Bio-Works

DATA SHEET

WorkBeads affimAb Edge GoBio prepacked columns

WorkBeads™ affimAb Edge is a next-generation resin in the affimAb series, engineered for efficient purification of monoclonal antibodies and any Fc-containing fragment or fusion product from laboratory to process scale. Built on an improved macroporous agarose-based matrix and coupled with an alkali-stable protein A ligand, WB affimAb Edge combines high dynamic binding capacity (DBC) with superior pressure flow characteristics. The result is faster processing, reduced elution volumes, and robust performance across multiple purification cycles. With its cost-effective design and competitive performance, WB affimAb Edge enables greater process efficiency and improved overall economics for antibody purification workflows.

The resin is also available in several different ready-to use prepacked column sizes, such as GoBio™ Mini 1 mL, GoBio Mini 5 mL, GoBio Screen 7x100 (3.8 mL), GoBio Prep 16x100 (20 mL), GoBio Prep 26x100 (53 mL) and GoBio Prod columns starting from 1 L.

- High DBC, with > 60 mg/mL at 4 min and > 70 mg/mL at 6 min residence time, enabling efficient purification and lower process costs.
- Superior pressure flow properties supporting high flow velocities at low back pressure, enabling faster cycle times and robust scale-up.
- Strong alkali stability enables repeated cleaning and sanitization using 0.5 M NaOH for extended resin lifetime and improved process economy.
- Cost-effective performance, competitive with leading Protein A resins while delivering improved process economics.

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 Edge ideal for purification of monoclonal antibodies (mAb) and any Fc-containing fragment or fusion product.

The main characteristics of WorkBeads affimAb Edge resin are shown in Table 1. For the main characteristics of all different prepacked columns formats, see Tables 2 and 3.

Table 1. Main characteristics of WorkBeads affimAb resin.

	WorkBeads affimAb Edge
Target substance	Antibodies (IgG), bound via the F_c-region
Matrix	Rigid, highly cross-linked agarose
Average particle size (D_{V50})[1]	50 – 60 µm
Ligand	Recombinant protein A expressed in *E. coli* using animal-free medium
Dynamic binding capacity (DBC)[2]	> 70 mg human IgG/mL resin (RT: 6 min) > 60 mg human IgG/mL resin (RT: 4 min)
Sample load	> 50% of DBC3
Flow velocity[4,5]	300 cm/h at 0.1 MPa
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 – 12 (Operational range)
2 – 14 (CIP range)	
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 HIgG in PBS, pH 7.4 at 0.33 mL/min (100 cm/h, 6 minutes residence time) in a column packed with WorkBeads affimAb Edge, column bed 5 × 100 mm.
3 It is recommended to load 50-80% of resin's DBC to achieve maximum yield.
4 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).
5 Recommended flow rate determined in a 10 × 200 mm column.

GoBio prepacked column family

GoBio prepacked column family is developed for convenient, reproducible and fast results and includes columns with different sizes and formats.

GoBio Mini 1 mL and GoBio Mini 5 mL for small scale purification and screening using a shorter packed bed.

GoBio Screen 7x100 (3.8 mL) for reproducible process development including fast and easy optimization of methods and parameters.

GoBio Prep 16x100 (20 mL) and GoBio Prep 26x100 (53 mL) for lab-scale purifications and scaling up.
GoBio Prep 16x600 (120 mL) and GoBio Prep 26x600 (320 mL) for preparative lab-scale size exclusion chromatography.

GoBio Prod 80x200 (1 L), GoBio Prod 130x200 (2.7 L), GoBio Prod 200x200 (6 L), GoBio Prod 240x200 (9 L) and GoBio Prod 330x250 (21.4 L) for production-scale purifications.

Table 2. Main characteristics of GoBio Mini, GoBio Screen and GoBio Prep columns.

