WorkBeads 40S
WorkBeads 40Q

The WorkBeads™ 40S and WorkBeads 40Q resins for ion exchange chromatography are designed for research and industrial scale purification of proteins, peptides and oligonucleotides by utilizing the difference in their surface charge. WorkBeads 40S resin is a strong cation exchanger derivatized with sulfonate ligands, and WorkBeads 40Q resin is a strong anion exchanger derivatized with quaternary amine ligands. These resins demonstrate the property of high-resolution separation while giving low backpressure facilitate both capture and polishing purification applications in standard bioprocess columns.

- High throughput and purity
- Reliable and reproducible results
- High chemical stability for easy cleaning-in-place

Resin description

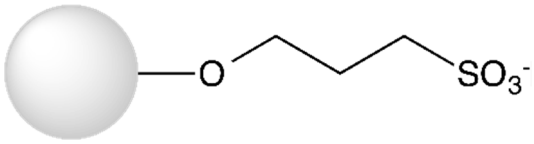
WorkBeads are agarose-based chromatographic resins manufactured using a proprietary method that results in porous beads with a tight size distribution and exceptional mechanical stability. Agarose based matrices have been successfully used for decades in biotechnology purification, from research to production 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.

WorkBeads 40S is a strong cation exchange resin derivatized with sulfonates as functional groups. WorkBeads 40Q is a strong anion exchanger derivatized with quarternary amines as functional groups.

The functional groups are coupled to the resin via chemically stable linkages. The structures of the ligands used in WorkBeads 40S and WorkBeads 40Q are shown in Figure 1.

The main characteristics of WorkBeads 40S and WorkBeads 40Q resins are shown in Table 1. For more details, please see IN 40 100 010.

(A)



(B)

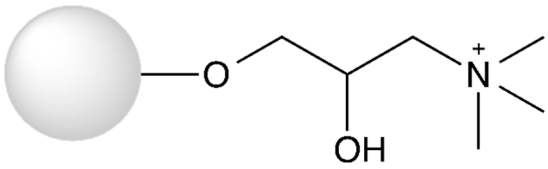


Figure 1. Structure of the ligand used in (A) WorkBeads 40S and (B) WorkBeads 40Q.

Table 1. Main characteristics of the WorkBeads 40S and WorkBeads 40Q resins.

	WorkBeads 40S	WorkBeads 40Q
Target substances	Proteins and peptides	Proteins, peptides and oligonucleotides
Matrix	Rigid, highly cross-linked agarose	Rigid, highly cross-linked agarose
Average particle size[1] (D_{v50})	45 µm	45 µm
Ionic group (ligand)	Sulfonate ($-SO_3^-$)	Quarternary amine ($-N^+(CH_3)_3$)
Ionic capacity	180 - 250 µmol H^+/ml resin	180 - 250 µmol Cl^-/ml resin
Dynamic binding capacity (DBC)	130 mg BSA/ ml resin[2]	47 mg BSA/ml resin[3]
Max flow rate (20 cm bed height and 5 bar)	600 cm/h	600 cm/h
Chemical stability	Compatible with all standard aqueous buffers used for protein purification, 1 M NaOH, 30% isopropanol or 70% ethanol. Should not be stored at < pH 3 for prolonged time	
pH stability	2 - 13	2 - 13
Storage	2 to 25°C in 20% ethanol with 0.2 M sodium acetate	2 to 25°C in 20% ethanol

1. The median particle size of the cumulative volume distribution.
2. Dynamic binding capacity determined at 4-minutes residence time in 20 mM Na-citrate, pH 4.0.
3. Dynamic binding capacity determined at 2.5-minutes residence time in 50 mM Tris-HCl, 50 mM NaCl, pH 8.0.

Applications

WorkBeads 40S and WorkBeads 40Q resins are designed for ion exchange chromatography (IEX). The resins can be used for research- and industrial-scale purification of proteins, peptides and oligonucleotides. The particle size has been selected to enable high-resolution separations at moderate backpressure. The resins can therefore be used in capture step purification as well as for polishing. For purification requiring exceptionally high flow rates in order to handle large sample volumes WorkBeads 100S and WorkBeads 100Q should be considered for the capture step.

Principle

Ion exchange chromatography separates biomolecules according to surface charge. For example, protein interact with different affinities with opposite charged groups on the resin that depend both on the number of charges involved in the interaction and on the distribution of the charges on the protein. The surface charge of proteins depends on the pH of their environment. When the pH is equal to the isoelectric point (pI) of the protein the net charge is zero. At pH values below the pI the net charge will be positive, and at a pH above the pI the net charge will be negative. It should be noted that the interaction of the protein depends on the presence and distribution of both positive and negative charged groups on the surface. A protein may therefore also interact with an ion exchange resin at the isoelectric point.

The likelihood of binding to either the cation or the anion exchange resin will increase when the pH moves away from the protein pI.

IEX is one of the most frequently used chromatography techniques because of its versatility and ability to separate proteins even with small differences in charge and is a concentration step. It is one of the more cost-effective chromatography techniques and is excellent for scale-up.

For additional information about the ion exchange chromatography principle, see instruction IN 40 100 010.

Protein selectivity

Below, two examples are presented. In Figure 2, the basic proteins, concanavalin A, ribonuclease A, α-chymotrypsinogen A and lysozyme, are separated using WorkBeads 40S, packed in a glass column of 10 cm bed height. In Figure 3, the acidic proteins apo-transferrin, α-lactalbumin and soybean trypsin inhibitor are separated using WorkBeads 40Q, packed in a glass column with 100 mm bed height.

Column:	WorkBeads 40S in 6.6 × 100 mm, 3.42 ml
Binding buffer:	50 mM MES, pH 6.0
Elution buffer:	50 mM MES, 1 M NaCl, pH 6.0
Sample:	1 ml of 1.5 mg/ml of concanavalin A, 1.5 mg/ml ribonuclease A, 0.5 mg/ml of α-chymotrypsinogen A and 0.5 mg/ml lysozyme in binding buffer
Flow rate:	150 cm/h, 4-minute residence time
Gradient:	0 - 50% elution buffer in 20 CV

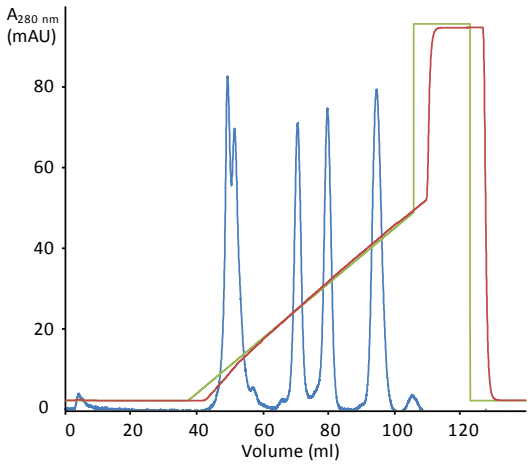


Figure 2. Separation on a cation exchange chromatography column of, peaks from left to right, concanavalin A, α-chymotrypsinogen A, ribonuclease A and lysozyme. A 1-ml sample was applied onto WorkBeads 40S column, 100 mm bed height. The blue line corresponds to the absorbance at 280 nm, the red line to the conductivity and the green line to the percentage of the elution buffer.

Column:	WorkBeads 40Q in 6.6 × 100 mm, 3.42 ml
Binding buffer:	50 mM Tris-HCl, pH 7.4
Elution buffer:	50 mM Tris-HCl, 1 M NaCl, pH 7.4
Sample:	10 ml of 0.3 mg/ml apo-transferrin, 0.2 mg/ml α-lactalbumin and 0.6 mg/ml soybean trypsin inhibitor in binding buffer
Flow rate:	150 cm/h, 4-minutes residence time
Gradient:	0 - 40% elution buffer in 20 CV

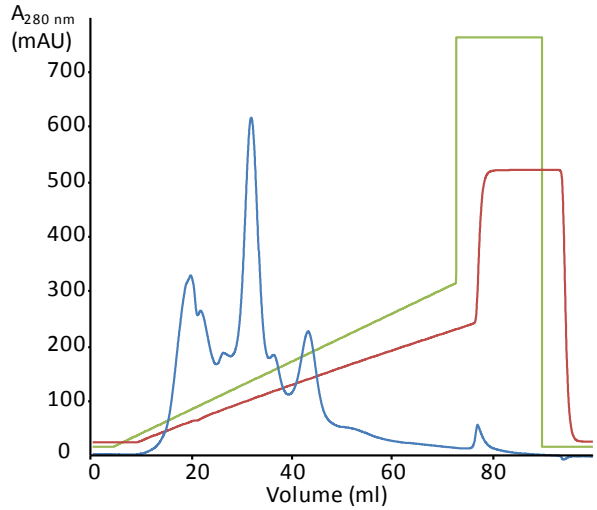


Figure 3. Separation on anion exchange chromatography column of, peaks from left to right, apo-transferrin, α-lactalbumin and soybean trypsin inhibitor. 10 ml sample applied onto a column packed with WorkBeads 40Q resin. 100 mm bed height. The blue line corresponds to the absorbance at 280 nm, the red line to the conductivity and the green line to the percentage of the elution buffer.

Flow properties

WorkBeads 40S and WorkBeads 40Q ion exchange chromatography resins are designed for high throughput protein separations under a variety of conditions. The high resolution that can be obtained at high protein loadings and high flow rates makes it ideal for process applications. Pressure/flow properties for WorkBeads 40S is shown in Figure 4. The measurements were carried out with an open bed (adaptor not pushed against the bed).

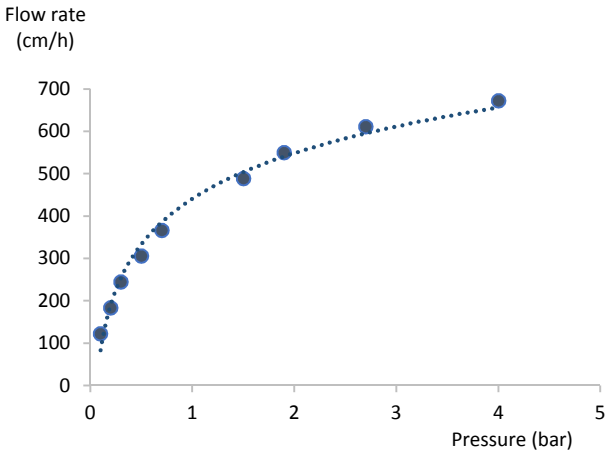


Figure 4. Pressure to flow rate properties of WorkBeads 40S determined with deionized water, 25 x 200-mm glass column.

Cleaning-in-place

During purification impurities such as cell debris, lipids, nucleic acids and protein precipitates from the samples may gradually build up in the resin bed. Fouling is typical even for well-clarified samples. The severity of this process depends on the composition of sample applied to the column. These adsorbed impurities will reduce the performance of the column over time. Regular cleaning (Cleaning-in-place, CIP) keeps the resin clean, reduces the rate of further fouling, and maintains the capacity, resolution and flow properties of the column. Cleaning of a column using 1 M NaOH using a low reversed flow for 2 hours or overnight is often sufficient.

Sanitization (reduction of microorganisms) can be carried out using combinations of NaOH and ethanol (e.g., incubation with a mixture of 0.5 M NaOH and 40% ethanol for 3 hours). The sanitization procedure and its effectiveness will depend on the microorganisms to be sanitized and needs to be evaluated for each case.

Storage

Store at 2 to 25°C in 20% ethanol. For WorkBeads 40S it is recommended to include 0.2 M sodium acetate in the storage solution.

Related products

Product name	Pack size[1]	Article number
Prepacked columns		
BabyBio S 1 ml	1 ml x 1	45 200 101
BabyBio Q 1 ml	1 ml x 1	45 100 101
BabyBio DEAE 1 ml	1 ml x 1	45 150 101
BabyBio Dsalt 5 ml	5 ml x 1	45 360 105
OptioBio 40S 10x100	7.9 ml x 1	55 420 011
OptioBio 40Q 10x100	7.9 ml x 1	55 410 011
Bulk resins		
WorkBeads 40 DEAE	25 ml	40 150 001
WorkBeads 100S	25 ml	10 200 001
	200 ml	10 200 002
	500 ml	10 200 005
	1 L	10 200 010
	5 L	10 200 050
	10 L	10 200 060
WorkBeads 100Q	25 ml	10 210 001
	200 ml	10 210 002
	500 ml	10 210 005
	1 L	10 210 010
	5 L	10 210 050
	10 L	10 210 060

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 40S	25 ml	40 200 001
	200 ml	40 200 002
	1 L	40 200 010
	5 L	40 200 050
	10 L	40 200 060
WorkBeads 40Q	25 ml	40 100 001
	200 ml	40 100 002
	1 L	40 100 010
	5 L	40 100 050
	10 L	40 100 060

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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Bio-Works

WorkBeads 100S
WorkBeads 100Q

The WorkBeads™ 100S and WorkBeads 100Q resins for ion exchange chromatography are designed for industrial purification applications, which have high flow rate and low backpressure requirements. The products are intended for the purification of proteins, peptides and oligonucleotides by utilizing the difference in surface charge. WorkBeads 100S is a strong cation exchanger with sulfonate ligands and WorkBeads 100Q is a strong anion exchanger with quaternary amine ligands.

