WorkBeads affimAb
BabyBio affimAb 1 ml
BabyBio affimAb 5 ml

WorkBeads™ affimAb resin is an alkaline-stable resin designed for purification of monoclonal and polyclonal antibodies in laboratory to process scale. This resin has a superior basematrix in combination with an optimized alkaline-stable protein A ligand. This results in high dynamic binding capacity also at short residence times, and stabile capacity over multiple purification cycles with cleaning-in-place using 0.5 M NaOH.

Prepacked BabyBio affimAb 1 ml and 5 ml columns are available for small-scale purifications and condition screening in process development. WorkBeads affimAb resin can also be used for purifications in other formats, such as batch and centrifugation purifications.

- Top performance dynamic binding capacity also at short residence times
- Outstanding alkaline stability with 0.5 M NaOH, extends the number of purification cycles
- Excellent purity, recovery and reproducibility
- Negligible protein A leakage
- Convenient prepacked 1 ml and 5 ml BabyBio™ columns

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 alkaline stabile recombinant protein A attached to the optimized basematrix is produced in *E. coli* under conditions free of components of animal origin and purified to high purity before coupling. This combination gives both high dynamic binding capacities for antibodies and the possibility for efficient cleaning-in-place with 0.5 M NaOH.

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 high capacity, chemical stability and the optimized agarose matrix make WorkBeads affimAb ideal for purification of monoclonal antibodies (mAb) as well as polyclonal antibodies. For convenient small-scale purifications of antibodies WorkBeads affimAb is available prepacked in BabyBio affimAb 1 ml and 5 ml columns.

The main characteristics of WorkBeads affimAb resin are shown in Table 1. For more details, please see instructions, IN 40 800 010 AA.

Table 1. Main characteristics of WorkBeads affimAb resin.

	WorkBeads affimAb
Target substance	Antibodies (IgG), bound via the F_c-region
Matrix	Rigid, highly cross-linked agarose
Average particle size[1] (D_{v50})	50 µm
Ligand	Recombinant protein A expressed in *E. coli* using animal-free medium
Dynamic binding capacity[2] (DBC)	> 40 mg human IgG/ml resin
Max recommended flow rate[3]	300 cm/h
Chemical stability	Compatible with all standard aqueous buffers used for protein purification, 10 mM HCl (pH 2), 0.5 M NaOH (pH 12), 0.1 M sodium citrate buffer (pH 3), 6 M guanidine-HCl, 20% ethanol. Should not be stored at low pH for prolonged time.
pH stability	3 - 10
Cleaning-in-place stability	Up to 0.5 M NaOH
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 ($Q_{B10\%}$) by frontal analysis with 1 mg/ml human polyclonal IgG in PBS, pH 7.4 at 1.4 ml/min (245 cm/h, 2.5 minutes residence time) in a column packed with WorkBeads affimAb resin, column bed 6.6 x 100 mm.
3. Max recommended flow rate at 20 °C using aqueous buffers. 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 flow rate when operating at 4 °C), or by additives (e.g., use half of the max flow rate for 20% ethanol).

BabyBio column description

The BabyBio column hardware's are 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 fittings and tubings).

The main characteristics of BabyBio affimAb columns are shown in Table 2. For more details, please see instructions IN 45 800 010 AA.

Table 2. Main characteristics of BabyBio affimAb 1 ml and BabyBio affimAb 5 ml columns.

