WorkBeads affimAb
BabyBio affimAb 1 ml
BabyBio affimAb 5 ml

WorkBeads™ affimAb resin is an alkali-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 alkali-stable protein A ligand. This results in high dynamic binding capacity also at short residence times, and stable 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 purification 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 alkali 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 alkali-stable recombinant protein A attached to the optimized base matrix 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 purification 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
Maximum recommended flow rate[3,4]	300 cm/h
Chemical stability	Compatible with all standard aqueous buffers used for protein purification and with 10 mM HCl (pH 2), 0.5 M NaOH (pH 12), 0.1 M sodium citrate buffer (pH 3), 6 M guanidine-HCl, and 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 (240 cm/h, 2.5 minutes residence time) in a column packed with WorkBeads affimAb, column bed 6.6 x 100 mm.
3. Maximum recommended 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. Maximum recommended flow rate determined in a 25 x 200 mm column.

BabyBio column description

The BabyBio column hardware 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 fittings and tubing).

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
Dynamic binding capacity (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[3]	
BabyBio affimAb 1 ml	0.2 - 1 ml/min (28 - 150 cm/h)
BabyBio affimAb 5 ml	0.9 - 4 ml/min (38 - 180 cm/h)
Maximum flow rate[4]	
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 (240 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. Recommended flow rates include the flow rates in all steps; cleaning, equilibration, applying sample, washing, elution, etc.
4. 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).

Applications

High alkali stability

The alkali 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.

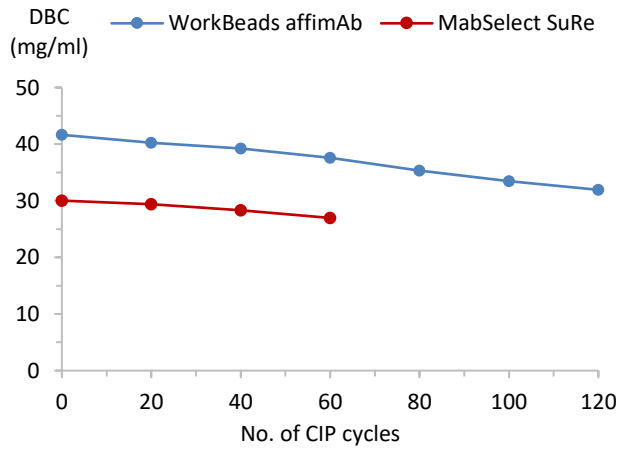

Figure 1. DBC for polyclonal human IgG on WorkBeads affimAb (blue) and MabSelect™ SuRe (GE Healthcare) (red) determined by frontal analysis at 2.4 minutes residence time after 120 resp. 60 CIP cycles with 0.5 M NaOH at 15 minutes contact time.

High dynamic binding capacity

The optimized density of the alkali-stable protein A ligand immobilized on the matrix allows high dynamic binding capacity for antibodies also 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 2.4 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.

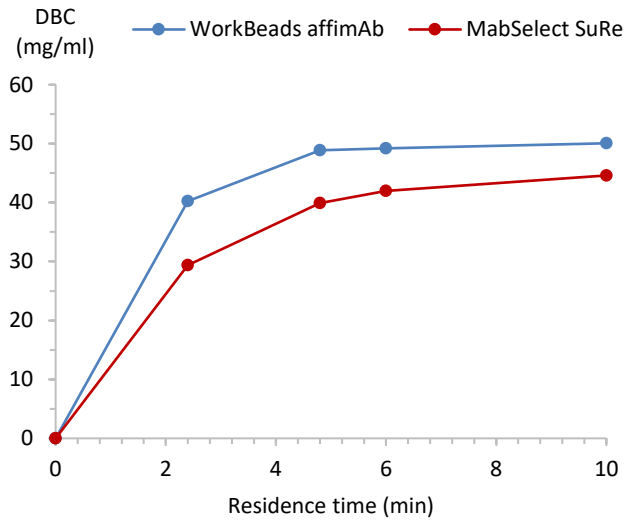

Figure 2. Dependency of dynamic binding capacity on residence time. Frontal analysis using 1 mg/ml human polyclonal IgG in PBS, pH 7.4 was performed in 6.6 x 100 mm packed bed.

BabyBio affimAb is prepacked with WorkBeads affimAb resin. The prepacked columns are designed for small-scale purification and condition screening during process development. The DBC was determined at 10% breakthrough ($Q_{B10\%}$) by frontal analysis with 1 mg/ml human polyclonal IgG in PBS, pH 7.4 at different residence time for BabyBio affimAb 1 m (1, 2.4 and 4 minutes). A comparison with BabyBio affimAb 5 ml and WorkBeads affimAb packed in a column with 100 mm bed height at 2.4 minutes residence time is shown in Figure 3. Notice that the dynamic binding capacity of BabyBio columns is slightly lower. This is expected for this type of column dimensions. BabyBio is during process development best used for initial estimation of residence time/dynamic binding capacity. For more accurate determinations a longer bed height, e.g., 100 mm is recommended.