- High throughput and scalability
- Reliable and reproducible results
- High chemical stability for easy cleaning-in-place

Resin description

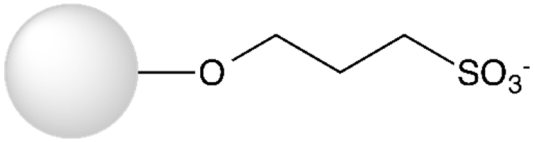
WorkBeads are agarose-based chromatographic resins manufactured using a proprietary method that results in porous beads with a tight size distribution and exceptional mechanical stability. Agarose based matrices have been successfully used for decades in biotechnology purification, from research to production 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.

WorkBeads 100S is a strong cation exchange resin derivatized with sulfonates as functional groups. WorkBeads 100Q is a strong anion exchanger derivatized with quaternary amines as functional groups.

The functional groups are coupled to the resin via chemically stable linkages. The structures of the ligands used in WorkBeads 100S and WorkBeads 100Q are shown in Figure 1.

The main characteristics of WorkBeads 100S and WorkBeads 100Q resins are shown in Table 1. For more details, please see IN 10 200 010.

(A)



(B)

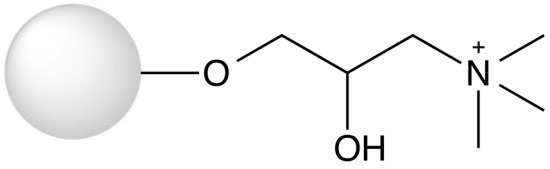


Figure 1. Structure of the ligand used in (A) WorkBeads 100S and (B) WorkBeads 100Q.

Table 1. Main characteristics of WorkBeads 100S and WorkBeads 100Q resins.

	WorkBeads 100S	WorkBeads 100Q
Target substances	Proteins and peptides	Proteins, peptides and oligonucleotides
Matrix	Rigid, highly cross-linked agarose	Rigid, highly cross-linked agarose
Average particle size[1] (D_{v50})	90 - 110 µm	90 - 110 µm
Ionic group (ligand)	Sulfonate ($-SO_3^-$)	Quarternary amine ($-N^+(CH_3)_3$)
Ionic capacity	180 - 250 µmol H^+/ml resin	140 - 200 µmol Cl^-/ml resin
Dynamic binding capacity (DBC)	>100 mg BSA/ml resin[2]	>40 mg BSA/ml resin[3]
Pressure flow characteristic	2 bar at 900 cm/h, 25 mm diameter column, 20 cm bed height	
Chemical stability	Compatible with all standard aqueous buffers used for protein purification, 1 M NaOH, 30% isopropanol or 70% ethanol. Should not be stored at < pH 3 for prolonged time	
pH stability	2 - 13	2 - 13
Storage	2 to 25°C in 20% ethanol with 0.2 M sodium acetate	2 to 25°C in 20% ethanol

1. The median particle size of the cumulative volume distribution.
2. Dynamic binding capacity determined at 4-minutes residence time in the presence of 20 mM Na-citrate, pH 4.0.
3. Dynamic binding capacity determined at 4-minutes residence time in the presence of 50 mM Tris-HCl, 50 mM NaCl, pH 8.0.

Applications

WorkBeads 100S and WorkBeads 100Q are designed for ion exchange chromatography (IEX). The resins can be used for industrial purification of proteins, peptides and oligonucleotides when high flow rate and low back-pressure is required. The flow properties of these resins make them suitable for capture step purification where large volumes need to be processed. Following the capture step, during the enhance and polishing purification steps, there is less need for high flow rates, as the important requirement is high-resolution separation. Accordingly, it is recommended to select WorkBeads 40S and WorkBeads 40Q for these purification steps.

Principle

Ion exchange chromatography separates biomolecules according to surface charge. For example, protein interact with different affinities with opposite charged groups on the resin that depend both on the number of charges involved in the interaction and on the distribution of the charges on the protein. The surface charge of proteins depends on the pH of their environment. When the pH is equal to the isoelectric point (pI) of the protein the net charge is zero. At pH values below the pI the net charge will be positive, and at a pH above the pI the net charge will be negative. It should be noted that the interaction of the protein depends on the presence and distribution of both positive and negative charged groups on the surface. A protein may therefore also interact with an ion exchange resin at the isoelectric point.

The likelihood of binding to either the cation or the anion exchange resin will increase when the pH moves away from the protein pI.

IEX is one of the most frequently used chromatography techniques because of its versatility and ability to separate proteins even with small differences in charge, and is a concentration step. It is one of the more cost effective chromatography techniques and is excellent for scale-up.

Protein resolution

A comparison between WorkBeads 40Q and WorkBeads 100Q resolution shows a similar selectivity pattern with an improved resolution for WorkBeads 40Q (see Figure 2). Corresponding chromatograms for WorkBeads 100S compared to WorkBeads 40S is shown in Figure 3. This is due to the different size of the beads.

The larger WorkBeads 100 resins are designed for capture steps where the focus is rapid enrichment of the target substance, rather than high resolution. Elution is routinely carried out using step gradients. Further enhancement and polishing of the pure target protein can be optimized as required using WorkBeads 40 resins designed for higher resolution.

The smaller bead size of WorkBeads 40 resins gives higher resolution and more narrow peaks, whereas WorkBeads 100 resins gives the same selectivity but broader peaks. The advantage of WorkBeads 100 resins are their low backpressure allowing higher flow rates and longer columns.

Column:	(A) WorkBeads 40Q, (B) WorkBeads 100Q, 7.9 ml
Binding buffer:	50 mM Tris-HCl, pH 7.4
Elution buffer:	50 mM Tris HCl, 1 M NaCl, pH 7.4
Sample:	10 ml 0.45 mg/ml apo-transferrin, 0.7 mg/ml α-lactalbumin, 1.4 mg/ml soybean trypsin inhibitor in binding buffer
Flow rate:	2.0 ml/min (150 cm/h), 4 minutes residence time
Gradient:	0 - 40% elution buffer, 20 column volume (CV)

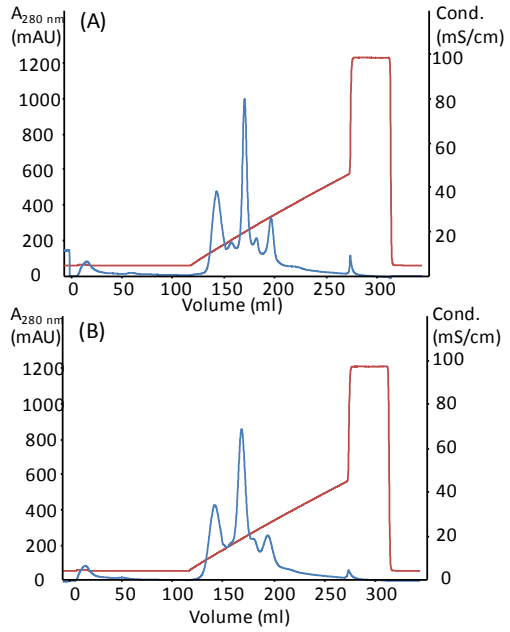


Figure 2. Chromatogram from resolution comparison between WorkBeads 40Q (A) and WorkBeads 100Q (B).

Column:	(A) WorkBeads 40S, (B) WorkBeads 100S, 7.9 ml
Binding buffer:	50 mM MES, pH 6.0
Elution buffer:	50 mM MES, 1 M NaCl, pH 6.0
Sample:	2.5 ml 1.5 mg/ml concanavalin A, 1.5 mg/ml α-chymotrypsinogen A, 1.5 mg/ml ribonuclease A, 0.5 mg/ml lysozyme in binding buffer
Flow rate:	2.0 ml/min (150 cm/h), 4 min residence time
Gradient:	0 - 50% elution buffer, 20 CV

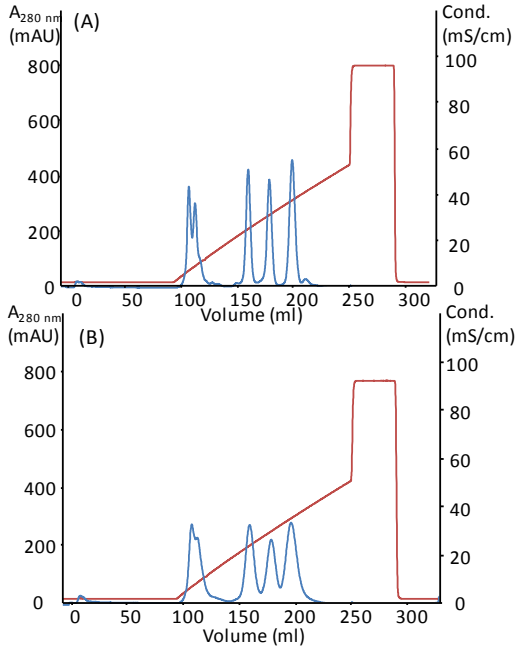


Figure 3. Chromatogram of selectivity on two different cation exchange resins, WorkBeads 40S (A) and WorkBeads 100S (B).

Large sample loading and volume

The WorkBeads 100 resins have very strong mechanical resistance and generate low backpressure due to the relatively large particle size (see Figure 4 and Figure 5). The resins therefore represent an excellent choice for purification when high flow rate is required to handle large sample volumes and to minimise processing time. Increasing the flow rate means that the contact time is reduced. To adjust for this a longer column can be used while keeping the backpressure acceptably low. This is a critical feature for large processes where short cycle times are important.

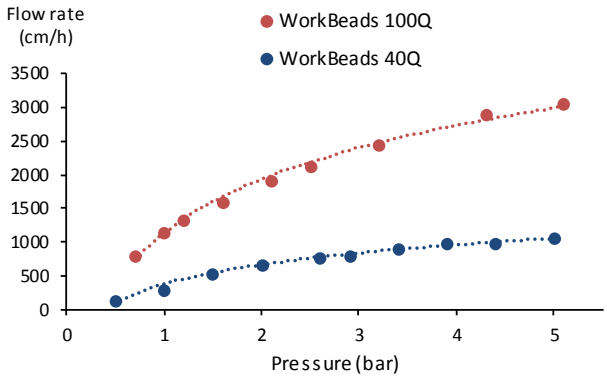


Figure 4. Pressure-flow properties of WorkBeads 100Q and WorkBeads 40Q. The data was obtained using distilled water passed through a 10 mm (i.d.) × 200 mm bed of resin in a glass column. The bed was open, i.e., the top adaptor was not pushed towards the chromatography bed.

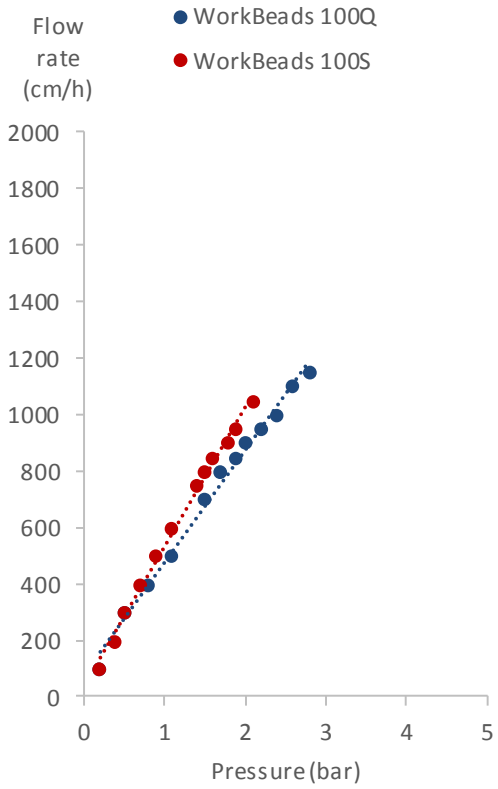


Figure 5. Pressure to flow rate properties of WorkBeads 100Q and WorkBeads 100S. Pressure-flow properties obtained in distilled water, glass column dimension of 20 cm bed height, diameter 25 mm.

Cleaning-in-place

During purification impurities such as cell debris, lipids, nucleic acids and protein precipitates from the samples may gradually build up in the resin bed. Fouling is typical even for well-clarified samples. The severity of this process depends on the composition of sample applied to the column. These adsorbed impurities will reduce the performance of the column over time. Regular cleaning (Cleaning-in-place, CIP) keeps the resin clean, reduces the rate of further fouling, and maintains the capacity, resolution and flow properties of the column. Cleaning of a column using 1 M NaOH using a low reversed flow for 2 hours or overnight is often sufficient.