	BabyBio affimAb
Target substance	Antibodies (IgG), bound via the F_c-region
Resin	WorkBeads affimAb
Matrix	Rigid, highly cross-linked agarose
Average particle size[1] (D_{v50})	50 µm
Ligand	Recombinant protein A expressed in *E. coli* using animal-free medium
DBC[2]	> 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 affimAb 1 ml	0.5 - 1 ml/min (75 - 150 cm/h)
BabyBio affimAb 5 ml	1 - 4 ml/min (45 - 180 cm/h)
Maximum flow rate[3]	
BabyBio affimAb 1 ml	4 ml/min (620 cm/h)
BabyBio affimAb 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, 10 mM HCl (pH 2), 0.5 M NaOH (pH 12), 0.1 M sodium citrate buffer (pH 3), 6 M guanidine-HCl, 20% ethanol. Should not be stored at low pH for prolonged time.
pH stability	3 - 10
Cleaning-in-place stability	Up to 0.5 M NaOH
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 ($Q_{B10\%}$) by frontal analysis with 1 mg/ml human polyclonal IgG in PBS, pH 7.4 at 1.4 ml/min (245 cm/h, 2.5 minutes residence time)) in a column with a WorkBeads affimAb bed height of 100 mm. Notice that the dynamic binding capacity at corresponding flow rate in BabyBio columns is slightly lower due to their shorter 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 flow rate when operating at 4 °C), or by additives (e.g., use half of the max flow rate for 20% ethanol).

Applications

High alkaline stability

The alkaline stability of WorkBeads affimAb has been tested by dynamic binding capacity after multiple cleaning-in-place (CIP) cycles, Figure 1.

Each CIP cycle includes equilibration in PBS pH 7.4, then 0.5 M NaOH at 15 minutes contact time, wash with PBS, pH 7.4 followed by a wash with 100 mM glycine-HCl, pH 2.7. The DBC was determined at every 20th CIP cycle, at 10% breakthrough by frontal analysis at 2.4 minutes residence time in a 6.6 x 100 mm glass column using a solution of 1 mg/ml polyclonal IgG in the presence of PBS, pH 7.4.

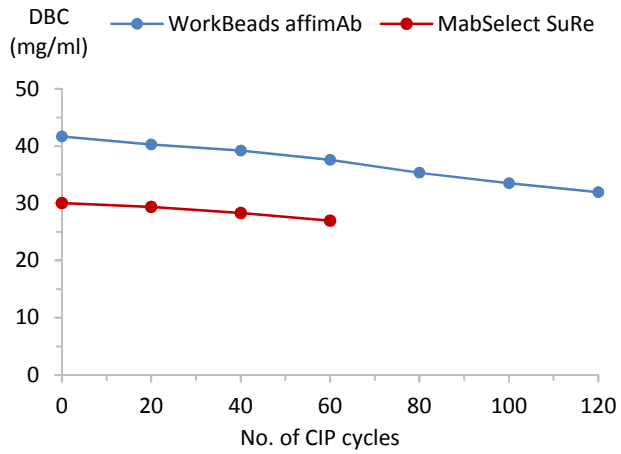

Figure 1. DBC for polyclonal human IgG on WorkBeads affimAb (blue) and MabSelect SuRe™ (GE Healthcare) (red) determined by frontal analysis at 2.4 minutes residence time after 120 resp. 60 CIP cycles with 0.5 M NaOH at 15 minutes contact time.

High dynamic binding capacity

The optimized density of the alkaline stable protein A ligand immobilized on the matrix allows high dynamic binding capacity for antibodies even at short residence times. WorkBeads affimAb has a dynamic binding capacity of typically more than 40 mg IgG/ml resin under standard binding conditions (PBS, pH 7.4 and 2.5 minutes residence time), see Figure 2. The dynamic binding capacity is essentially the same at 4.8 and 6 minutes residence times, and most binding capacity is utilized at 4 minutes residence time. This indicates a static binding capacity of 50 mg IgG/ml resin.

