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)

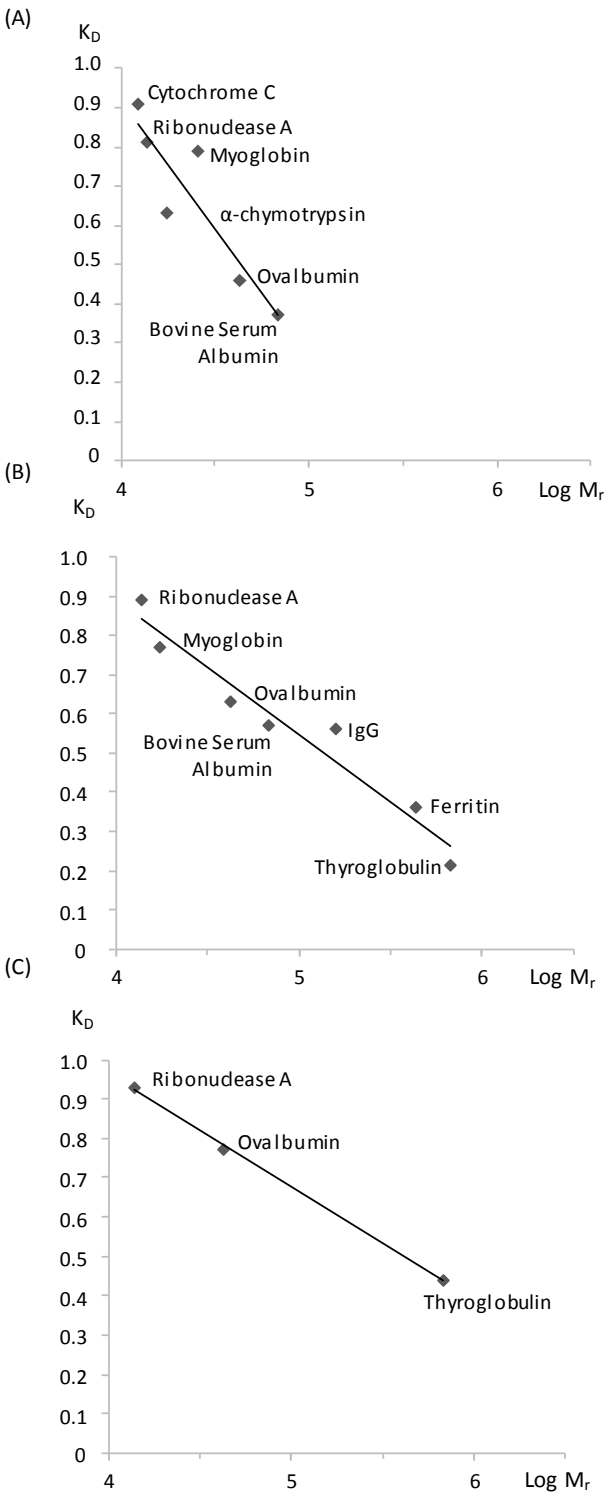

Figure 1. K_D-log M_r plots. Standard proteins applied on a 10x300 mm column packed with (A) WorkBeads 40/100 SEC, (B) WorkBeads 40/1000 SEC and (C) WorkBeads 40/10 000 SEC.

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

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