Sanitization (reduction of microorganisms) can be carried out using combinations of NaOH and ethanol (e.g., incubation with a mixture of 0.5 M NaOH and 40% ethanol for 3 hours). The sanitization procedure and its effectiveness will depend on the microorganisms to be sanitized, and needs to be evaluated for each case.

Storage

Store at 2 to 25°C in 20% ethanol. For WorkBeads 100S it is recommended to include 0.2 M sodium acetate in the storage solution.

Related product

Product name	Pack size[1]	Article number
Prepacked columns		
BabyBio S 1 ml	1 ml x 5	45 200 103
BabyBio Q 1 ml	1 ml x 5	45 100 103
BabyBio Dsalt 5 ml	5 ml x 5	45 360 107
OptioBio 40S 10x100	7.9 ml x 1	55 420 011
OptioBio 40Q 10x100	7.9 ml x 1	55 410 011
Bulk resins		
WorkBeads 40S	25 ml	40 200 001
WorkBeads 40Q	25 ml	40 100 001

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 100S	25 ml	10 200 001
	200 ml	10 200 002
	500 ml	10 200 005
	1 L	10 200 010
	5 L	10 200 050
	10 L	10 200 060
WorkBeads 100Q	25 ml	10 210 001
	200 ml	10 210 002
	500 ml	10 210 005
	1 L	10 210 010
	5 L	10 210 050
	10 L	10 210 060

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

Bio-Works
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WorkBeads 40/100 SEC
WorkBeads 40/1000 SEC
WorkBeads 40/10 000 SEC

The WorkBeads™ 40/100 SEC, WorkBeads 40/1000 SEC and WorkBeads 40/10 000 SEC resins are size exclusion chromatography (SEC) resins for laboratory and process-scale separations of proteins, peptides, nucleic acids, viruses and other biomolecules by utilizing the differences in their size. The resins are based on agarose, which is well established and well known in the biotech industry.

- Excellent resolution and high-flow compatibility
- Robust separation across a wide range of molecular weights
- Chemical stable resins

The WorkBeads SEC resins allows purifications over a large range of molecular weights. The combination of excellent resolution and flow-pressure properties make these resins suitable for both lab-scale and process-scale separations in standard columns from low to high flow rates. The chemical resistance of the resins allow purification over a broad range of conditions.

The main characteristics of WorkBeads SEC resins are shown in Table 1. For more details, please see instruction, IN 40 300 010.

Resin Description

WorkBeads are agarose-based chromatographic resins manufactured by a proprietary method that results in porous beads with a tight size distribution and exceptional 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 that require optimal purity and flow properties.

Table 1. Main characteristics of WorkBeads SEC resins.

	WorkBeads 40/100 SEC	WorkBeads 40/1000 SEC	WorkBeads 40/10 000 SEC
Matrix	Rigid, highly cross-linked agarose	Rigid, highly cross-linked agarose	Rigid, highly cross-linked agarose
Separation range[1]	10 - 150 kD	10 - 1200 kD	10 - 10 000 kD
Exclusion limit	150 kD	1200 kD	10 000 kD
Average particle size[2] (D_{V50})	45 µm	45 µm	45 µm
Recommended flow rate	20 - 100 cm/h	20 - 100 cm/h	20 - 50 cm/h
Max flow rate[3, 4]	300 cm/h (600 cm/h)	300 cm/h (600 cm/h)	300 cm/h (600 cm/h)
Chemical stability	Compatible with all standard aqueous buffers used for protein purification. Should not be stored at low pH for prolonged time.		
pH stability	2 - 13	2 - 13	2 - 13
Storage in 20% ethanol	2 to 25 °C	2 to 25 °C	2 to 25 °C

1. Globular proteins.
2. The median particle size of the cumulative volume distribution.
3. 15 mm (i.d.) x 900 mm column, or 25 x 200 mm (values within brackets)
4. **Note**: Make sure that the column hardware max pressure is not exceeded.

Principle

Size Exclusion Chromatography, also called gel filtration (GF), is a simple and reliable technique for separation of molecular components according to their size. The technique is based on the relative retardation of substances of different sizes when passed through a packed bed of porous beads. Very large substances in the applied sample will be eluted first since they will not enter the pores of the beads (larger than the size cut-off of that resin). These substance will only access the volume outside the beads, the void volume, V_0. Very small substance such as salt and buffer components will elute close to the geometrical volume of the packed bed, since they can enter essentially all pores of the beads, the total volume, V_t. Substances of intermediate sizes will elute at different volumes depending on their size relative to the pore sizes of the resin. The three available resins have different porosities giving them different separation ranges.

The packed column is prepared by equilibration with a suitable buffer, usually an aqueous buffer, before loading the sample. The composition of the buffer should be selected for the best stability of the target substance. It is in general recommended to include 150 mM NaCl in the buffer to eliminate electrostatic interactions between substance to be separated, and between the substances and the resin. Elution should be done with approx. 1.3 column volumes (CV) to allow all applied material to pass through the column, and to make sure that salt and low-molecular weight substances from the sample have been eluted from the column. A new sample can be applied directly.

An advantage with the SEC technique is the combined purification and buffer exchange or salt removal of the target substance material. This is one of the reasons for that SEC is a frequently used final step (the polishing step) in protein purification. A drawback with SEC is the low flow rate required, and this is one of the reasons to use SEC in the final step when the target substance has been concentrated during the previous step. An important benefit of SEC is that it can remove aggregates of the target substance that is co-purified in earlier purification steps.

The WorkBeads 40/100 SEC, WorkBeads 40/1000 SEC and WorkBeads 40/10 000 SEC resins are suitable for preparative purifications owing to the 45-µm particle size. For standard purifications of proteins, peptides and nucleic acids the flow rate should be low, 20 - 50 cm/h. Higher flow rates are possible (up to 300 cm/h), but will reduce the resolution between peaks. The recommended sample volume for a preparative SEC column for receiving the highest resolution is 1% - 4% of the column volume. The high rigidity of the resins allows the use of high flow rates in applications were the target substance is eluted in the void fraction, (e.g., virus purifications).

Separation ranges

Figure 1 shows the K_D-curve determination for WorkBeads 40/100 SEC, WorkBeads 40/1000 SEC and WorkBeads 40/10 000 SEC. The K_D-curves are determined using standard proteins applied onto a 10x300 mm glass column. The void volume (V_0) was determined by Keyhole Limpet Hemocyanin (KLH) and the total volume (V_t) was determined by acetone.
We recommend not to use Blue Dextran as a molecular weight marker due to it may give unspecific binding to the resin.

Resin:	(A) WorkBeads 40/100 SEC (B) WorkBeads 40/1000 SEC (C) WorkBeads 40/10 000 SEC
Column:	10x300 mm, 24 ml
Sample volume:	50 µl
Elution buffer:	20 mM Na-phosphate, 150 mM NaCl, pH 7.4 (PBS)
Flow rate:	0.79 ml/min (60 cm/h)
Samples (A):	5 mg/ml Keyhole Limpet Hemocyanin (KLH, M_r approx. 8 000 000) 5 mg/ml thyroglobulin from bovine thyroid (M_r 669 000) 15 mg/ml bovine serum albumin (M_r 66 500) 5 mg/ml ovalbumin (M_r 43 000) 5 mg/ml α-chymotrypsin from bovine pancreas (M_r 25 656) 1.5 mg/ml myoglobin equine skeletal muscle (M_r 17 200) 15 mg/ml ribonuclease A from bovine pancreas (M_r 13 700) 5 mg/ml cytochrome C from equine heart (M_r 12 400) 10% (v/v) acetone in distilled water (M_r 58.08)
Samples (B):	5 mg/ml Keyhole Limpet Hemocyanin (KLH, M_r approx. 8 000 000) 5 mg/ml thyroglobulin from bovine thyroid (M_r 669 000) 1.5 mg/ml ferritin from equine spleen (M_r 440 000) 6 mg/ml human polyclonal IgG (M_r 150 000) 5 mg/ml bovine serum albumin (M_r 66 500) 5 mg/ml ovalbumin (M_r 43 000) 1.5 mg/ml myoglobin equine skeletal muscle (M_r 17 200) 5 mg/ml ribonuclease A from bovine pancreas (M_r 13 700) 10% (v/v) acetone in distilled water (M_r 58.08)
Samples (C):	5 mg/ml Keyhole Limpet Hemocyanin (KLH, M_r approx. 8 000 000) 5 mg/ml thyroglobulin from bovine thyroid (M_r 669 000) 5 mg/ml ovalbumin (M_r 43 000) 5 mg/ml ribonuclease A from bovine pancreas (M_r 13 700) 10% (v/v) acetone in distilled water (M_r 58.08)

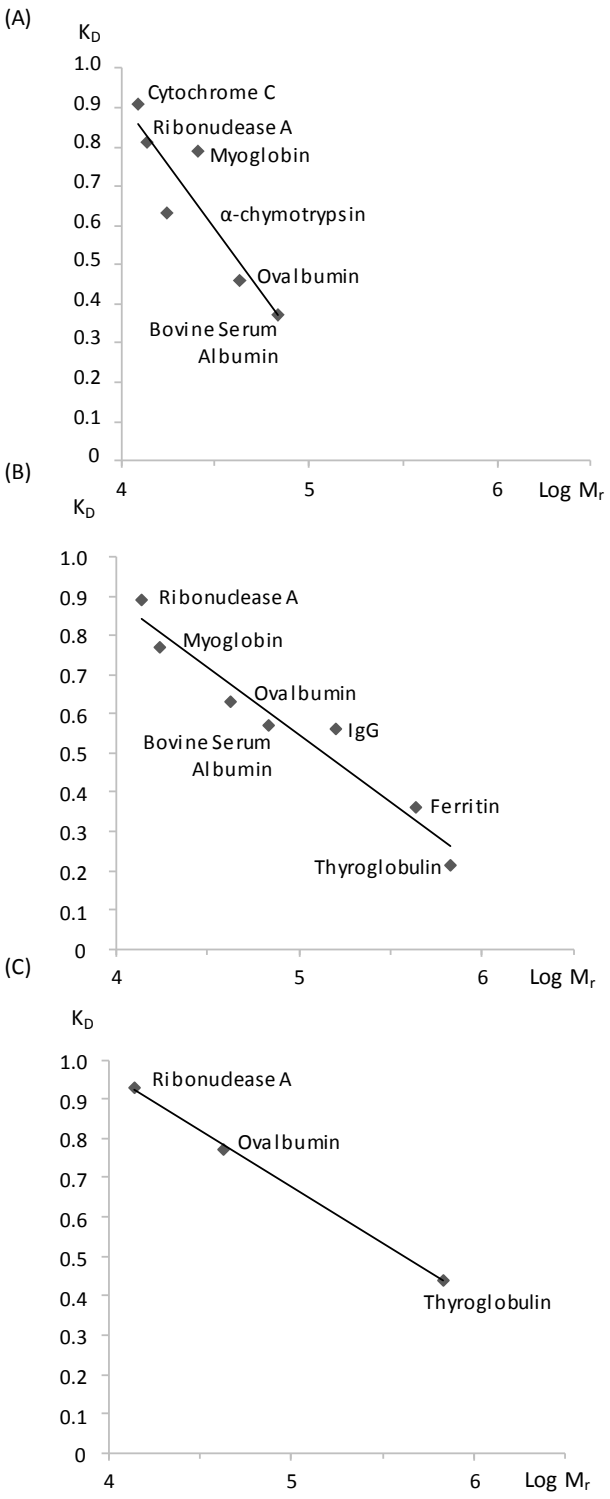

Figure 1. K_D-log M_r plots. Standard proteins applied on a 10x300 mm column packed with (A) WorkBeads 40/100 SEC, (B) WorkBeads 40/1000 SEC and (C) WorkBeads 40/10 000 SEC.

Cleaning-in-place

During purification impurities such as cell debris, lipids, nucleic acids and protein precipitates from the samples may 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 (Cleaning-in-place, CIP) keeps the resin clean, reduces the rate of further contamination, and prolongs the capacity, resolution and flow properties of the column. Cleaning of a column using 1 M NaOH applied by a low reversed flow for 2 hours or overnight is often sufficient.

Sanitization (reduction of microorganism) can be done using combinations of NaOH and ethanol (e.g., incubation with a mixture of 0.5 M NaOH and 40% ethanol for 3 hours). The sanitization procedure and its effectiveness will depend on the microorganism to be removed, and needs to be evaluated for each case.

Storage

Store at 2 to 25°C in 20% ethanol.