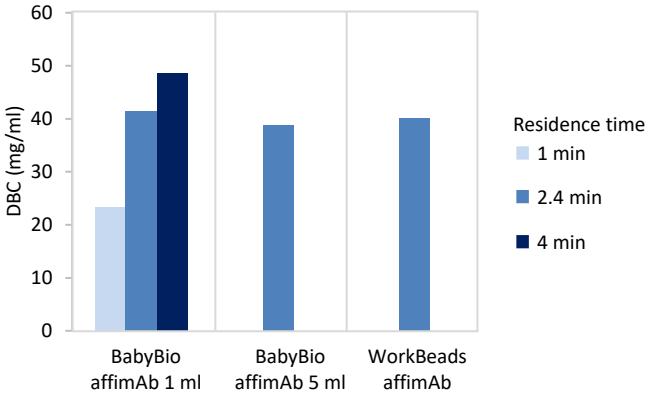

Figure 3. Dependency of dynamic binding capacity on residence time. Frontal analysis using 1 mg/ml human polyclonal IgG in PBS, pH 7.4 was performed in BabyBio affimAb 1 ml (28 mm bed height), BabyBio affimAb 5 ml (38 mm bed height) and WorkBeads affimAb in a 6.6 x 100 mm packed bed (100 mm bed height).

Particle size distribution

WorkBeads resin is manufactured with a narrow particle size distribution, exemplified in Figure 4. The optimized rigidity of the base matrix results in low back-pressure even at higher flow rates, while the narrow particle size distribution of the resin allows for packed columns with higher efficiency.

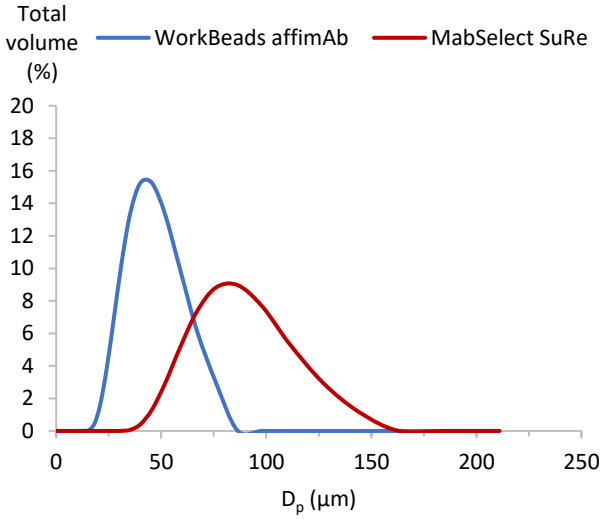

Figure 4. Particle size distribution comparison of WorkBeads affimAb (blue) and MabSelect SuRe (red).

Resin rigidity

WorkBeads affimAb is designed for process-scale purification of monoclonal antibodies. Pressure-flow properties for the base matrix is shown in Figure 5. 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.

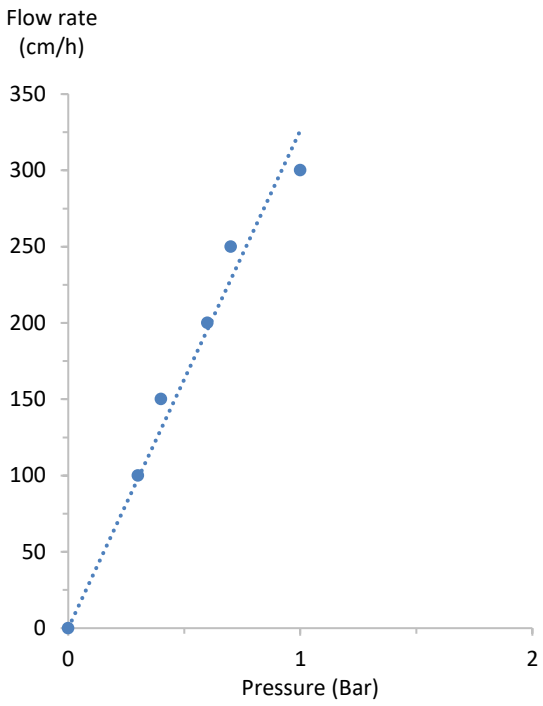

Figure 5. Pressure-flow data on WorkBeads base matrix in water obtained in a 25 x 200 mm open bed glass column. The pressure over the bed at low flow rates is often too low to detect.

