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.

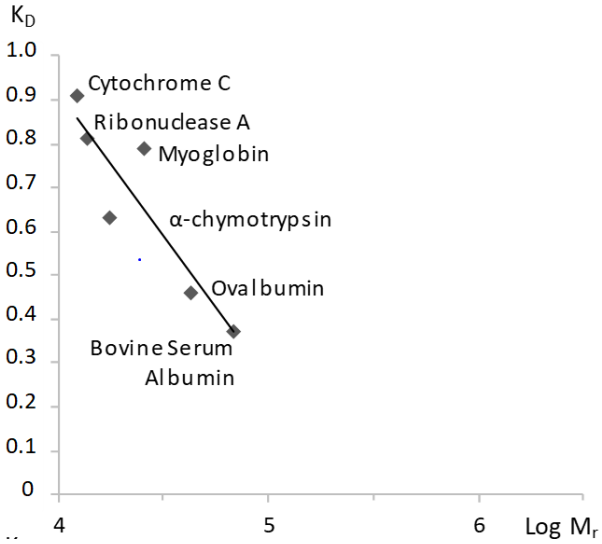
We recommend not to use Blue Dextran as a molecular weight marker as it may cause unspecific binding to the resin.

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)

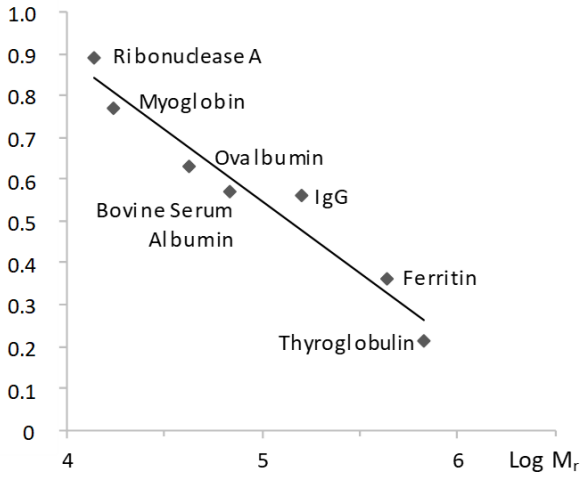
Samples (A): 5 mg/ml Hemocyanin Keyhole Limpet (HKL), M_r approx. 8 000 000
5 mg/ml thyroglobulin (bovine thyroid), M_r 669 000
5 mg/ml bovine serum albumin (BSA), M_r 66 500
5 mg/ml ovalbumin, M_r 43 000
5 mg/ml α-chymotrypsin (bovine pancreas), M_r 25 656
1.5 mg/ml myoglobin (equine skeletal muscle), 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



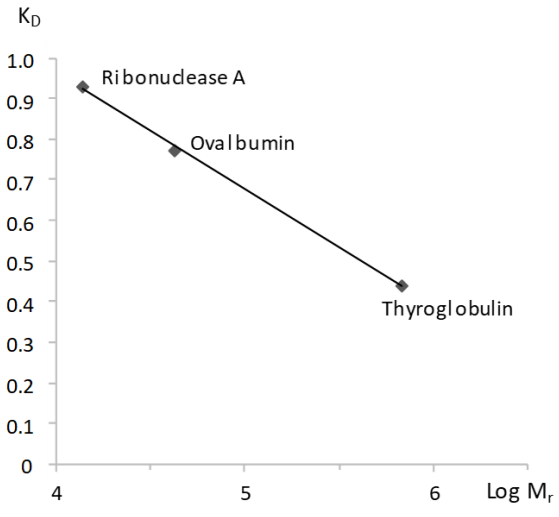
Samples (B): 5 mg/ml Hemocyanin Keyhole Limpet (HKL), M_r approx. 8 000 000
5 mg/ml thyroglobulin (bovine thyroid), M_r 669 000
1.5 mg/ml ferritin (equine spleen), M_r 440 000
6 mg/ml human polyclonal IgG, M_r 150 000
5 mg/ml bovine serum albumin (BSA), M_r 66 500
5 mg/ml ovalbumin, M_r 43 000
1.5 mg/ml 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

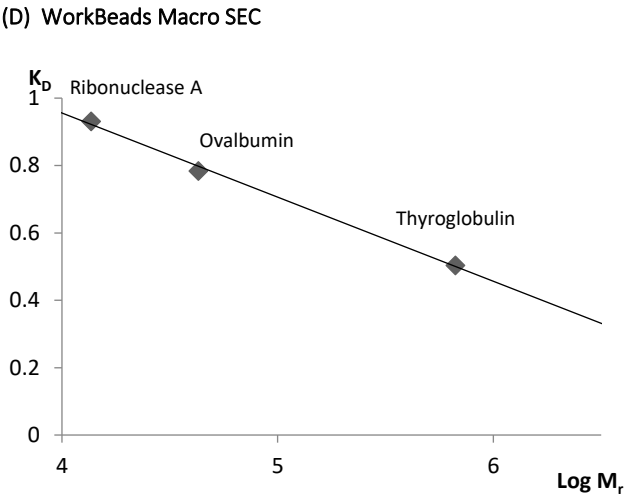


Samples (C): 5 mg/ml Hemocyanin Keyhole Limpet (HKL), M_r approx. 8 000 000
5 mg/ml thyroglobulin (bovine thyroid), M_r 669 000
5 mg/ml ovalbumin, M_r 43 000
15 mg/ml ribonuclease A (bovine pancreas), M_r 13 700
10% (v/v) acetone in distilled water, M_r 58.08

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

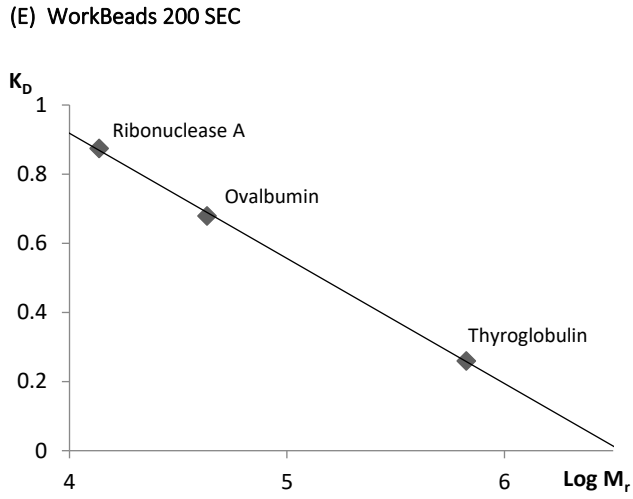


Figure 1. K_D-log M_r plots. Standard proteins applied on a 10 x 300 mm column packed with (A) WorkBeads 40/100 SEC, (B) WorkBeads 40/1000 SEC, (C) WorkBeads 40/10 000 SEC and (D) WorkBeads Macro SEC. (E) WorkBeads 200 SEC is packed in a 16 x 900 mm column.

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

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