	GoBio Mini 1 mL & 5 mL	GoBio Screen 7x100	GoBio Prep 16x100	GoBio Prep 26x100
Column hardware	Polypropylene	Acrylic	Acrylic	Acrylic
Top and bottom filters	Polyethylene	Polyamide	Polyamide	Polyamide
Top and bottom plugs	Polypropylene	Polypropylene	Polypropylene	Polypropylene
Connections	1/16" female (top) 1/16" male (bottom)	1/16" female (both ends)	1/16" female (both ends)	1/16" female (both ends)
Column volumes	1 mL 5 mL	3.8 mL	20 mL	53 mL
Column dimensions	7 × 28 mm (1 mL) 13 x38 mm (5 mL)	7 × 100 mm	16 × 100 mm	26 × 100 mm
Max. column hardware pressure[1]	0.3 MPa, 3 bar, 43 psi	5 bar, 0.5 MPa, 70 psi	5 bar, 0.5 MPa, 70 psi	5 bar, 0.5 MPa, 70 psi
Chemical stability	1 M NaOH, 30% isopropanol, 70% ethanol	1 M NaOH, 20% isopropanol, 20% ethanol	1 M NaOH, 20% isopropanol, 20% ethanol	1 M NaOH, 20% isopropanol, 20% ethanol

[1] The maximum pressure the packed bed can withstand depends on the sample/liquid viscosity and chromatography resin characteristics. The pressure also depends on the tubing used to connect the column and the system restrictions after the column outlet.

Table 3. Main characteristics of and GoBio Prod columns.

	GoBio Prod 80x200, GoBio Prod 130x200, GoBio Prod 200x200, GoBio Prod 280x200, GoBio Prod 330x250
Column hardware	Acrylic
Top and bottom filters	Polyamide
Top and bottom plugs	Polypropylene
Connections	TC-connections
Column volumes	1 L, 2.7 L, 6 L, 9 L, 21.4 L
Column dimensions	80 × 200 mm (1 L), 130 × 200 mm (2.7 L), 200 × 200 mm (6 L), 280 × 200 mm (9 L), 330 × 250 mm (21.4 L)
Max. column hardware pressure[1]	5 bar, 0.5 MPa, 70 psi
Chemical stability	1 M NaOH, 20% isopropanol, 20 % ethanol

[1] The maximum pressure the packed bed can withstand depends on the sample/liquid viscosity and chromatography resin characteristics. The pressure also depends on the tubing used to connect the column and the system restrictions after the column outlet.

Applications

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. Feeds with low target expression can be purified using WorkBeads affimAb Edge, provided that the loading exceeds 50% of the resin's dynamic binding capacity (DBC) to ensure optimal recovery.

Elution is performed at a lower pH, yielding a purified and concentrated antibody. The pH should then be neutralized to avoid affecting the antibody's bioactivity.

High alkali stability

The alkali stability of WorkBeads affimAb Edge has been tested by dynamic binding capacity after multiple cleaning-inplace (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 4 minutes residence time in a 5 × 100 mm glass column using a solution of 10 mg/mL HIgG in the presence of PBS, pH 7.4.

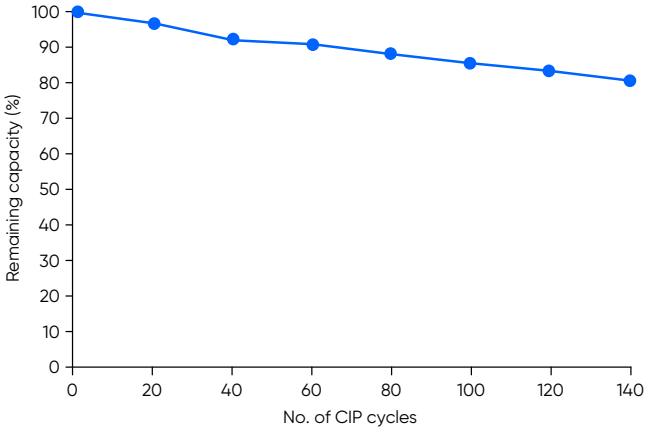

Figure 1. DBC for HIgG on WorkBeads affimAb Edge determined by frontal analysis at 4 minutes residence time after 140 CIP cycles with 0.5 M NaOH at 15 minutes contact time.

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 Edge has a dynamic binding capacity of typically more than 60 mg IgG/mL resin under standard binding conditions (PBS, pH 7.4 and 4-minutes residence time), see Figure 2. The most binding capacity of > 70 mg IgG/mL resin is utilized at 6 minutes residence time.