Related products

Product name	Pack size[1]	Article number
Bulk resins		
WorkBeads 40S	25 ml	40 200 001
WorkBeads 40Q	25 ml	40 100 001
WorkBeads 40 DEAE	25 ml	40 150 001
WorkBeads Protein A	10 ml	40 605 003
WorkBeads 40 Ni-NTA	25 ml	40 651 001

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 40/100 SEC	25 ml	40 340 001
	300 ml	40 340 003
	1 L	40 340 010
	5 L	40 340 050
WorkBeads 40/1000 SEC	25 ml	40 300 001
	300 ml	40 300 003
	1 L	40 300 010
	5 L	40 300 050
WorkBeads 40/10 000 SEC	25 ml	40 350 001
	300 ml	40 350 003
	1 L	40 350 010
	5 L	40 350 050

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

Bio-Works

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Virdings allé 18
754 50 Uppsala
Sweden

WorkBeads 40/100 SEC
WorkBeads 40/1000 SEC
WorkBeads 40/10 000 SEC
WorkBeads Macro SEC
WorkBeads 200 SEC

WorkBeads™ 40/100 SEC, WorkBeads 40/1000 SEC, WorkBeads 40/10 000 SEC, WorkBeads Macro SEC and WorkBeads 200 SEC resins are size exclusion chromatography (SEC) resins used for laboratory and process-scale separations of proteins, peptides, nucleic acids, viruses and other biomolecules by exploiting the differences in their size. The resins are based on agarose, a well-established and familiar material in the biotech industry.

Although the general recommendation for SEC is to use low flow rate for best purification, the rigidity and tight particle size distribution of WorkBeads allow for purification of viruses and other large substance at high flow rate for fast processing and high yields.

- Produced using a proprietary cross-linking method that results in highly porous and physically stable matrices
- Availability in several different porosities gives robust and wide separation ranges
- Alternative bead sizes for viscous samples
- Resistant to harsh cleaning agents (NaOH)

Resin description

WorkBeads are agarose-based chromatographic resins manufactured by a proprietary method that results in porous beads with a tight size distribution and exceptional 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 that require optimal purity and flow properties.

The different WorkBeads SEC resins allow purifications over a large range of molecular weights. The combination of excellent resolution and flow-pressure properties makes these resins suitable for both lab-scale and process-scale separations in standard columns from low to high flow rates. The chemical resistance of the resins allow purification over a broad range of conditions.

The main characteristics of WorkBeads SEC resins are shown in Tables 1 and 2. For more details, see instructions, IN 40 300 010 and IN 20 300 010.

Table 1. Main characteristics of WorkBeads 40 SEC resins.

	WorkBeads 40/100 SEC	WorkBeads 40/1000 SEC	WorkBeads 40/10 000 SEC	WorkBeads Macro SEC
Separation range[1]	10 - 150 kD	10 - 1200 kD	10 - 10 000 kD	10 - 30 000 kD
Exclusion limit	150 kD	1200 kD	10 000 kD	30 000 kD
Matrix	Highly cross-linked agarose	Highly cross-linked agarose	Highly cross-linked agarose	Highly cross-linked agarose
Average particle size[2] (D_{V50})	45 µm	45 µm	45 µm	45 µm
Recommended flow rate[3]	15 - 150 cm/h	15 - 150 cm/h	15 - 150 cm/h	15 - 150 cm/h
Max flow rate[4,5]	600 cm/h	600 cm/h	300 cm/h	300 cm/h
Chemical stability	Compatible with all standard aqueous buffers used for protein purification. Should not be stored at low pH for prolonged time.			
pH stability	2 - 13	2 - 13	2 - 13	2 - 13
Storage	2 to 25 °C in 20% ethanol	2 to 25 °C in 20% ethanol	2 to 25 °C in 20% ethanol	2 to 25 °C in 20% ethanol

1. Globular proteins.
2. The median particle size of the cumulative volume distribution.
3. The flow rate is important for the resolution and a lower flow rate often gives an increased resolution. A higher flow rate can be used during equilibration to speed up the separation.
4. Determined in water using a 25 × 200 mm column.
5. **Note**: Make sure that the column hardware max pressure is not exceeded.

Table 2. Main characteristics of WorkBeads 200 SEC resin.

	WorkBeads 200 SEC
Separation range[1]	10 - 6000 kD
Exclusion limit	6000 kD
Matrix	Highly cross-linked agarose
Average particle size[2] (D_{V50})	180 µm
Recommended flow rate[3]	15 - 150 cm/h
Max flow rate[4,5]	900 cm/h
Chemical stability	Compatible with all standard aqueous buffers used for protein purification. Should not be stored at low pH for prolonged time.
pH stability	2 - 13
Storage	2 to 25 °C in 20% ethanol

1. Globular proteins.
2. The median particle size of the cumulative volume distribution.
3. The flow rate is important for the resolution and a lower flow rate often gives an increased resolution. A higher flow rate can be used during equilibration to speed up the separation.
4. Determined in water using a 25 × 200 mm column.
5. **Note:** Make sure that the column hardware max pressure is not exceeded.

Principle

Size Exclusion Chromatography (SEC), also called gel filtration (GF), is a simple and reliable technique for separation of molecular components according to their size. The technique is based on the relative retardation of substances of different sizes when passed through a packed bed of porous beads. Very large substances in the applied sample will be eluted first, since they will not enter the pores of the beads (larger than the size cut-off of that resin). These substances will only access the volume outside the beads, the void volume, V_0. Very

small substance such as salt and buffer components will elute close to the geometrical volume of the packed bed, since they can enter essentially all pores of the beads, the total volume, V_t. Substances of intermediate sizes will elute at different volumes depending on their size relative to the pore sizes of the resin. The five available resins have different porosities and bead sizes resulting in different separation ranges.

The packed column is prepared by equilibration with a suitable buffer, usually an aqueous buffer, before loading the sample. The composition of the buffer should be selected to give the best stability of the target substance. A general recommendation is to include 150 mM NaCl in the buffer to eliminate electrostatic interactions in the substance to be separated, and between substances and the resin. Elution should be done with approx. 1.3 column volumes (CV) to allow all applied material to pass through the column, and to make sure that salt and low-molecular weight substances from the sample have been eluted from the column. A new sample can be applied directly.
An inherent advantage with SEC is the combined purification and buffer exchange or salt removal of the target substance material. This is one of the reasons that SEC is a frequently used final step (polishing step) in protein purification. A drawback with SEC is the relative low flow rate required, and this is one of the reasons to use SEC in the final step when the target substance has often been concentrated during the previous step. An important benefit of SEC is that it can remove aggregates of the target substance that are co-purified in earlier purification steps.

WorkBeads resins are all based on the same base matrix and therefore have the same characteristics. WorkBeads 40/100 SEC, WorkBeads 40/1000 SEC and WorkBeads 40/10 000 SEC and WorkBeads Macro SEC resins have the same bead sizes 45 µm, but different porosities which makes it easy to change from one to the other when different fractionation ranges are desired.
WorkBeads 200 SEC has the same characteristics but a mean bead size of 180 µm which makes this resin suitable to use with viscous samples, for example serum and whole blood. In these cases the larger beads will have a positive effect on the backpressure.
See Table 3 for a comparison of the different WorkBeads SEC resins.

For standard purifications of proteins, peptides and nucleic acids the flow rate should be low, 15 - 150 cm/h. Higher flow rates are possible (up to 300 cm/h) but will reduce the resolution between peaks. The recommended sample volume for a preparative SEC column for receiving the highest resolution is 1% - 4% of the column volume. The high rigidity of the resins allows the use of high flow rates for applications in which the target substance is eluted in the void fraction, (e.g., virus purifications).

Table 3. Comparison of WorkBeads SEC resins.

	Average bead size, µm	Separation range, kD	Exclusion limit, kD	Separation range, D 10^4 10^5 10^6 10^7 10^8
WorkBeads 40/100 SEC	45	10 – 150	150	
WorkBeads 40/1000 SEC	45	10 – 1200	1200	
WorkBeads 40/10 000 SEC	45	10 – 10 000	10 000	
WorkBeads Macro SEC	45	10 – 30 000	30 000	
WorkBeads 200 SEC	180	10 – 6000	6000	

Separation ranges

Figure 1 shows the K_D-curve determination for WorkBeads 40/100 SEC, WorkBeads 40/1000 SEC, WorkBeads 40/10 000 SEC, WorkBeads Macro SEC and WorkBeads 200 SEC. The K_D-curves are determined using standard proteins applied onto a 10 x 300 mm glass column. The void volume (V_0) was determined by Hemocyanin Keyhole Limpet (HKL) and the total volume (V_t) was determined by acetone.

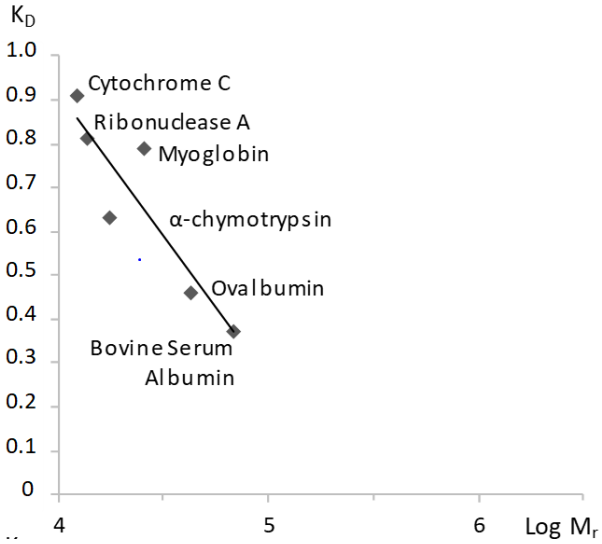
We recommend not to use Blue Dextran as a molecular weight marker as it may cause unspecific binding to the resin.

Resins: (A) WorkBeads 40/100 SEC
(B) WorkBeads 40/1000 SEC
(C) WorkBeads 40/10 000 SEC
(D) WorkBeads Macro SEC
(E) WorkBeads 200 SEC

Columns: 10 x 300 mm, 24 ml
16 x 900-950 mm, 181-191 ml (WorkBeads 200 SEC)
Sample volume: 50 µl
Elution buffer: 20 mM sodium phosphate, 150 mM NaCl, pH 7.4 (PBS)
Flow rate: 0.8 ml/min (60 cm/h)
0.8 ml/min (25 cm/h) (WorkBeads 200 SEC)

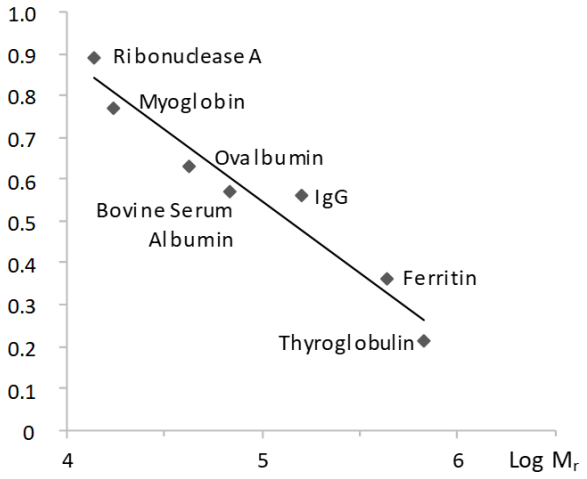
Samples (A): 5 mg/ml Hemocyanin Keyhole Limpet (HKL), M_r approx. 8 000 000
5 mg/ml thyroglobulin (bovine thyroid), M_r 669 000
5 mg/ml bovine serum albumin (BSA), M_r 66 500
5 mg/ml ovalbumin, M_r 43 000
5 mg/ml α-chymotrypsin (bovine pancreas), M_r 25 656
1.5 mg/ml myoglobin (equine skeletal muscle), M_r 17 200
15 mg/ml ribonuclease A (bovine pancreas), M_r 13 700
5 mg/ml cytochrome C (equine heart), M_r 12 400
10% (v/v) acetone in distilled water, M_r 58.08

(A) WorkBeads 40/100 SEC



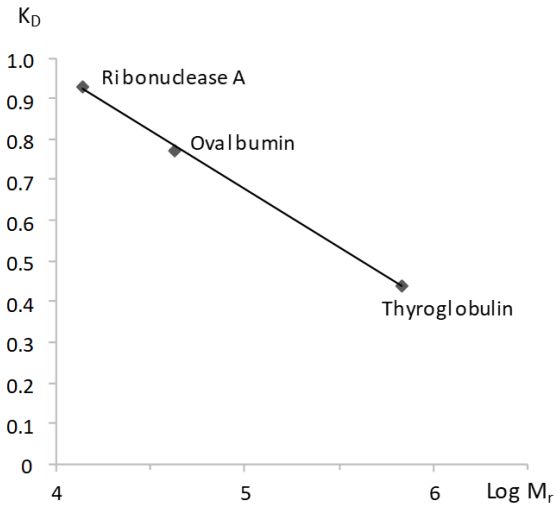
Samples (B): 5 mg/ml Hemocyanin Keyhole Limpet (HKL), M_r approx. 8 000 000
5 mg/ml thyroglobulin (bovine thyroid), M_r 669 000
1.5 mg/ml ferritin (equine spleen), M_r 440 000
6 mg/ml human polyclonal IgG, M_r 150 000
5 mg/ml bovine serum albumin (BSA), M_r 66 500
5 mg/ml ovalbumin, M_r 43 000
1.5 mg/ml myoglobin (equine skeletal muscle), M_r 17 200
15 mg/ml ribonuclease A (bovine pancreas), M_r 13 700
10% (v/v) acetone in distilled water, M_r 58.08