Purification of monoclonal antibodies

Figure 6 presents a comparison of purity results for a monoclonal antibody expressed in Chinese Hamster Ovary (CHO) cells purified on WorkBeads affimAb and MabSelect SuRe. Purity analysis presented in Figure 6B, includes results from a corresponding purification run on MabSelect SuRe made under identical conditions.

Resins:	WorkBeads affimAb
	MabSelect SuRe (chromatogram not shown)
Column:	3.4 ml (6.6 x 100 mm)
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)

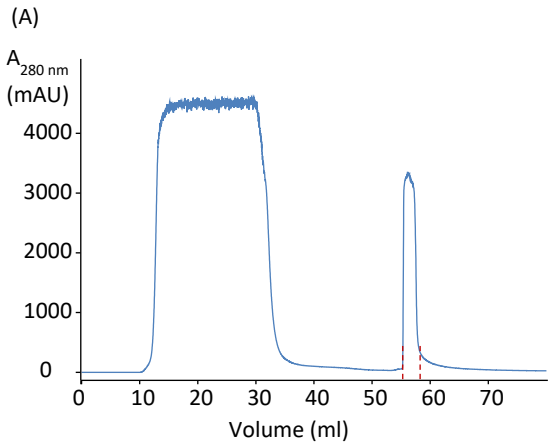

Figure 6A. Purification of a monoclonal IgG from CHO cells using WorkBeads affimAb. The blue line corresponds to the absorbance at 280 nm.

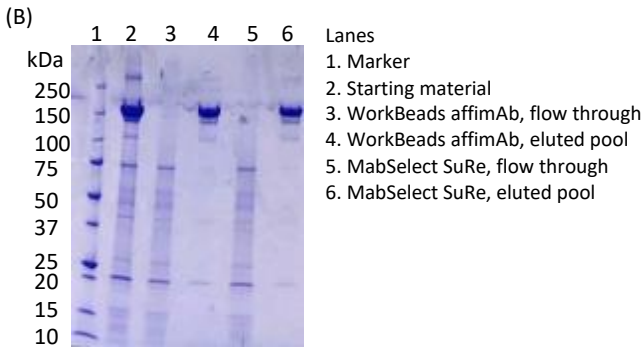

Figure 6B. Analysis of the purified mAb by SDS-PAGE, non-reduced conditions. Comparison of mAb purified by identical method on WorkBeads affimAb and MabSelect SuRe resins.

Low protein A leakage

WorkBeads affimAb is designed to have low leakage of the immobilized protein A ligand. The protein A leakage is similar to other protein A resins on the market. A series of 50 purification runs at 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. The elution profile from different cycles is shown in Figure 7. Fractions from the eluted sample were analysed by enzyme-linked immunosorbent assay (ELISA) using Protein A ELISA kit (#9333-1, Repligen). Levels of ligand leakage were determined using ligand-specific derived standard curves, i.e. WorkBeads affimAb ligand and MabSelect SuRe ligand used as separate standards. 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

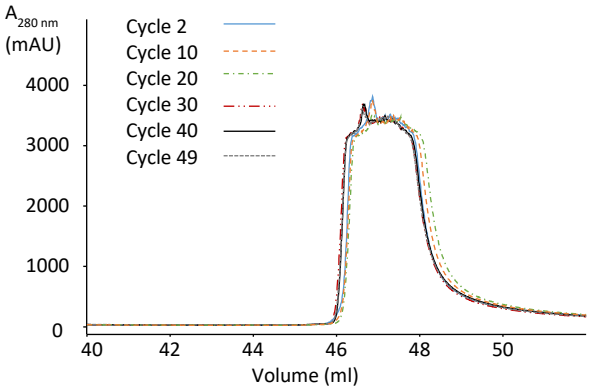

Figure 7. Elution profiles from purifications on WorkBeads affimAb after repeated CIP cycles.

Table 3. Protein A ligand leakage levels in eluates analysed by ELISA for WorkBeads affimAb and MabSelect SuRe.

Purification cycle	Leakage of Protein A ligand (ppm*)	
	WorkBeads affimAb	MabSelect SuRe
6	13.0	2.1
9	10.7	2.2
13	13.3	4.2
23	9.3	7.7
36	7.5	3.8
43	9.5	3.8
49	8.3	3.3

*ppm is measured in ng leached protein A ligand per mg of eluted IgG. The level of leakage ligand is dependent on the experimental set up as well as the sample used. All levels are below the expected level of 20 ppm of protein A ligand leakage.