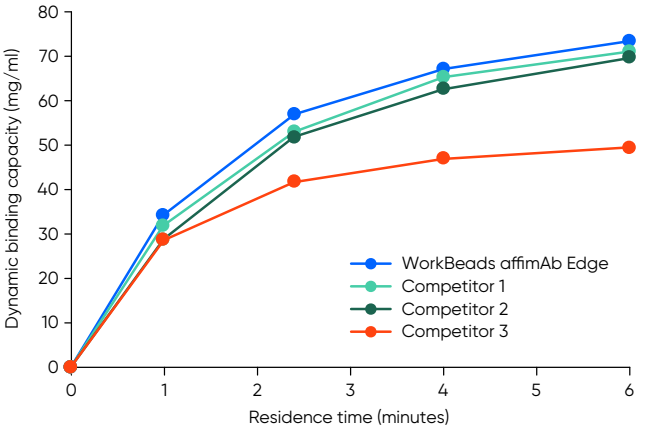

Figure 2. Dependency of dynamic binding capacity on residence time. Frontal analysis using 1 mg/mL HIgG in PBS, pH 7.4 was performed in 5 × 100 mm packed bed.

Particle size distribution

WorkBeads resin is manufactured with a narrow particle size distribution, exemplified in Figure 3. The optimized rigidity of the base matrix results in low backpressure even at higher flow rates, while the narrow particle size distribution of the resin allows for packed columns with higher efficiency.

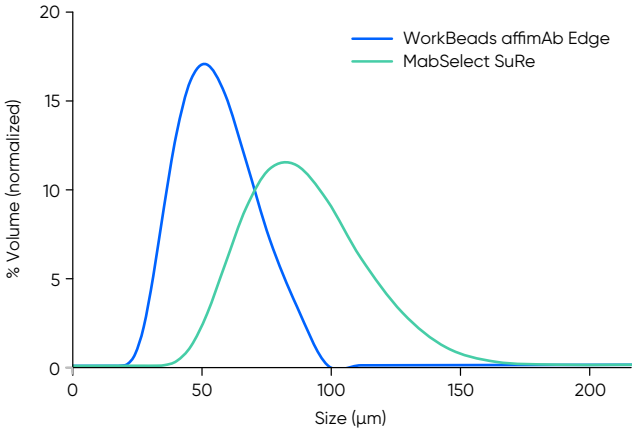

Figure 3. Particle size distribution comparison pf WorkBeads affimAb Edge (blue) and MabSelect SuRe (green).

Resin rigidity

WorkBeads affimAb Edge is designed for process-scale purification of monoclonal antibodies. Pressure-flow properties for the base matrix 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.

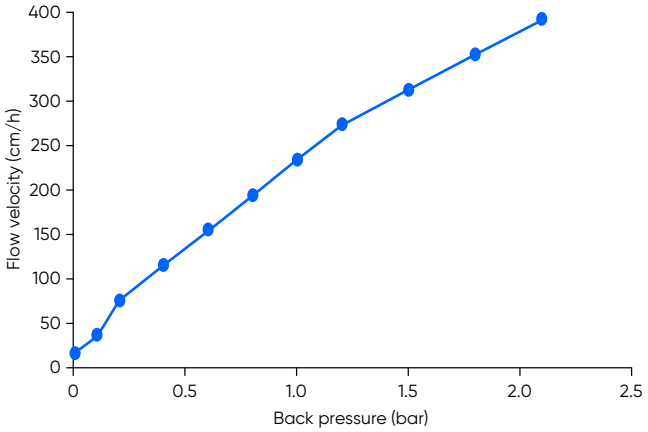

Figure 4. Pressure-flow data on WorkBeads affimAb Edge base matrix in water obtained in a 140 × 200 mm open bed glass column. The pressure over the bed at low flow rates is often too low to detect.