(B) WorkBeads 40/1000 SEC

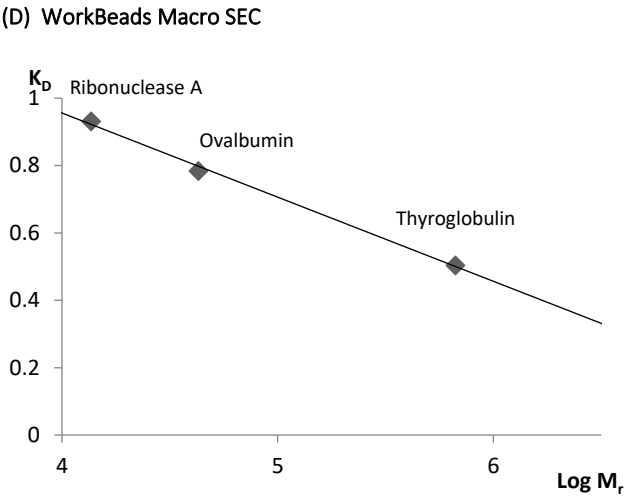


Samples (C): 5 mg/ml Hemocyanin Keyhole Limpet (HKL), M_r approx. 8 000 000
5 mg/ml thyroglobulin (bovine thyroid), M_r 669 000
5 mg/ml ovalbumin, M_r 43 000
15 mg/ml ribonuclease A (bovine pancreas), M_r 13 700
10% (v/v) acetone in distilled water, M_r 58.08

C) WorkBeads 40/10 000 SEC



Samples (D): 5 mg/ml Hemocyanin Keyhole Limpet (HKL), M_r approx. 8 000 000
5 mg/ml thyroglobulin (bovine thyroid), M_r 669 000
5 mg/ml ovalbumin, M_r 43 000
15 mg/ml ribonuclease A (bovine pancreas), M_r 13 700
10% (v/v) acetone in distilled water, M_r 58.08



Samples (E): 5 mg/ml Hemocyanin Keyhole Limpet (HKL), M_r approx. 8 000 000
5 mg/ml thyroglobulin (bovine thyroid), M_r 669 000
5 mg/ml ovalbumin, M_r 43 000
15 mg/ml ribonuclease A (bovine pancreas), M_r 13 700
10% (v/v) acetone in distilled water, M_r 58.08

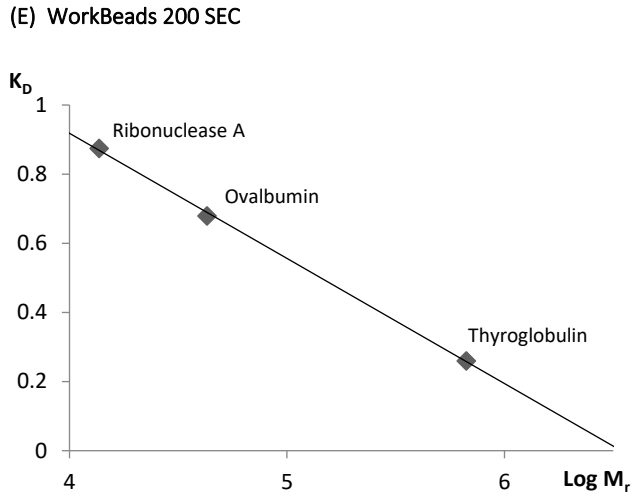


Figure 1. K_D-log M_r plots. Standard proteins applied on a 10 x 300 mm column packed with (A) WorkBeads 40/100 SEC, (B) WorkBeads 40/1000 SEC, (C) WorkBeads 40/10 000 SEC and (D) WorkBeads Macro SEC. (E) WorkBeads 200 SEC is packed in a 16 x 900 mm column.

Cleaning-in-place

During purification, impurities such as cell debris, lipids, nucleic acids and protein precipitates from the samples may 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 (Cleaning-in-place, CIP) keeps the resin clean, reduces the rate of further contamination, and prolongs the capacity, resolution and flow properties of the column. Cleaning of a column using 1 M NaOH applied by a low reversed flow for 2 hours or overnight is often sufficient.

Sanitization (reduction of microorganism) can be done using combinations of NaOH and ethanol (e.g., incubation with a mixture of 0.5 M NaOH and 40% ethanol for 3 hours). The sanitization procedure and its effectiveness will depend on the microorganism to be removed, and needs to be evaluated for each case.

Storage

Store at 2 to 25°C in 20% ethanol.

Related products

Product name	Pack size[1]	Article number
Pre-packed columns		
BabyBio Dsalt 1 ml	1 ml x 5	45 360 103
BabyBio Dsalt 5 ml	5 ml x 5	45 360 107
Bulk resins		
WorkBeads Dsalt	300 ml	40 360 003
	1 L	40 360 010
	5 L	40 360 050
	10 L	40 360 060

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 40/100 SEC	25 ml	40 340 001
	300 ml	40 340 003
	1 L	40 340 010
	5 L	40 340 050
	10 L	40 340 060
WorkBeads 40/1000 SEC	25 ml	40 300 001
	300 ml	40 300 003
	1 L	40 300 010
	5 L	40 300 050
	10 L	40 300 060
WorkBeads 40/10 000 SEC	25 ml	40 350 001
	300 ml	40 350 003
	1 L	40 350 010
	5 L	40 350 050
	10 L	40 350 060
WorkBeads Macro SEC	25 ml	40 370 001
	300 ml	40 370 003
	1 L	40 370 010
	5 L	40 370 050
	10 L	40 370 060
WorkBeads 200 SEC	300 ml	20 300 003
	1 L	20 300 010
	5 L	20 300 050
	10 L	20 300 060

Orders: sales@bio-works.com or contact your local distributor.
For more information about local distributor and products visit www.bio-works.com or contact us at info@bio-works.com

Bio-Works
Virdings allé 18
754 50 Uppsala
Sweden

WorkBeads 40 TREN
BabyBio TREN 1 ml
BabyBio TREN 5 ml

WorkBeads™ 40 TREN resin for multimodal ion exchange chromatography (IEX) has a ligand that is positively charged below approx. pH 9. This resin can be used for several different applications, especially due to its higher salt tolerant properties, *e.g.,* for alternative IEX selectivity, for sample cleanup in monoclonal antibody (mAb) purification processes to guard the protein A column from chromatins, viruses, endotoxins and other host cell impurities, or as a polishing step in the mAb purification process. This unique resin is also available in prepacked BabyBio™ 1 ml and 5 ml columns for fast and easy small-scale purifications as well as fast screening for optimizing purification conditions.

- Differential selectivity due to higher salt tolerance and multimodal properties
- Reduced fouling of *e.g.* protein A resins by chromatin, viruses, endotoxins and host cell impurity removal
- High binding capacity and purity
- Available in prepacked BabyBio 1 ml and 5 ml columns

Resin description

WorkBeads are agarose-based chromatographic resins manufactured using a proprietary method that results in porous beads with a tight size distribution and high mechanical stability. Agarose based matrices have been successfully used for decades in biotechnology purification, from research 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.

WorkBeads 40 TREN resin contains ligands based on Tris(2-aminoethyl)amine (TAEA). The structure of the ligand used in WorkBeads 40 TREN is shown in Figure 1.

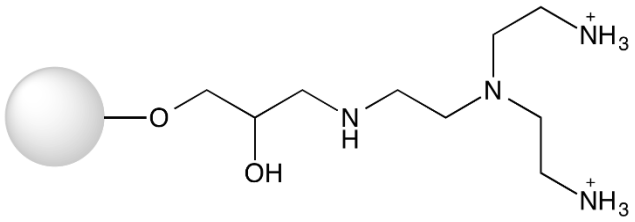


Figure 1. Structure of the ligand used in WorkBeads 40 TREN.

WorkBeads 40 TREN resin can be used for the separation of biomolecules exploiting surface charge to purify proteins, peptides and oligonucleotides. It can also be used in flow through mode to adsorb impurities while letting the target pass through the column (negative chromatography mode).

The main characteristics of WorkBeads 40 TREN resin are shown in Table 1. For more detailed instructions of how to use WorkBeads 40 TREN, see instruction IN 40 600 020.

Table 1. Main characteristics of WorkBeads 40 TREN resin.

	WorkBeads 40 TREN
Target substances	Proteins, peptides, oligonucleotides, viruses, endotoxins and chromatin fragments
Matrix	Rigid, highly cross-linked agarose
Average particle size[1] (D_{v50})	45 µm
Ligand	Tris(2-ethylaminoethyl)amine (TAEA)
Dynamic binding capacity	50 mg BSA/ml resin[2]
Max flow rate (20 cm bed height and 5 bar)[3]	600 cm/h
Chemical stability	Compatible with all standard aqueous buffers used for protein purification. Should not be stored at low pH for prolonged time.
pH stability	2 - 13
Storage	2 to 25 °C in 20% ethanol

1. The median particle size of the cumulative volume distribution.
2. Dynamic binding capacity determined at 4 minutes residence time (0.25 ml/min in 1 ml column) in 50 mM Tris-HCl, 50 mM NaCl, pH 8.0.
3. Optimal flow rate during binding is depending on the sample.

BabyBio description

The column 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 filters in the top and the bottom of the column have a pore size optimized to allow loading of semi-crude feed with minimal clogging.

The ready-to-use BabyBio prepacked 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 tubing), with a female and a male connection at the top and bottom respectively. BabyBio columns can be connected in series providing a convenient way to perform smaller scale-up experiments.

The main characteristics of BabyBio TREN columns are shown in Table 2. For additional information, see instruction IN 45 655 030.

Table 2. Main characteristics of BabyBio TREN columns.

	BabyBio TREN
Target substance	Proteins, peptides, oligonucleotides, viruses, endotoxins, and chromatin fragments.
Resin	WorkBeads 40 TREN
Matrix	Rigid, highly cross-linked agarose
Average particle size[1] (D_{v50})	45 µm
Ligand	Tris(2-aminoethyl)amine (TAEA)
Dynamic binding capacity	50 mg BSA/ml resin[2]
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 1 ml	0.25 - 1 ml/min (37 - 150 cm/h)
BabyBio 5 ml	1.25 - 5 ml/min (56 - 225 cm/h)
Maximum flow rate[4]	
BabyBio 1 ml	5 ml/min (780 cm/h)
BabyBio 5 ml	20 ml/min (900 cm/h)
Maximum back pressure	0.3 MPa, 3 bar, 43 psi
Chemical stability	Compatible with all standard aqueous buffers used for protein purification. Do not keep the column at low pH for prolonged time.
pH stability	2 - 13
Storage	2 to 25°C in 20% ethanol

1. The median particle size of the cumulative volume distribution.
2. Dynamic binding capacity determined at 4 minutes residence time (0.25 ml/min in 1 ml column) in 50 mM Tris-HCl, 50 mM NaCl, pH 8.0.
3. Optimal flow rate during binding is depending on the sample. During column wash and elution, a flow rate of 1 ml/min and 5 ml/min can be used for 1 ml and 5 ml columns, respectively. Note: 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.
4. Aqueous buffers at 20°C. 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 at 4°C), or by additives (e.g. use half of the maximum flow rate for 20% ethanol).

Applications

Multimodal ion exchange chromatography

WorkBeads 40 TREN resin can be used for similar applications to those when using ion exchange chromatography resins. In Figure 2, an example of separation of the acidic proteins apo-transferrin, α-lactalbumin and soybean trypsin inhibitor are separated on BabyBio TREN 1 ml, prepacked with WorkBeads 40 TREN. Figure 3 shows a separation comparison between BabyBio TREN 1 ml and BabyBio DEAE 1 ml (a weak anion exchange chromatography column). As can be seen in the chromatograms, the selectivity is different. WorkBeads 40 TREN is a high-salt-tolerant multimodal AIEX resin.