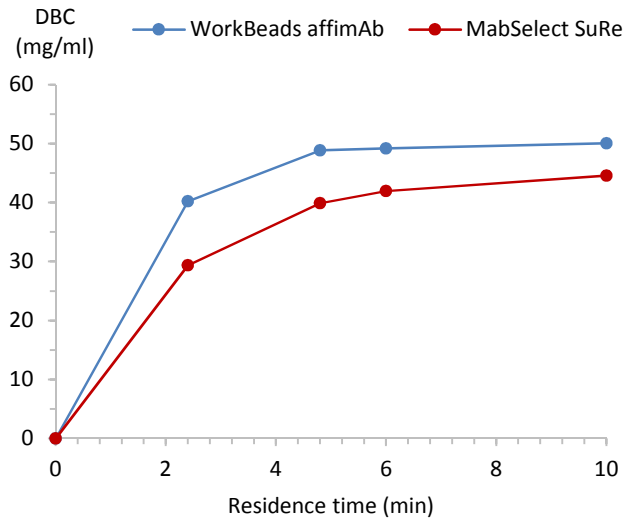

Figure 2. Dependency of dynamic binding capacity on residence time. Frontal analysis using 1 mg/ml human polyclonal IgG in PBS, pH 7.4 was performed in 6.6 x 100 mm glass column.

Particle size distribution

WorkBeads resin is manufactured with a narrow particle size distribution, exemplified in Figure 3.

The combination of the optimized basematrix rigidity and the narrow particle size distribution gives low backpressure even at higher flow rates in combination with more efficient packed columns.

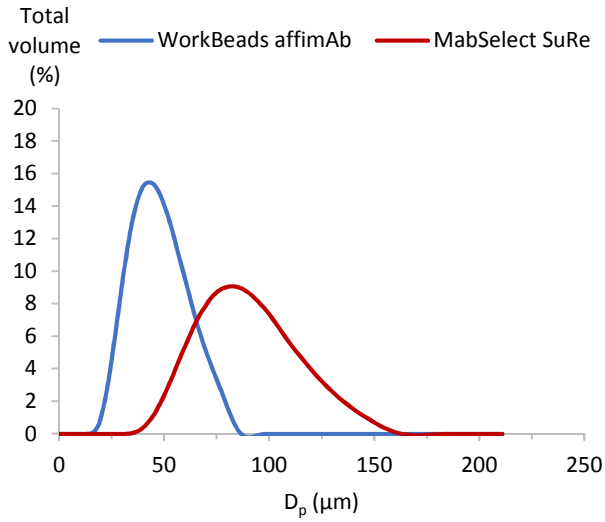

Figure 3. Particle size distribution comparison of WorkBeads affimAb (blue) and MabSelect SuRe (red).

Resin rigidity

WorkBeads affimAb is designed for process scale purification of monoclonal antibodies. Pressure/flow properties for the basematrix is shown in Figure 4. The measurements were carried out with an open bed (adaptor not pushed against the bed). The high rigidity of the agarose beads allows for increased flow rates and increased process economy.

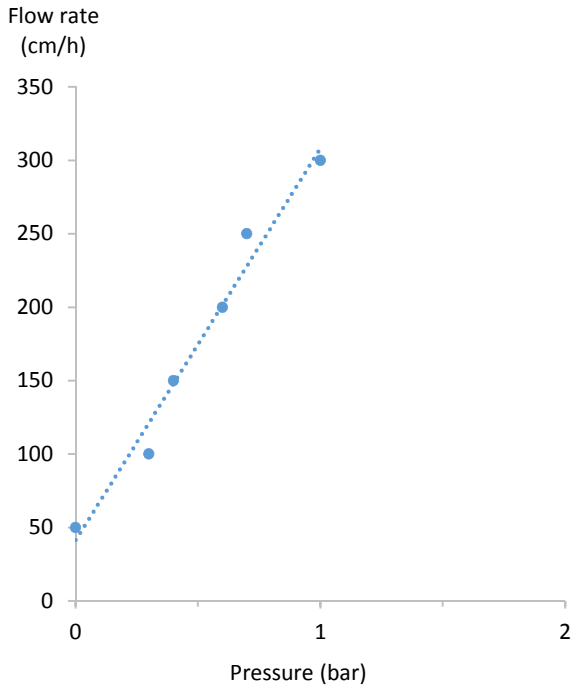

Figure 4. Pressure/flow data on WorkBeads basematrix in water obtained in a 25x200 mm open bed glass column.