Purification of monoclonal antibodies

Figure 5 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 5B, 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 × 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)

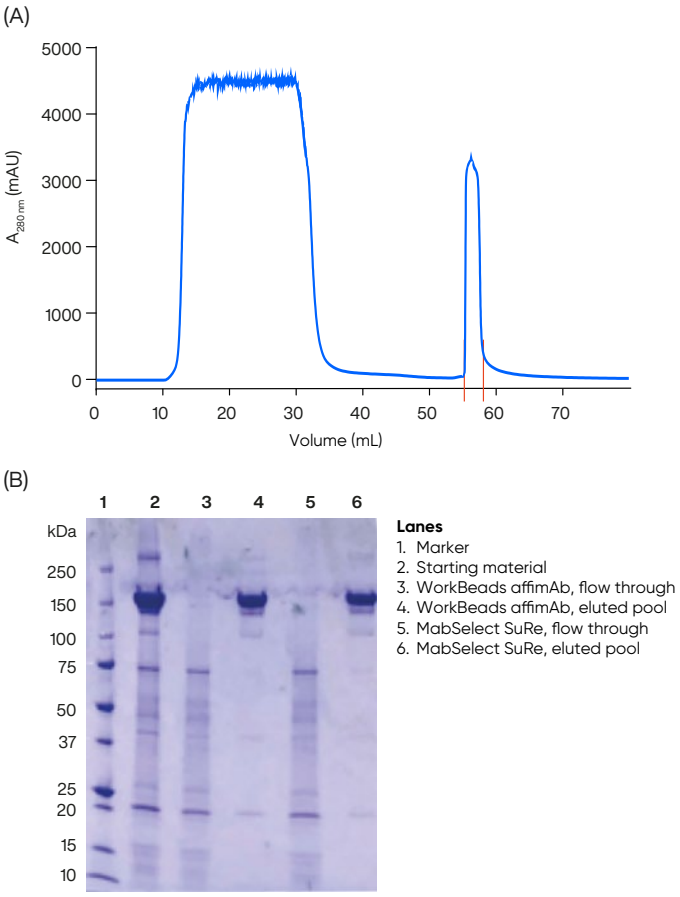

Figure 5. (A) Purification of a monoclonal IgG from CHO cells using WorkBeads affimAb. The blue line corresponds to the absorbance at 280 nm. (B) Analysis of the purified mAb by SDS-PAGE, non-reduced conditions. Comparison of mAb purified by identical method on WorkBeads affimAb and MabSelect SuRe resins.

Low protein A leakage

WorkBeads affimAb Edge 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 150 purification runs at laboratory scale applying 52.8 g mAb/L sample on WorkBeads affimAb Edge was performed. Following each run, the column was treated with 0.15 M phosphoric acid (Strip1), 0.5 M citric acid (Strip 2), and finally 0.5 M sodium hydroxide (CIP), each applied for a contact time of 18 minutes. The elution profile from the first and the last cycle is shown in Figure 6.

Fractions from the eluted sample were analysed by enzyme-linked immunosorbent assay (ELISA) using Protein A ELISA kit (Cygnus Technologies). Levels of ligand leakage were determined using ligand-specific derived standard curves. The ligand leakage is shown in Fig 7.

Resin:	WorkBeads affimAb Edge
Column:	6.8 mL (6.6 × 250 mm)
Sample load:	52.8 g monoclonal antibody per liter of resin (200 cm/h)
Binding/wash buffer:	PBS and NaCl, pH 7.4 (200 cm/h)
Elution buffer:	0.2 M glycine-HCl, pH 3.2 (200 cm/h)
Strip 1:	0.15 M Phosphoric acid (200 cm/h) (18 min contact time in each cycle)
Strip 2:	0.5 M Citric acid (200 cm/h) (18 min contact time in each cycle)
CIP:	0.5 M NaOH (200 cm/h) (18 min contact time in each cycle)