Column:	BabyBio TREN 1 ml
Binding buffer:	50 mM Tris-HCl, pH 7.4
Elution buffer:	50 mM Tris-HCl, 1 M NaCl, pH 7.4
Sample:	2.5 ml of 0.3 mg/ml apo-transferrin, 0.2 mg/ml α-lactalbumin, 0.6 mg/ml soybean trypsin inhibitor in binding buffer
Flow rate:	1 ml/min (150 cm/h)
Gradient:	0 - 100% elution buffer in 20 column volumes (CV)

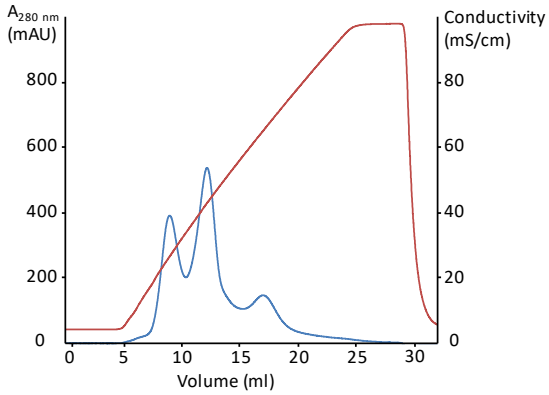


Figure 1. Separation of a protein mix on a BabyBio TREN 1 ml column. The peaks from left to right corresponds to apo-transferrin, α-lactalbumin and soybean trypsin inhibitor. The blue line corresponds to the absorbance at 280 nm and the red line to the conductivity.

Column:	(A) BabyBio TREN 1 ml (B) BabyBio DEAE 1 ml
Binding buffer:	50 mM Tris-HCl, pH 7.4
Elution buffer:	50 mM Tris-HCl, 1 M NaCl, pH 7.4
Sample:	2.5 ml of 0.3 mg/ml apo-transferrin, 0.2 mg/ml α-lactalbumin, 0.6 mg/ml soybean trypsin inhibitor in binding buffer
Flow rate:	1 ml/min (150 cm/h)
Gradient:	0 - 40% elution buffer in 20 CV

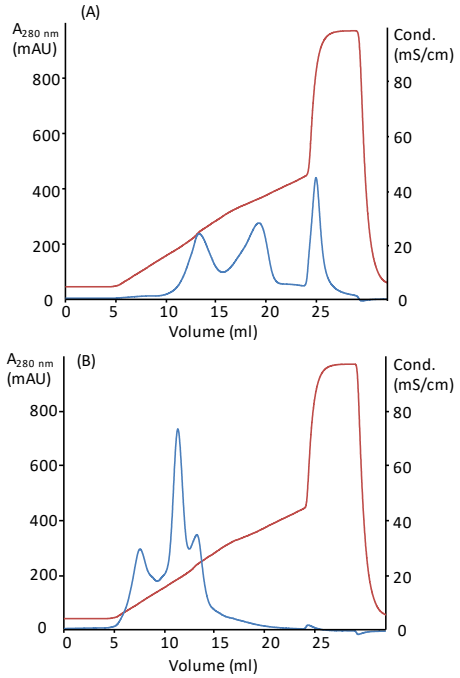


Figure 2. Comparison of separation of a protein mix on a BabyBio TREN 1 ml (A) and BabyBio DEAE 1 ml (B). A more shallow salt gradient was used compared to the separation in Fig. 2. The peaks from left to right corresponds to apo-transferrin, α-lactalbumin and soybean trypsin inhibitor. The blue line corresponds to the absorbance at 280 nm and the red line to the conductivity.

Viruses bind to WorkBeads 40 TREN

For biopharmaceutical processes that result in products for use in humans, virus clearance studies need to be conducted. Viruses in the final product can prove to be fatal, and therefore need to be removed in the purification process. This virus removal can be performed in a pre-treatment step using WorkBeads 40 TREN as shown below.

A feed (clarified supernatant containing mAbs expressed from CHO cells) was spiked with two viruses, MVM and X-MuLV (which may be present in CHO cells), one at a time and in duplicates.
MVM, Minute Virus of Mice, is a small (18-26 nm) non-enveloped parvovirus and X-MuLV, Xenotropic Murine Leukemia Virus is a moderately large (80-120 nm) enveloped retrovirus. The virus titers pre- and post-purification on WorkBeads 40 TREN were measured and compared to control samples that showed the amount of virus applied to the columns. A logarithmic reduction value (LRV) was calculated.

The capacity of WorkBeads 40 TREN resin for reduction of virus in the flow through was high for both viruses tested. The average logarithmic reduction factor (LRV) of the parvovirus MVM in two runs was 4.89 Log10 TCID50 units which is deemed effective removal. The average LRV of X-MuLV in two runs was > 3.67 Log10 TCID50 units. This is moderately effective to effective removal, see Table 3 below.

Table 3. Virus removal using WorkBeads 40 TREN

Virus type	Reduction of MVM (Log_{10} $TCID_{50}$)	Reduction of X-MuLV (Log_{10} $TCID_{50}$)
Run 1	4.87	3.44
Run 2	4.91	3.82
Average	4.9	3.7

Two different viruses were here shown to efficiently bind to WorkBeads 40 TREN, showing it can be used for virus clearance from feeds or for enrichment of viruses to be used for gene therapy and characterization studies etc.

Loading capacities for WorkBeads 40 TREN

WorkBeads 40 TREN has been shown to bind chromatin and other host-cell derived impurities in a mammalian cell culture (Nian et al., J. Chromatogr. A, 1431 (2016) 1-7; Chen et al., J. Biotechnol., 236 (2016) 128-140). In Table 4 loading capacities (determined at 4 minutes residence times) for proteins, peptides, viruses and ssDNA, all molecules that bind to this resin, are shown.

Table 4. Loading capacities of different molecules on WorkBeads 40 TREN

Molecule	Dynamic binding capacity (mg/mL resin)	Column tested
MVM	4.9[1]	WorkBeads 40 TREN (10 x 100 mm)
X-MuLV	3.7[1]	WorkBeads 40 TREN (10 x 100 mm)
BSA	50	BabyBio TREN 1 mL
Peptide[3]	31	BabyBio TREN 1 mL
ssDNA[4]	21	WorkBeads 40 TREN (6.6 x 100 mm)

1. Log10 TCID50 in complex mixtures/feeds.
2. Dynamic binding capacity determined at 4 minutes residence time (0.25 ml/min in 1 ml column) in 50 mM Tris-HCl, 50 mM NaCl, pH 8.0.
3. Synthesized acidic 39 aa-long peptide.
4. Synthesized 20 nt-long ssDNA.

Use of WorkBeads 40 TREN in mAb purification

Purification of monoclonal antibodies usually involves purification on chromatography resins with protein A ligands followed by polishing steps based on anion- or cation exchange chromatography. The presence of chromatin fragments (fragments of the chromosomes, based on histone proteins and DNA) is a major cause for fouling of protein A columns, and is also a key impurity after the protein A step. Chromatin particles are heavily charged structures with massive negative net charges. Due to this, they can easily be adsorbed on WorkBeads 40 TREN at neutral or low pH, which has proved to be useful for removal of chromatin and other impurities.

The use of WorkBeads 40 TREN in binding or flow through mode will also facilitate removal of nucleic acids, endotoxins, viruses, host cell proteins and other cell-derived impurities. As protein A ligands may be cleaved by proteases, leached protein A ligands can be removed by a polishing step using a WorkBeads 40 TREN column after the protein A purification step. Notice that the majority of mAbs are basic, thus are mainly positively charged at neutral pH, and therefore do not bind to the resin.

Figure 4 and 5 shows that the of majority of HCD, HMWS and HCP from a CHO cell feed containing mAbs were adsorbed to the WorkBeads 40 TREN and thus removed from the sample before it was loaded onto the WorkBeads affimAb column. Viruses are also efficiently removed as has been shown above. No significant yield loss of mAbs was detected.

The characteristics of WorkBeads 40 TREN can be exploited in several ways in a mAb purification process, see Figure 6 and below.

1. As a precipitation agent added to the feed to induce chromatin precipitation for easy removal by continuous centrifugation followed by depth filtration before the protein A step. 0.5 - 5% g resin/ml cell supernatant is often enough for chromatin removal from cell supernatant feed.

2. As a guard column for removal of chromatin and other impurities before the protein A column.

3. In a polishing step after the Protein A purification step.

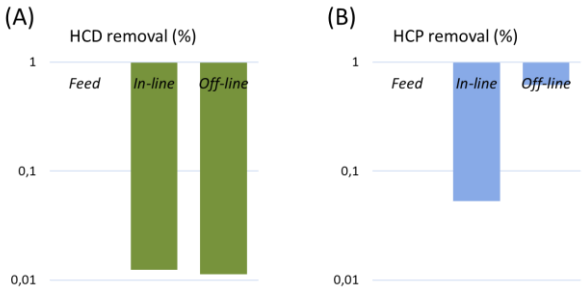


Figure 4. Removal of host cell impurities. (A) HCD removal pre- and post-WorkBeads 40 TREN for inline *vs* offline purifications. Feed value is set to 1. (B) HCP removal pre- and post-WorkBeads 40 TREN for inline *vs* offline purifications. Feed value is set to 1.

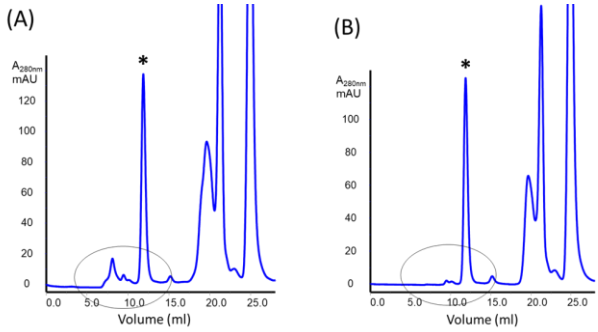


Figure 5. Analytical SEC profiles of CHO cell supernatants. (A) Before pre-treatment with WorkBeads 40 TREN and after (B). The asterisk (*) highlights the target mAb, and the high-molecular weight substances (HMWS) are marked with a circle.

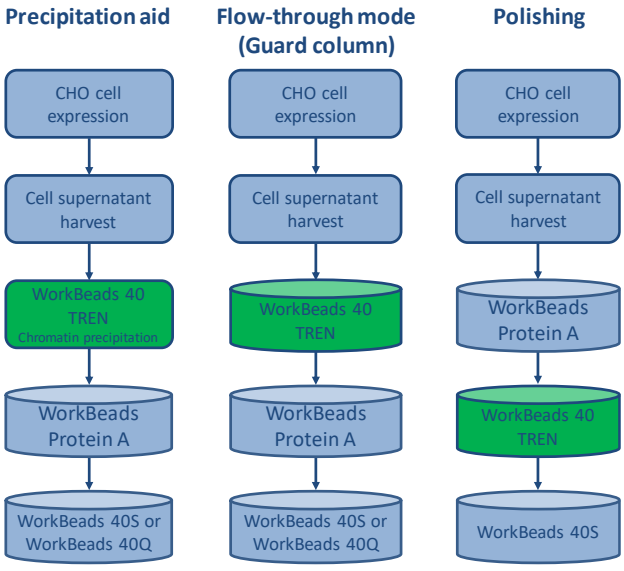


Figure 6. Use of WorkBeads 40 TREN in mAb purification processes.

Cleaning-in-place

After chromatin removal from cell supernatant/feeds WorkBeads 40 TREN can usually not be cleaned but must be discarded. During other purification schemes, impurities such as cell debris, lipids, nucleic acids and protein precipitates from the samples may gradually build-up in the resin, (cause fouling). The severity of this process depends on the type of sample applied to the column, and the pre-treatment of the sample. The impurities covering the resin may reduce the performance of the column over time. Regular cleaning (Cleaning-in-place, CIP) keeps the resin clean, reduces the rate of further fouling, and prolongs the capacity, resolution and flow properties of the column. Cleaning of a column using 1 M NaOH applied by a low reversed flow for 2 hours or overnight is often sufficient.

Storage

Store WorkBeads 40 TREN resin and BabyBio TREN columns at 2 to 25°C in 20% ethanol.

Related products

Product name	Pack size[1]	Article number
Prepacked columns		
BabyBio IEX Screening Kit[2]	1 ml x 4	45 900 001
BabyBio S 1 ml	1 ml x 5	45 200 103
BabyBio S 5 ml	5 ml x 5	45 200 107
BabyBio Q 1 ml	1 ml x 5	45 100 103
BabyBio Q 5 ml	5 ml x 5	45 100 107
BabyBio affimAb 1 ml	1 ml x 5	45 800 103
BabyBio affimAb 5 ml	5 ml x 5	45 800 107
BabyBio Dsalt 1 ml	1 ml x 5	45 360 103
BabyBio Dsalt 5 ml	5 ml x 5	45 360 107
Bulk resins		
WorkBeads 40S	25 ml	40 200 001
	200 ml	40 200 002
WorkBeads 40Q	25 ml	40 100 001
	200 ml	40 100 002
WorkBeads affimAb	25 ml	40 800 001
	200 ml	40 800 002
WorkBeads Dsalt	300 ml	40 360 003

1. All different pack sizes are available on www.bio-works.com
2. BabyBio IEX Screening Kit includes one of each: BabyBio S 1 ml, BabyBio Q 1 ml, BabyBio DEAE 1 ml and BabyBio TREN 1 ml.