Purification of monoclonal antibodies

Figure 5 presents a comparison of purity results for a monoclonal antibody expressed in CHO cells purified either on WorkBeads affimAb or MabSelect SuRe (chromatogram not shown). Purity analysis presented in Fig. 5B. The two resins are packed in similar columns, 6.6 x 100 mm, and the purifications are done in exactly the same way.

Resins:	WorkBeads affimAb
Column:	3.4 ml (6.6 x 100mm)
Sample:	18 ml clarified cell supernatant from CHO cells
Binding buffer:	PBS, pH 7.4
Elution buffer:	100 mM glycine-HCl, pH 2.7
Flow rates:	
Equilibration/wash:	1.7 ml/min (300 cm/h)
Sample load:	0.6 ml/min (100 cm/h)
Elution:	0.9 ml/min (150 cm/h)

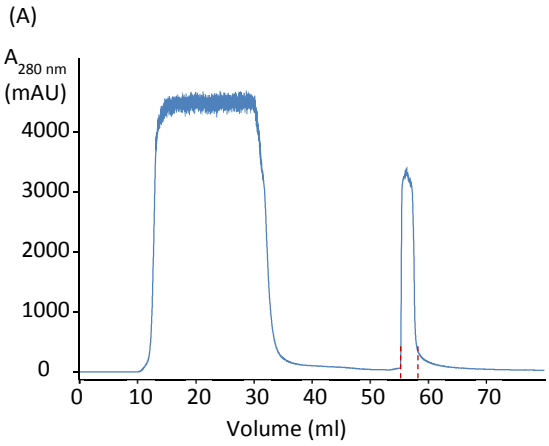

Figure 5A. Purification of a monoclonal IgG from CHO cells using WorkBeads affimAb. The blue line corresponds to the absorbance at 280 nm.

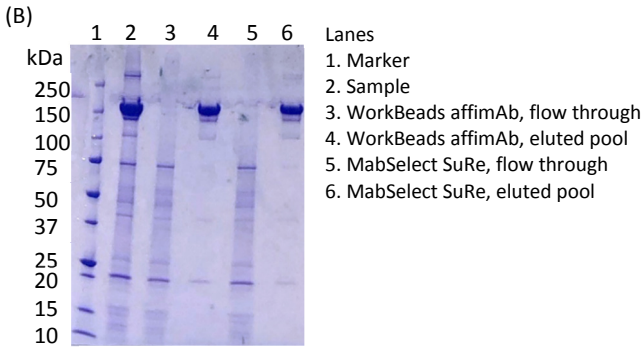

Figure 5B. Analysis of the purified mAb by SDS-PAGE, non-reduced conditions. Comparison of mAb purified in the same way on MabSelect SuRe resin from GE Healthcare.

Minimal protein A leakage

WorkBeads affimAb is designed to have low leakage of the immobilized protein A-ligand. The protein A leakage is similar compared to other protein A resins on the market.

A series of 50 purification runs in laboratory scale applying CHO cell supernatant on WorkBeads affimAb was performed. Each run was followed by a CIP using 0.5 M NaOH and 10 minutes contact time. Elution profile from different cycles is shown in Figure 6. Fractions from the eluted sample were analyzed by enzyme-linked immunosorbent assay (ELISA) using Protein A ELISA kit (#9333-1, Repligen). The ligand leakage is shown in Table 3.

Resin:	WorkBeads affimAb
Column:	1.7 ml (6.6 x 50 mm)
Sample load:	18 ml clarified cell supernatant from CHO cells (100 cm/h)
Binding/wash buffer:	PBS, pH 7.4 (300 cm/h)
Elution buffer:	100 mM glycine-HCl, pH 2.7 (150 cm/h)
CIP:	0.5 M NaOH, (100 cm/h) 10 min contact time in each cycle

