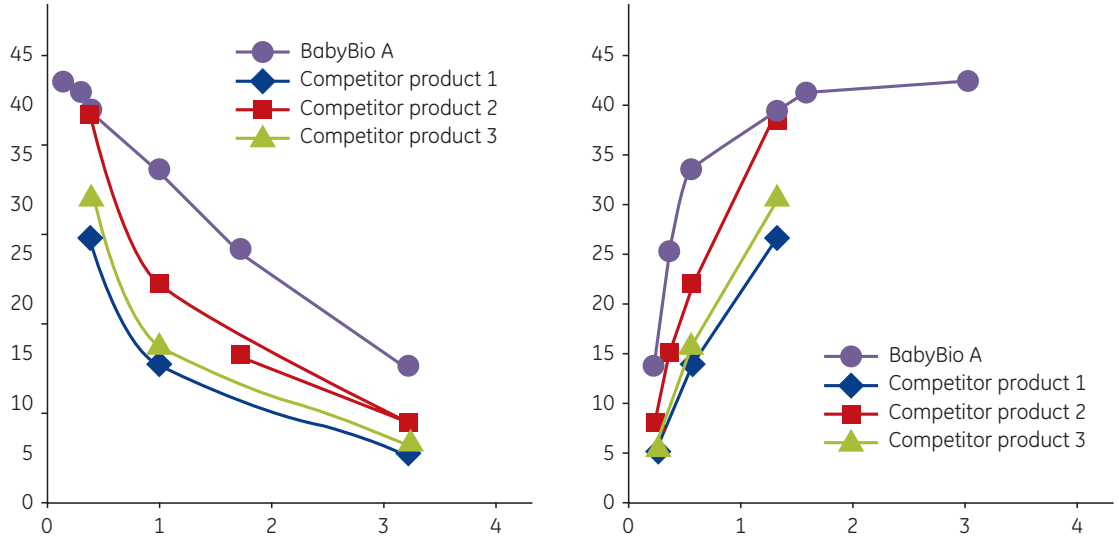

Figure 2. Dynamic binding capacity ($Q_{b,10}$)for human serum IgG at different flow rates by frontal analysis of 1 mg/ml IgG in PBS, pH 7.4. Competitor values are obtained from corresponding data sheet and are based on experiments under similar conditions.

Applications

BabyBio A columns can be used for purification of antibodies in research as well as for bioprocess development. For labscale purification of IgG a set of standard conditions can often be used without further optimization. For very high purity requirements in lab-scale and for most bioprocesses it is common to add a second purification step to remove any leachables, impurities from the source material or antibody dimers or aggregates. For large processes polishing is usually performed using ion exchange chromatography. An example of purification of a monoclonal antibody is shown in Figure 3.

Column:	BabyBio Protein 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

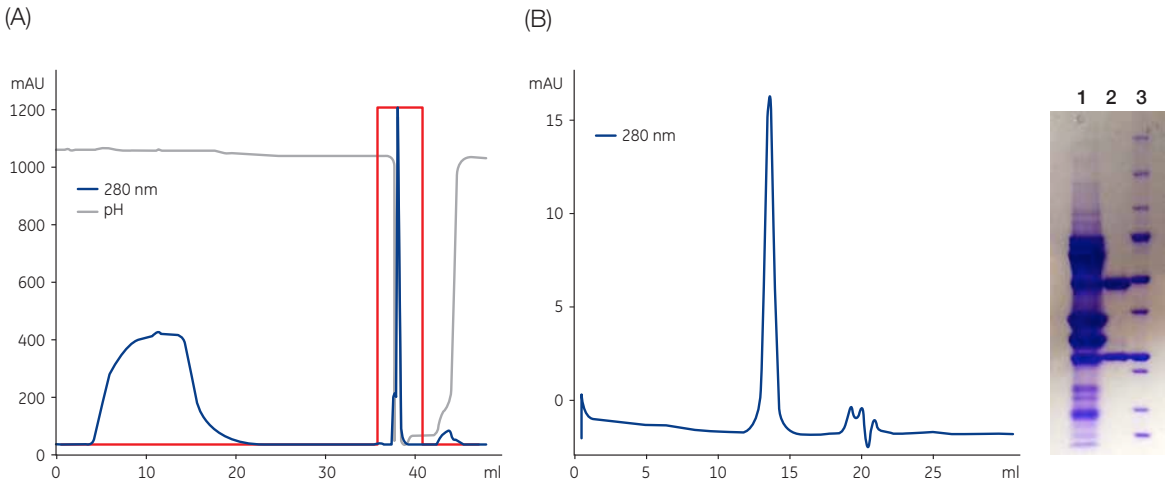

Figure 3. (A) Purification of a monoclonal IgG from CHO cell supernatant. (B) Analysis of the purified MAb by SEC and SDS-PAGE (1. Sample, 2. Purified MAb, 3. Mr markers; 250, 150, 100, 75, 50, 37, 25, 21. 6.9 kD).