Ordering information

Product name	Pack size	Article number
WorkBeads 40 TREN	25 ml	40 603 001
	150 ml	40 603 003
	1 L	40 603 010
BabyBio TREN 1 ml	1 ml x 1	45 655 211
	1 ml x 2	45 655 212
	1 ml x 5	45 655 213
	1 ml x 10	45 655 214
BabyBio TREN 5 ml	5 ml x 1	45 655 215
	5 ml x 2	45 655 216
	5 ml x 5	45 655 217
	5 ml x 10	45 655 218

Orders: sales@bio-works.com or contact your local distributor.

For more information about local distributor and products visit www.bio-works.com
or contact us at info@bio-works.com

Bio-Works
Virdings allé 18
754 50 Uppsala
Sweden

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.

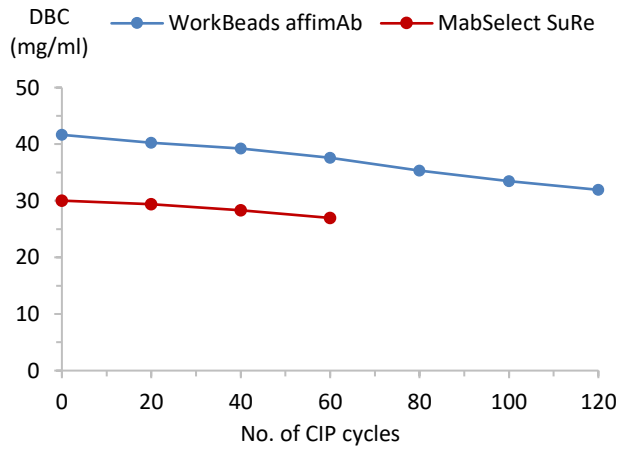


Figure 1. DBC for polyclonal human IgG on WorkBeads affimAb (blue) and MabSelect™ SuRe (GE Healthcare) (red) determined by frontal analysis at 2.4 minutes residence time after 120 resp. 60 CIP cycles with 0.5 M NaOH at 15 minutes contact time.

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.

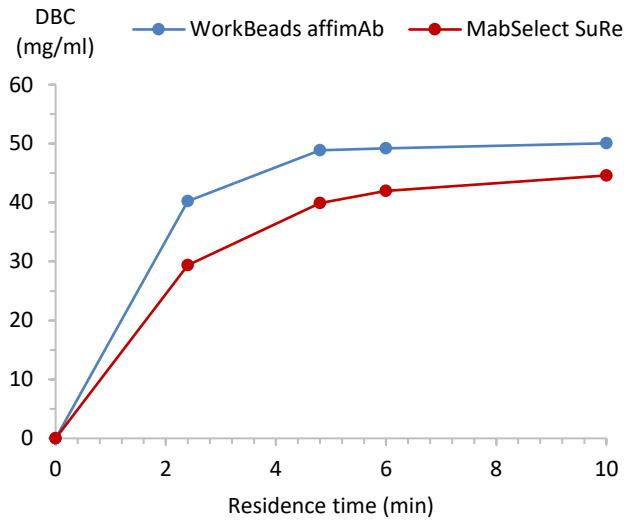


Figure 2. Dependency of dynamic binding capacity on residence time. Frontal analysis using 1 mg/ml human polyclonal IgG in PBS, pH 7.4 was performed in 6.6 x 100 mm packed bed.

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.

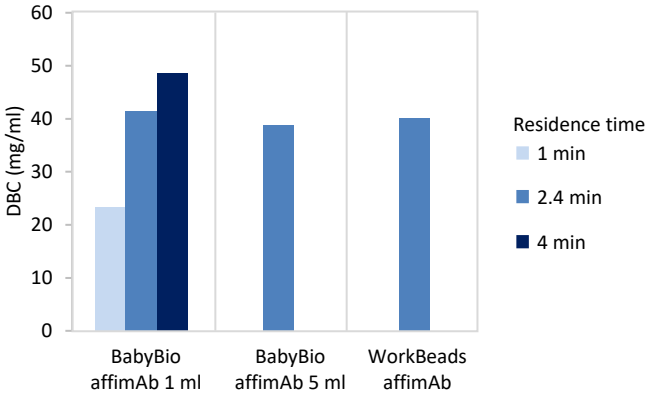


Figure 3. Dependency of dynamic binding capacity on residence time. Frontal analysis using 1 mg/ml human polyclonal IgG in PBS, pH 7.4 was performed in BabyBio affimAb 1 ml (28 mm bed height), BabyBio affimAb 5 ml (38 mm bed height) and WorkBeads affimAb in a 6.6 x 100 mm packed bed (100 mm bed height).

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.

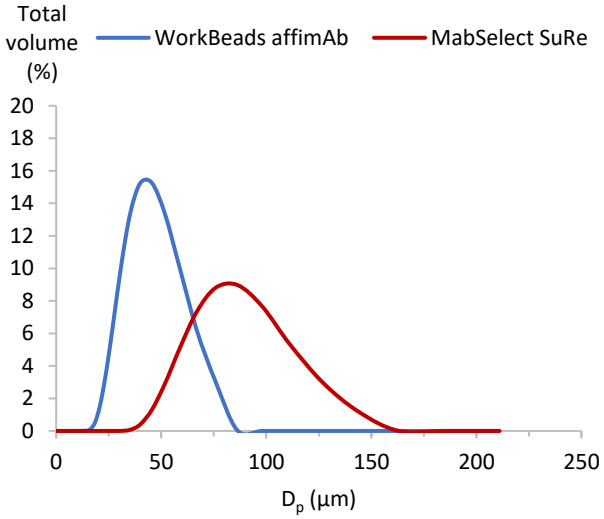


Figure 4. Particle size distribution comparison of WorkBeads affimAb (blue) and MabSelect SuRe (red).

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.

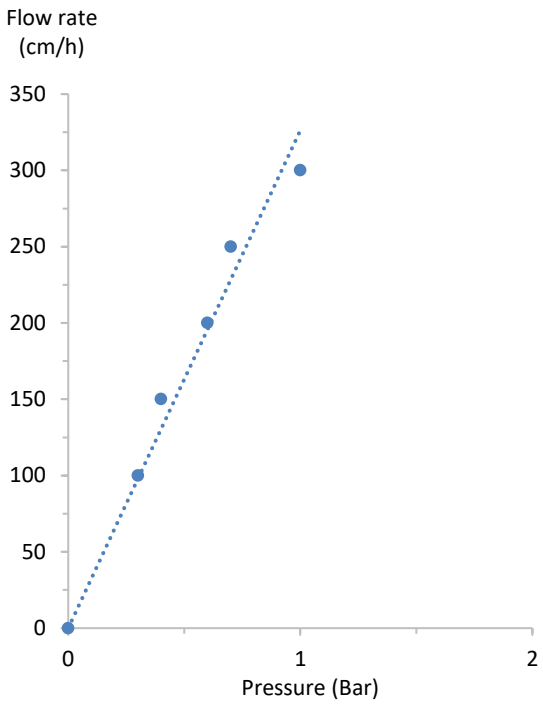


Figure 5. Pressure-flow data on WorkBeads base matrix in water obtained in a 25 x 200 mm open bed glass column. The pressure over the bed at low flow rates is often too low to detect.

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)

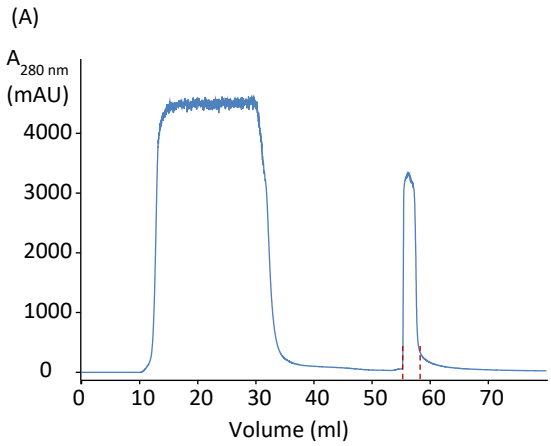


Figure 6A. Purification of a monoclonal IgG from CHO cells using WorkBeads affimAb. The blue line corresponds to the absorbance at 280 nm.

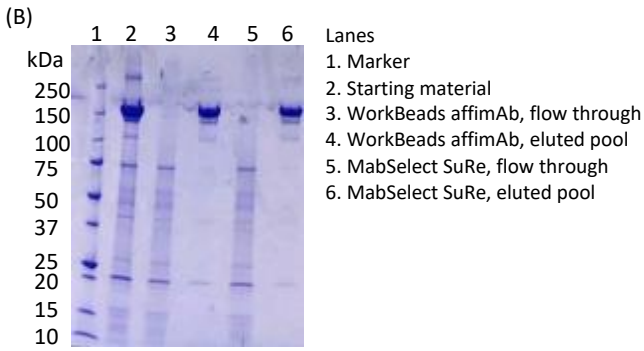


Figure 6B. Analysis of the purified mAb by SDS-PAGE, non-reduced conditions. Comparison of mAb purified by identical method on WorkBeads affimAb and MabSelect SuRe resins.

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

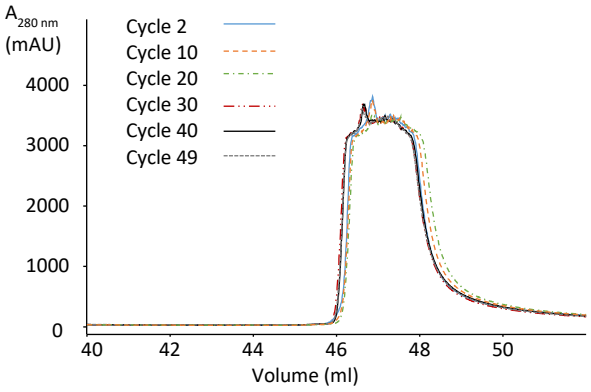


Figure 7. Elution profiles from purifications on WorkBeads affimAb after repeated CIP cycles.

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.

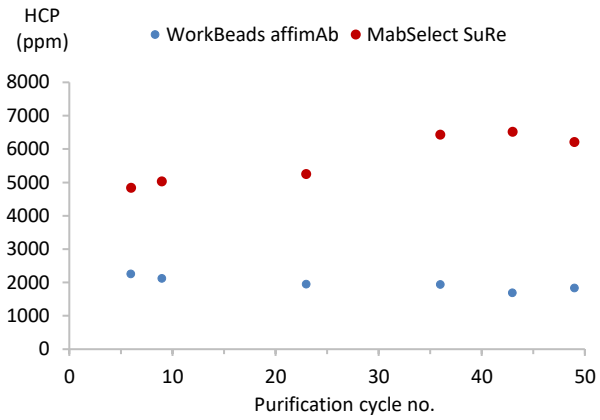


Figure 9. HCP levels in eluates analysed by ELISA for WorkBeads affimAb (blue) and MabSelect SuRe (red).

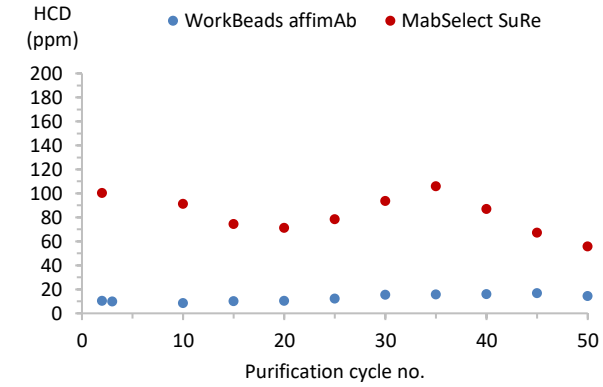


Figure 10. HCD levels in eluates analysed by Quant-iT PicoGreen dsDNA Assay Kit for WorkBeads affimAb (blue) and MabSelect SuRe (red).

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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OptioBio 40S 10×100
OptioBio 40Q 10×100

The prepacked OptioBio™ glass columns are designed for small-scale purification as well as screening and optimization in bioprocess development and scale-up. OptioBio 40S 10×100 and OptioBio 40Q 10×100 columns are prepacked with WorkBeads™ 40S and WorkBeads 40Q resins for ion exchange chromatography (IEX). The resins are designed for research and industrial scale purification of proteins, peptides and nucleic acids and utilise the difference in their surface charge. WorkBeads 40S is a strong cation exchange resin with sulfonate ligands, and WorkBeads 40Q is a strong anion exchange resin with quaternary amine ligands. The property of high-resolution separation in combination with low backpressure facilitates both capture and polishing purification applications.

- Prepacked for reliable and reproducible results
- Optimal for high-performance small-scale purification and method optimization in bioprocess development
- High throughput and purity

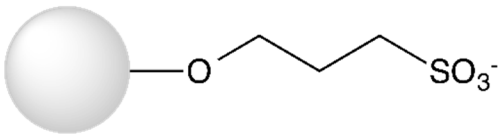
Resin description

WorkBeads are agarose based chromatographic resins manufactured using a proprietary method that results in porous beads with a tight size distribution and high mechanical stability. Agarose based matrices have been successfully used for decades in biotechnology, from research to production scale purifications, due to their exceptional compatibility with biomolecules including proteins, peptides, nucleic acids and carbohydrates. WorkBeads resins are designed for separations that require optimal capacity and purity.