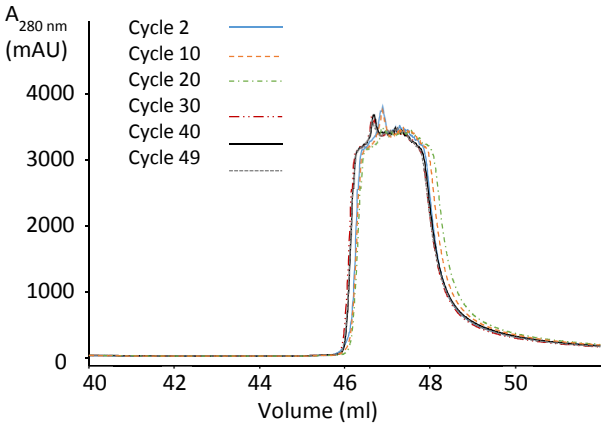

Figure 6. Elution profiles from purification on WorkBeads affimAb after repeated CIP cycles.

Table 3. ELISA results regarding leakage of WorkBeads affimAb ligand.

Purification cycle	Leakage of protein A from WorkBeads affimAb (ng/ml)
2	4.1
5	2.7
10	0.7
30	1.2
35	2.3
40	1.8
41*	0.2
45	1.9
50	1.3

*Cycle 41 corresponds to a blank run with PBS, pH 7.4.

A similar study was performed with 20 cycles IgG sample load and CIP with with 0.5 M NaOH on both WorkBeads affimAb and MabSelect SuRe. Fractions from the eluted IgG were analyzed by ELISA. The ligand leakage comparison is shown in Table 4.

Table 4. ELISA results regarding ligand leakage of WorkBeads affimAb compared to MabSelect SuRe..

Purification cycle	Leakage of protein A (ng/ml)	
	WorkBeads affimAb	MabSelect SuRe
10	1.2	0.6
20	0.6	0.7
21*	0.1	0.2

*Cycle 21 corresponds to a blank run with PBS, pH 7.4.

Process optimization

The primary aim of process method optimization is to find the suitable binding and elution conditions for best purity and yield, and to minimizing denaturation and aggregation of the antibody. The binding affinity for IgG to protein A varies depending on what species the IgG originates from and which subclass it belongs to. There is also 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_1.

Elution is normally performed at reduced pH, down to pH 2.7 but depends 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 one or two polishing purification steps based on, e.g., ion exchange chromatography is often added to the process in order to remove aggregates, traces of leached protein A and impurities from the feed. After optimizing the eluent composition, the process is scaled up by keeping the linear flow rate and sample to bed volume ratio constant and only increasing the column diameter. In case the column bed height needs to be increased the set residence time should be kept the same, which means that the linear flow rate can be increased correspondingly.

Cleaning-in-place

During purification, impurities such as cell debris, lipids, nucleic acids and protein precipitates from the samples, 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, CIP, reduces the contamination, and prolongs the binding capacity, resolution and flow properties of the column. CIP of WorkBeads affimAb can been done using NaOH of concentrations of up to 0.5 M over 15 minutes or more.

Storage

Store WorkBeads affimAb and BabyBio affimAb columns at 2 to 8 °C in 20% ethanol.

Related products

Product name	Pack size[1]	Article number
Prepacked columns		
BabyBio S 5 ml	5 ml x 5	45 200 107
BabyBio Q 5 ml	5 ml x 5	45 100 107
BabyBio TREN 5 ml	5 ml x 5	45 655 217
BabyBio Dsalt 5 ml	5 ml x 5	45 360 107
Bulk resins		
WorkBeads 40S	25 ml	40 200 001
WorkBeads 40Q	25 ml	40 100 001
WorkBeads 40 TREN	25 ml	40 603 001

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 affimAb	25 ml	40 800 001
	200 ml	40 800 002
	1 L	40 800 010
	5 L	40 800 050
	10 L	40 800 060
BabyBio affimAb 1 ml	1 ml x 1	45 800 101
	1 ml x 2	45 800 102
	1 ml x 5	45 800 103
	1 ml x 10	45 800 104
BabyBio affimAb 5 ml	5 ml x 1	45 800 105
	5 ml x 2	45 800 106
	5 ml x 5	45 800 107
	5 ml x 10	45 800 108

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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