Effective reduction of HCP and HCD

The design of WorkBeads affimAb gives higher purity of the eluted mAb, with reduced amounts of both host cell proteins (HCP) and host cell DNA (HCD) in the eluate.

HCP in eluates from the series of laboratory scale purifications on WorkBeads affimAb and MabSelect SuRe were analysed using a CHO HCP ELISA kit (#F550, Cygnus Technologies), shown in Figure 9. HCD in the eluates were analysed using Quant-iT™ PicoGreen ™ dsDNA Assay Kit (#P7589, ThermoFisher), shown in Figure 10.

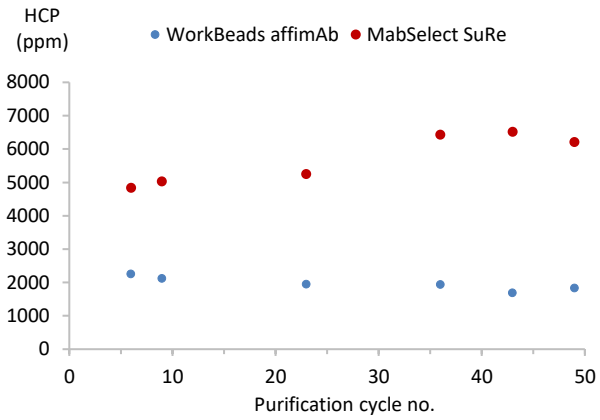

Figure 9. HCP levels in eluates analysed by ELISA for WorkBeads affimAb (blue) and MabSelect SuRe (red).

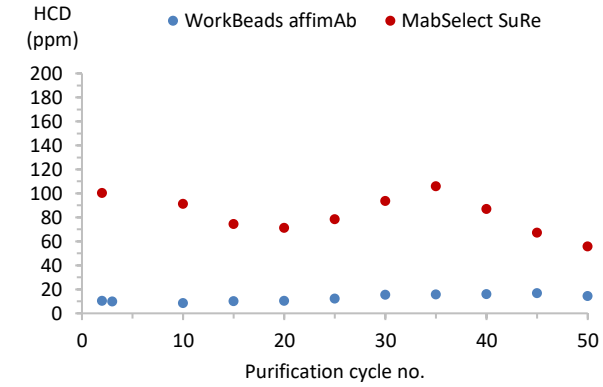

Figure 10. HCD levels in eluates analysed by Quant-iT PicoGreen dsDNA Assay Kit for WorkBeads affimAb (blue) and MabSelect SuRe (red).

Removing impurities from the host cell, such as HCP and HCD, is a key quality attribute during downstream process purification of monoclonal antibodies. WorkBeads affimAb shows low levels of both HCP (Fig. 9) and HCD (Fig. 10) compared with MabSelect SuRe. The low levels of impurities are also maintained over the 50 purification cycles.

Increased lifetime of protein A resin

Purification of antibodies or F_c-fusion proteins from mammalian host cells, such as CHO, results in extensive bioburden on the protein A resin. Chromatins, together with host cell proteins in general, cause damage to the protein A resin. Regular cleaning-in-place (CIP) is mandatory in the purification process, but accumulative fouling of the column will still occur. Maximized lifetime of the protein A resin is thus an important requirement during the purification process development.

Introducing WorkBeads 40 TREN upstream of the protein A resin, as a guard column, is a new important tool during process purification of monoclonal antibodies. Clarified cell extract is passed through the guard column to remove a majority of impurities such as host cell DNA, host cell proteins, and if bacterial host cells are used, endotoxins. Early removal of these impurities eliminates bioburden on the protein A resin and extends its lifetime. Reduction of impurities early in the purification process further enhances the final purity of the product.

The impurities in the sample feed applied onto the protein A resin can be reduced by using WorkBeads 40 TREN, as a guard column. Reduction of up to 99% of host cell DNA and 95% of host cell protein impurities from the sample feed has been shown. For more information about protection of protein A resin, see application note AN40 603 001.

Process optimization

The primary aim of process method optimization is to find the most suitable binding and elution conditions for best purity and yield, and to minimize denaturation or 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 differences between individual IgG species.

Typical binding conditions are low salt concentration buffers at neutral pH. For efficient capture of weakly bound antibodies, it is sometimes 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 this 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, are 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. If 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 extent 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) removes impurities and prolongs the life time of the column. CIP of WorkBeads affimAb can be done using NaOH of concentrations of up to 0.5 M during 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 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
Bulk resins		
WorkBeads 40S	25 ml	40 200 001
WorkBeads 40Q	25 ml	40 100 001
WorkBeads 40 TREN	25 ml	40 603 001
WorkBeads 40 TREN	150 ml	40 603 003

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 distributors and products please visit www.bio-works.com or contact us at info@bio-works.com

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