WorkBeads 40S is a strong cation exchange resin derivatized with sulfonates as functional groups. WorkBeads 40Q is a strong anion exchanger derivatized with quarternary amines as functional groups.

The functional groups are coupled to the resin via chemically stable linkages. The structures of the ligands used in WorkBeads 40S and WorkBeads 40Q are shown in Figure 1.

(A)



(B)

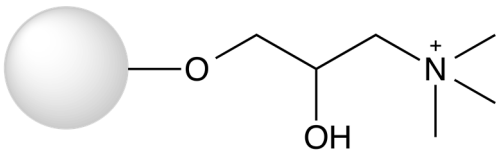


Figure 1. Structure of the ligand used in (A) WorkBeads 40S and (B) WorkBeads 40Q.

The main characteristics of OptioBio prepacked glass columns for ion exchange chromatography are shown in Table 1. For more details, please see instruction IN 55 410 010.

Table 1. Main characteristics of OptioBio 40S 10×100 and OptioBio 40Q 10×100 columns.

	OptioBio 40S 10×100	OptioBio 40Q 10×100
Target substance	Proteins and peptides	Protein, peptides and oligonucleotides
Resin	WorkBeads 40S	WorkBeads 40Q
Matrix	Rigid, highly cross-linked agarose	Rigid, highly cross-linked agarose
Average particle size (DV_{50})[1]	45 µm	45 µm
Ionic group (ligand)	Sulfonate ($-SO_3^-$)	Quarternary amine ($-N^+(CH_3)_3$)
Ionic capacity	180 - 250 µmol H^+/ml resin	180 - 250 µmol Cl^-/ml resin
Dynamic binding capacity (DBC)	150 mg BSA/ml resin[2]	47 mg BSA/ml resin[3]
Column volume (CV)	7.9 ml	7.9 ml
Column dimension	10 x 100 mm	10 x 100 mm
Recommended flow rate	2 - 4 ml/min (150 - 300 cm/h)	2 - 4 ml/min (150 - 300 cm/h)
Maximum flow rate[4]	6 ml/min (450 cm/h)	6 ml/min (450 cm/h)
Column hardware pressure limit	2.1 MPa, 21 bar, 305 psi	2.1 MPa, 21 bar, 305 psi
Chemical stability	Compatible with all standard buffers used for protein purification, 1 M NaOH, 30 % isopropanol or 70 % ethanol. Should not be stored at < pH 3 for prolonged time.	
pH stability	3 - 12 (working range) 2 - 13 (cleaning)	3 - 12 (working range) 2 - 13 (cleaning)
Storage	2 to 25 °C in 20% ethanol with 0.2 M sodium acetate	2 to 25 °C in 20% ethanol

1. The median particle size of the cumulative volume distribution.
2. Dynamic binding capacity determined in 20 mM Na-citrate, pH 4.0, at a flow of 2 ml/min (150 cm/h; 4 minutes residence time).
3. Dynamic binding capacity determined in 50 mM Tris-HCl, 50 mM NaCl, pH 8.0, at a flow of 2 ml/min (150 cm/h; 4 minutes residence time).
4. Maximum flow rate for aqueous buffers at 20 °C. 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 for 20% ethanol.

Column description

The column is made from borosilicate glass. The top and bottom filters are polyethylene and the adaptors are PEEK with 10/32 female connection for 1/16" tubing.

The ready-to-use glass columns are delivered with plugs in the inlet and outlet for storage. These columns can easily be connected to a pump or chromatography system using finger tight fittings (coned 10/32) for 1/16" o.d. tubing (standard HPLC PEEK tubing). The design of the columns, including the adaptors, have low dead volume and therefor low band broadening.

The prepacked OptioBio columns for ion exchange chromatography are designed for small scale purification as well as screening and optimization in bioprocess development and scale-up. The columns can be used for easy and reproducible scale-up purification after screening or small-scale purification on prepacked BabyBio™ columns. Alternatively used directly for screening and optimization in bioprocess development.

Applications

IEX principle

Ion exchange chromatography separates biomolecules according to surface charge.

Proteins interact with different affinities with opposite charged groups on the resin.

The interaction depends both on the number of charges involved and on the distribution of the charges on the protein. The surface charge of proteins depends on the pH of their environment. When the pH is equal to the isoelectric point (pI) of the protein the net charge is zero. At pH values below the pI the net charge will be positive, and at a pH above the pI the net charge will be negative. It should be noted that the interaction of the protein depends on the presence and distribution of both positive and negative charged groups on the surface. A protein may therefore also interact with an ion exchange resin at the isoelectric point. The likelihood of binding to either the cation or the anion exchange resin will increase when the pH moves away from the protein pI.

IEX is one of the most frequently used chromatography techniques because of its versatility and ability to separate proteins even with small differences in charge. This feature, in combination with that IEX in binding mode, also works as a concentration step makes it a very efficient tool. IEX is one of the more cost-effective chromatography techniques and it is excellent for scale-up.

For additional information about the ion exchange chromatography principle, see instruction IN 55 410 010.

Protein selectivity

In Figure 2, a set of basic proteins are separated on OptioBio 40S 10x100. In Figure 3, a set of acidic proteins are separated using OptioBio 40Q 10x100.

Column:	OptioBio 40S 10×100
Binding buffer:	50 mM MES, pH 6.0
Elution buffer:	50 mM MES, 1 M NaCl, pH 6.0
Sample:	2.5 ml, 1.5 mg/ml of Concanavalin A, 0.5 mg/ml α-Chymotrypsinogen A, 1.5 mg/ml Ribonuclease A, 0.5 mg/ml Lysozyme in binding buffer
Flow rate:	2 ml/min, 150 cm/h, 4 minutes residence time
Gradient:	0 - 50% elution buffer in 20 column volumes (CV)

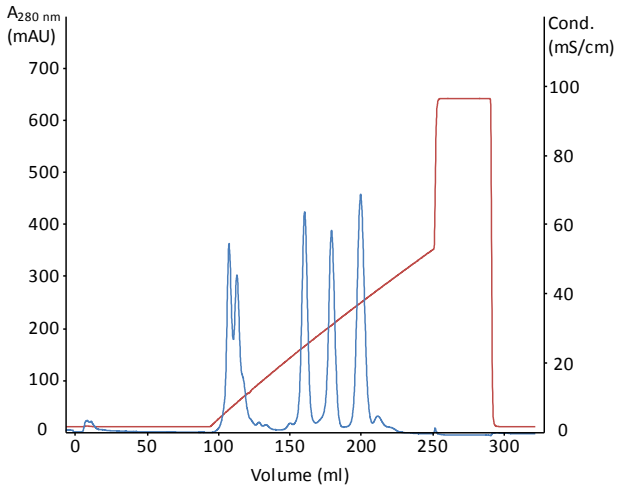


Figure 2. Separation on OptioBio 40S 10×100 prepacked strong cation exchange chromatography column. Peaks from left to right, Concanavalin A, α-Chymotrypsinogen A, Ribonuclease and Lysozyme. The blue line corresponds to the absorbance at 280 nm and the red line to the conductivity.

Column:	OptioBio 40Q 10×100
Binding buffer:	50 mM Tris-HCl, pH 7.4
Elution buffer:	50 mM Tris-HCl, 1 M NaCl, pH 7.4
Sample:	10 ml, 0.7 mg/ml apo-Transferrin, 0.45 mg/ml α-Lactalbumin, 1.4 mg/ml Soybean trypsin inhibitor in binding buffer
Flow rate:	2 ml/min, 150 cm/h, 4 minutes residence time
Gradient:	0 - 40% elution buffer in 20 CV

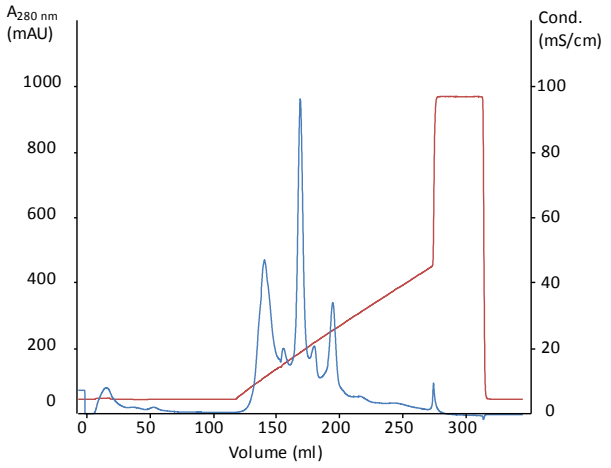


Figure 3. Separation on OptioBio 40Q 10×100 prepacked strong anion exchange chromatography column. Peaks from left to right, apo-Transferrin, α-Lactalbumin and Soybean trypsin inhibitor. The blue line corresponds to the absorbance at 280 nm and the red line to the conductivity.

Flow properties

WorkBeads 40S and WorkBeads 40Q ion exchange chromatography resins are designed for high throughput protein separation under various conditions. The high resolution obtainable even at high protein loadings and high flows makes it ideal for process applications when large volumes are processed.

In Figure 4, the operating pressure-flow curve for OptioBio 40S 10×100 column is shown in comparison with WorkBeads 40S pressure-flow curve in a 25×200 mm glass column with open bed (adaptor not pushed against the bed).

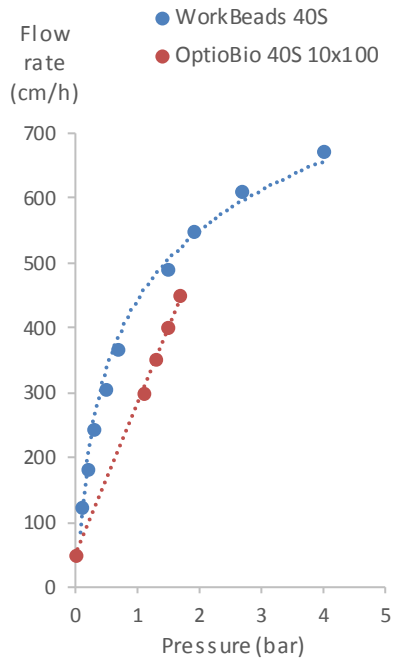


Figure 4. Pressure-flow properties of WorkBeads 40S determined with deionized water. Blue dots open bed in 25×200 mm glass column, red dots operating pressure-flow properties for OptioBio 40S 10×100.

Cleaning-in-place

During purification, impurities such as cell debris, lipids, nucleic acids and protein precipitates from the samples may gradually build up in the resin. Fouling is typical even for well-clarified samples. The severity of this process depends on the composition of the sample applied to the column. These adsorbed impurities will reduce the performance of the packed column over time. Regular cleaning (Cleaning-in-place, CIP) keeps the resin clean, reduces the rate of further fouling, and maintains the capacity, resolution and flow properties of the packed column. Cleaning of the packed column using 1 M NaOH applied by a low flow for 2 hours or overnight is often sufficient. If possible, perform the CIP using reversed flow to release any particles derived from the sample that may have collected on the top filter.

Sanitization (reduction of microorganisms) can be carried out using combinations of NaOH and ethanol (e.g., incubation with a mixture of 0.5 M NaOH and 40% ethanol for 3 hours). The sanitization procedure and its effectiveness will depend on the microorganisms to be removed and needs to be evaluated for each case.

Equipment

The prepacked OptioBio glass columns can be used with most standard liquid chromatography equipment.

Storage

Store the prepacked column at 2 to 25 °C in 20% ethanol. For OptioBio 40S 10×100 it is recommended to use 0.2 M sodium acetate and 20% ethanol. Make sure that the column is securely closed.

Related products

Product name	Pack size[1]	Article number
Prepacked columns		
BabyBio S 1 ml	1 ml × 5	45 200 103
BabyBio Q 1 ml	1 ml × 5	45 100 103
BabyBio DEAE 1 ml	1 ml × 5	45 150 103
BabyBio Dsalt 5 ml	5 ml × 5	45 360 107
Bulk resins		
WorkBeads 40S	25 ml	40 200 001
	200 ml	40 200 002
	1 L	40 200 010
	5 L	40 200 050
WorkBeads 40Q	25 ml	40 100 001
	200 ml	40 100 002
	1 L	40 100 010
	5 L	40 100 050
WorkBeads 40 DEAE	25 ml	40 150 001
WorkBeads 100S	25 ml	10 200 001
WorkBeads 100Q	25 ml	10 210 001

1. Other pack sizes can be found in the complete product list on our website www.bio-works.com.

Ordering information

Product name	Pack size	Article number
OptioBio 40S 10×100	7.9 ml × 1	55 420 011
OptioBio 40Q 10×100	7.9 ml × 1	55 410 011

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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