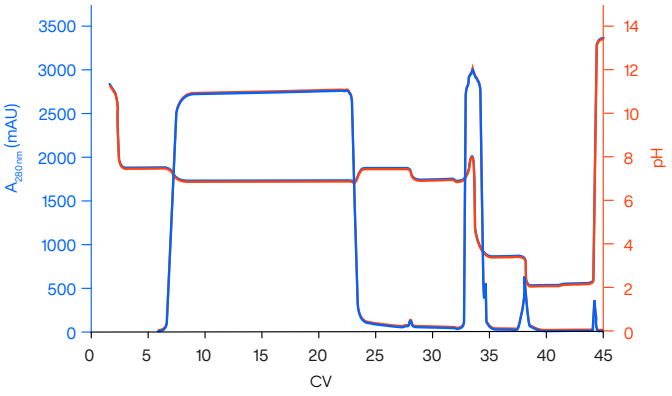

Figure 6. Protein A leakage study for WorkBeads affimAb Edge showing overlap between cycle 1 and cycle 150.

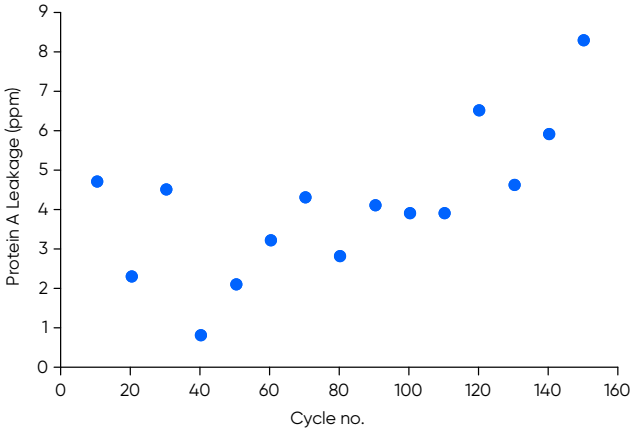

Figure 7. Protein A ligand leakage levels in eluates analysed by ELISA for WorkBeads affimAb Edge.

Effective reduction of HCP

The design of WorkBeads affimAb Edge gives higher purity of the eluted mAb, with reduced amounts of host cell proteins (HCP) in the eluate. HCP in eluates from the series of laboratory scale purifications on WorkBeads affimAb Edge were analysed using a HCP ELISA kit (Cygnus Technologies), shown in Figure 8. Removing impurities from the host cell, such as HCP, is a key quality attribute during downstream process purification of monoclonal antibodies. WorkBeads affimAb Edge shows low levels of HCP (Fig. 8), and these low impurity levels are maintained over 150 purification cycles.

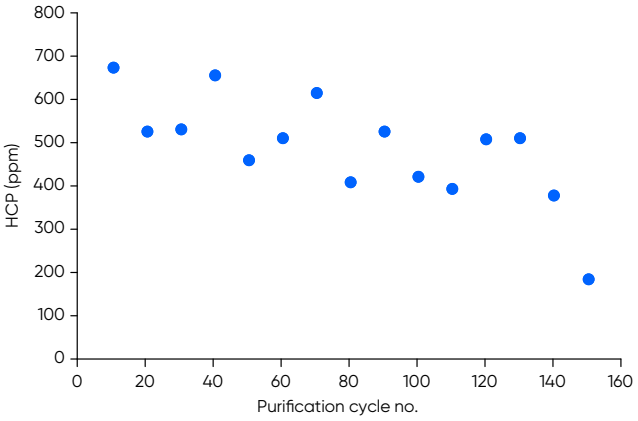

Figure 8. HCP levels in eluates anaylsed by ELISA for WorkBeads affimAb Edge.

Increased lifetime of protein A resin

Purification of antibodies or Fc-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 AN 40 603 001.

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 are also differences between individual IgG species. For the best results, loading 50-80% of the resin's dynamic binding capacity (DBC) generally maximizes yield.

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 IgG1. 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 lifetime of the column. CIP of WorkBeads affimAb Edge can be done using NaOH of concentrations of up to 0.5 M during 15 minutes or more.

Storage

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

For prolonged storage of the prepacked GoBio Screen and GoBio Prep columns connect the included transport syringe filled with storage solution to the bottom end of the column. Check stored columns every 6 months and replenish the syringe buffer (20% Ethanol) with up to 5 mL if needed.

