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

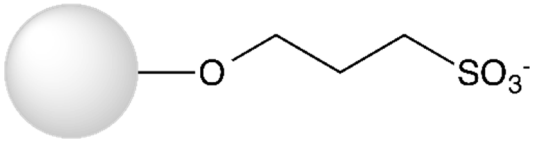
WorkBeads are agarose-based chromatographic resins manufactured using a proprietary method that results in porous beads with a tight size distribution and exceptional mechanical stability. Agarose based matrices have been successfully used for decades in biotechnology purification, from research to production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

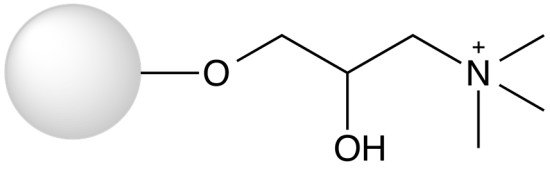


Figure 1. Structure of the ligand used in (A) WorkBeads 100S and (B) WorkBeads 100Q.

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)

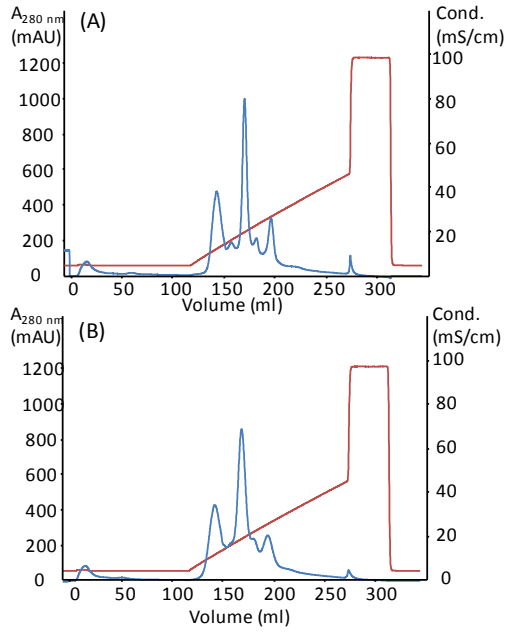


Figure 2. Chromatogram from resolution comparison between WorkBeads 40Q (A) and WorkBeads 100Q (B).

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

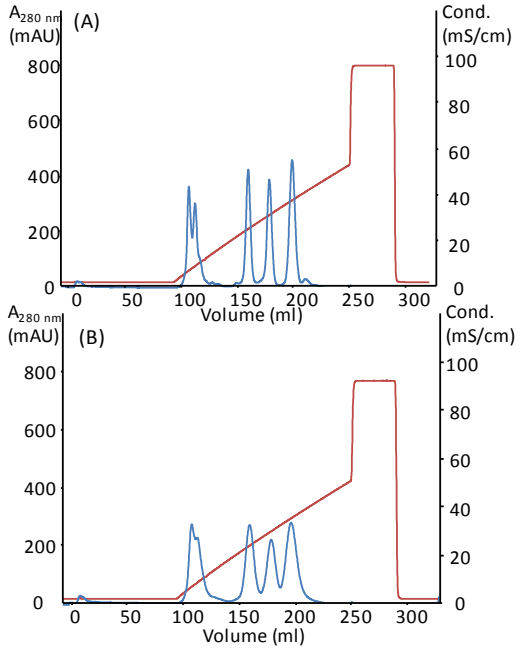


Figure 3. Chromatogram of selectivity on two different cation exchange resins, WorkBeads 40S (A) and WorkBeads 100S (B).

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.

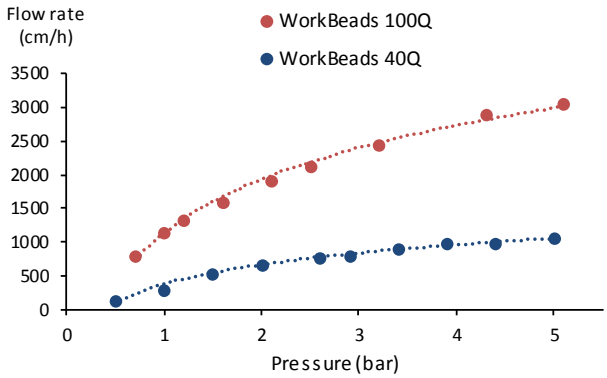


Figure 4. Pressure-flow properties of WorkBeads 100Q and WorkBeads 40Q. The data was obtained using distilled water passed through a 10 mm (i.d.) × 200 mm bed of resin in a glass column. The bed was open, i.e., the top adaptor was not pushed towards the chromatography bed.

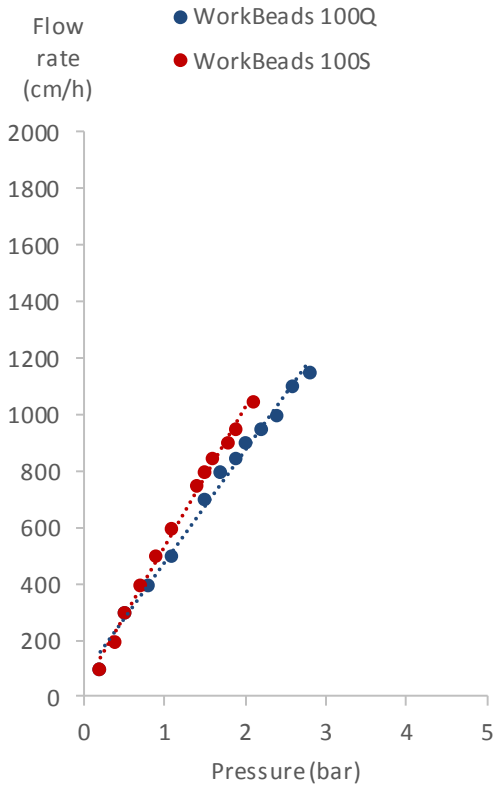


Figure 5. Pressure to flow rate properties of WorkBeads 100Q and WorkBeads 100S. Pressure-flow properties obtained in distilled water, glass column dimension of 20 cm bed height, diameter 25 mm.

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

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