Short instructions

1. Connect the column to the chromatography system.
2. Remove the storage solution using water or aqueous buffer.
3. Equilibrate (PBS, pH 7.4)
4. Apply the sample (often no preparation needed)
5. Wash (PBS, 7.4)
6. Elute at low pH (100 mM Gly-HCl, pH 2.7 or 100 mM Na-citrate, pH 3)
7. Re-equilibrate (PBS, pH 7.4) and if to be stored fill with 20% ethanol.

For details instructions and tips for optimization, see instructions for use.

Equipment

BabyBio A can be used with most standard liquid chromatography equipment. Purification can also be done using a syringe connected to the column by a luer/std HPLC connector.

Scale-up

Scale-up can conveniently be performed from a 1 ml column to a 5 ml column. For higher capacity columns can be connected in series. Note that the total backpressure will increase which may require a reduction of flow. Further scale-up can be done with bulk packages of WorkBeads Protein A packed in larger columns.

Cleaning

For repeated use it is recommended to perform cleaning 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. Alternatively, CIP can be performed by incubation 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. Store the column filled with 20% ethanol.

Low ligand leakage

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

Alkaline stability

The stability of the dynamic binding capacity (Q_{B10}) 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.

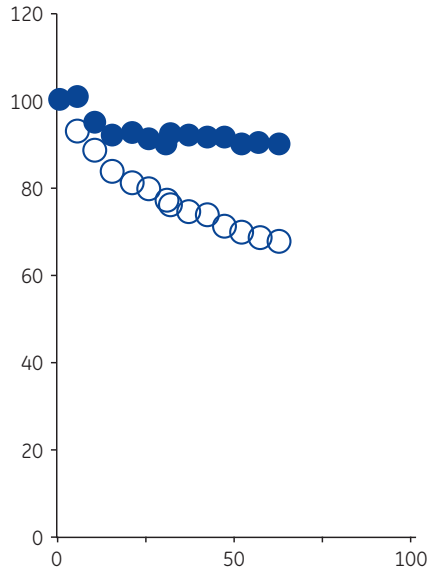

Figure 4. Alkaline stability of WorkBeads Protein A.

Column characteristics

Medium	WorkBeads Protein A
Matrix	Rigid, highly cross-linked agarose
Ligand	Recombinant protein A expressed in *E. coli* using animal-free medium
Coupling chemistry	Bromohydrin
Dynamic binding capacity[1]	>40 mg human IgG/ml medium
Column volumes	1 ml 5 ml
Column dimensions	7 × 28 mm (1 ml) 13 × 38 mm (5 ml)
Recomended flow rate BabyBio A 1 ml	0.5-1 ml/min
Recomended flow rate BabyBio A 5 ml	1-4 ml/min
Max flow rate BabyBio A 1 ml	5 ml/min
Max flow rate BabyBio A 5 ml	15 ml/min
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 2-10 cleaning
Storage	+4°C to +8°C in 20% ethanol

[1] Dynamic binding capacity was determined at 10% breakthrough (Q_{B10}) by frontal analysis with 1mg/ml human serum IgG in PBS, pH 7.4 at 240 cm/h in a column with a WorkBeads Protein A bed height of 100 mm (=2.5 minutes residence time). Notice that the dynamic binding capacity at corresponding flow in BabyBio columns is slightly lower due to their short length.

Ordering information

Product name	Pack size	Article number
BabyBio A 1 ml	1 × 1 ml	45605101
	2 × 1 ml	45605102
	5 × 1 ml	45605103
	10 × 1 ml	45605104
BabyBio A 5 ml	1 × 5 ml	45605105
	2 × 5 ml	45605106
	5 × 5 ml	45605107
	10 × 5 ml	45605108

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

info@bio-works.net
info@medicago.se

For more information about Bio-Works, please visit our website at:

www.bio-works.net
www.medicago.se

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