Related products

Product name	Pack size[1]	Article number
Prepacked columns		
GoBio Mini S 1 mL	1 mL × 5	45 2001 001
GoBio Mini S 5 mL	5 mL × 5	45 200 107
GoBio Mini Q 1 mL	1 mL × 5	45 100 101
GoBio Mini Q 5 mL	5 mL × 5	45 100 107
GoBio Mini TREN 1 mL	1 mL × 5	45 655 213
GoBio Mini TREN 5 mL	5 mL × 5	45 655 217
GoBio Mini Dsalt 5 mL	5 mL × 5	45 360 107
GoBio Screen 7x100 40S	3.8 mL × 1	55 420 001
GoBio Screen 7x100 40Q	3.8 mL × 1	55 410 001
GoBio Screen 7x100 40 TREN	3.8 mL × 1	55 463 001
GoBio Prep 16x100 40S	20 mL × 1	55 420 021
GoBio Prep 16x100 40Q	20 mL × 1	55 410 021
GoBio Prep 16x100 40 TREN	20 mL × 1	55 463 021
GoBio Prep 16x100 Dsalt[2]	20 mL × 1	55 700 021
GoBio Prep 26x100 40S	53 mL × 1	55 420 031
GoBio Prep 26x100 40Q	53 mL × 1	55 410 031
GoBio Prep 26x100 40 TREN[2]	53 mL × 1	55 463 031
GoBio Prep 26x100 Dsalt	53 mL × 1	55 700 031
Bulk resins		
WorkBeads 40S	25 mL	40 200 001
WorkBeads 40Q	25 mL	40 100 001
WorkBeads 40 TREN	25 mL 150 mL	40 603 001 40 603 003

[1] Other pack sizes can be found in the complete product list on www.bio- works.com
[2] Packed on request.

Ordering information

Product name	Pack size	Article number
Prepacked column		
GoBio Mini affimAb 1 mL	1 mL × 1 1 mL × 5 1 mL × 10	45 800 101 45 800 103 45 800 104
GoBio Mini affimAb edge 1 mL	1 mL × 1 1 mL × 5 1 mL × 10	45 810 101 45 810 103 45 810 104
GoBio Mini affimAb 5 mL	5 mL × 1 5 mL × 5 5 mL × 10	45 800 105 45 800 107 45 800 108
GoBio Mini affimAb Edge 5 mL	5 mL × 1 5 mL × 5 5 mL × 10	45 810 105 45 810 107 45 810 108
GoBio Screen 7x100 affimAb[1]	3.8 mL × 1	55 800 001
GoBio Screen 7x100 affimAb Edge[1]	3.8 mL × 1	55 810 001
GoBio Prep 16x100 affimAb[1]	20 mL × 1	55 800 021
GoBio Prep 16x100 affimAb Edge[1]	20 mL × 1	55 810 021
GoBio Prep 26x100 affimAb[1]	53 mL × 1	55 800 031
GoBio Prep 26x100 affimAb Edge[1]	53 mL × 1	55 810 031
GoBio Prod 80x200 affimAb[1]	1 L × 1	55 800 042
GoBio Prod 80x200 affimAb Edge[1]	1 L × 1	55 810 042
GoBio Prod 130x200 affimAb[1]	2.7 L × 1	55 800 062
GoBio Prod 130x200 affimAb[1]	2.7 L × 1	55 810 062
GoBio Prod 200x200 affimAb[1]	6 L × 1	55 800 072
GoBio Prod 200x200 affimAb[1]	6 L × 1	55 810 072
GoBio Prod 280x200 affimAb[1]	9 L × 1	55 800 082
GoBio Prod 280x200 affimAb[1]	9 L × 1	55 810 082
GoBio Prod 330x250 affimAb[1]	21.4 L × 1	55 800 093
GoBio Prod 330x250 affimAb[1]	21.4 L × 1	55 810 093
Bulk resin		
WorkBeads affimAb	25 mL 200 mL 1 L 5 L 10 L	40 800 001 40 800 002 40 800 010 40 800 050 40 800 060
WorkBeads affimAb Edge	25 mL 200 mL 1 L 5 L 10 L	40 810 001 40 810 002 40 810 010 40 810 050 40 810 060

[1] Packed on request.

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