Bio-Works

INSTRUCTION

WorkBeads SEC resins

WorkBeads 40/100 SEC, WorkBeads 40/1000 SEC,
WorkBeads 40/10 000 SEC and WorkBeads Macro SEC

WorkBeads™ 40/100 SEC, WorkBeads 40/1000 SEC, WorkBeads 40/10 000 SEC and WorkBeads Macro SEC resins are used for preparative size exclusion chromatography (SEC) in laboratory and process scale purification of proteins, virus and other biomolecules by utilizing the differences in their size. These resins are based on agarose, which is a biopolymer suitable for separation of biomolecules. WorkBeads resins are cross-linked using a proprietary method that results in a very rigid structure. Although the general recommendation for SEC is to use low flow rate for best purification, the rigidity and tight particle size distribution allow 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
- Resistant to harsh cleaning agents (NaOH)

Intended use

WorkBeads resins are developed and supported for both research and production-scale chromatography. WorkBeads resins are produced according to ISO 9001:2015, and Regulatory Support Files (RSF) are available to assist the process validation and submissions to regulatory authorities.

GoBio™ prepacked column family is developed for convenient, reproducible, and fast results and can be used from small scale purification through process development to full-scale manufacturing.

Safety

Please read the associated Safety Data Sheets (SDS) for WorkBeads 40/100 SEC, WorkBeads 40/1000 SEC, WorkBeads 40/10 000 SEC and WorkBeads Macro SEC and the safety instructions for any equipment to be used.

Unpacking and inspection

Unpack the shipment as soon as it arrives and inspect it for damage. Promptly report any damage or discrepancies to complaints@bio-works.com

Short protocol

This short protocol is for column packing and running WorkBeads 40 SEC resins. Detailed instructions and recommendations for optimization are given later in this instruction. Recommended flow rates are listed in Table 1 and examples of running buffers are listed in Table 2.

1. Make a slurry of the desired resin concentration.
2. Pour the slurry into the column.
3. Pack the resin with an appropriate flow rate.
4. Apply an axial compression of less than 2%.
5. Equilibrate the column with running buffer.
6. Apply sample.
7. Elute the target protein with running buffer.
8. Wash the column with deionized water.
9. Equilibrate the column with 20% ethanol for storage.

Principle

Size Exclusion Chromatography (SEC) is a simple and reliable technique for separation of molecular components according to their size. The technique relies on a separation resin of porous beads, packed closely together in a column. The different WorkBeads 40 SEC products have different pore size distribution, which give them different size separation ranges. The packed column is prepared by equilibration with a suitable running buffer, usually an aqueous buffer, before sample loading.

In SEC, large substances in the applied sample will elute earlier than small molecules, since large substances do not enter in the pores of the beads or they can only enter a fraction of the pores. Smaller substances can enter a larger fraction of the pores and they will therefore elute later. Intermediate size substances will enter the bead pores to different degrees, thus being eluted at different elution volumes. The eluent (buffer) that is used for equilibration of the column will enter completely into the pores of the beads. This means that the substances to be separated will be eluted in the eluent used for equilibration, thus allowing buffer exchange or salt removal (or addition). In addition to size, other characteristics e.g., shape, charge, hydrophobicity and interactions between substances or substances and resin, may affect the elution volume, thus the separation. Optimization of chromatography conditions (buffer composition, flow and sample volume) may be needed to obtain the required purification. The column is prepared for the next run by passing at least one column volume (CV) of buffer through the column. This will also ensure removal of possible remaining low molecule weight substances from the sample (e.g., salt, buffer, and low M_r impurities).

SEC is usually applied as the last purification step (polishing step) since it is limited in flow and sample volume, and since it allows removal of aggregates of the target substance, buffer exchange and salt removal.

Column packing

Follow both this general advice when packing a column and the column manufacturer's instructions. Preferably, use a column with an adjustable adaptor. In some instances, a packing reservoir or column extension may be needed. For standard SEC purification we recommend packing columns from 10 × 300 – 600 mm, 16 × 600 – 900 mm, 26 × 600 – 900 mm, and larger. Very large substances, e.g., viruses are eluted in the void volume and can be separated from substances (proteins and other impurities) that enter the bead pores, by using shorter columns, e.g., 200 – 300 mm bed heights.

Note: Always make sure that the maximum pressure of the column hardware is not exceeded. Backpressure caused by the chromatography system components connected downstream of the column may reduce the maximum flow that can be used. Wear eye protection.

1. Wash the resin

The resin is provided in 20% ethanol (in special cases 0.4% NaOH). To avoid undue backpressure when packing, wash the desired amount of resin with several column volumes of deionized water before packing.

2. Make a slurry

Add deionized water to the washed resin to obtain a 50% to 70% slurry concentration. Make approximately 15% extra slurry to compensate for the compression during packing. The amount of slurry to be prepared can be calculated according to:

$$\text{Slurry volume} = \frac{\text{bed volume} \times 100}{\%\ \text{slurry}} \times 1.15$$

3. Pour the slurry into the column

Pour the slurry slowly down the side of the column to avoid formation of air bubbles. Preferably, use a packing reservoir or an extra column tube (and connector between the columns) to extend the column volume to accommodate the entire slurry volume during packing.

4. Pack the bed

Pack the resin with a downward flow higher than the intended operational flow. We recommend 100 – 300 cm/h. A lower packing flow tend to give better column efficiency. Make sure the packing flow rate does not exceed the maximum pressure of the column hardware or the resin. The intended operational flow should not be more than 75% of the packing flow rate. For high-flow applications in shorter columns (200 – 300 mm bed height) a packing flow rate of 600 cm/h may be used.

5. Close the column

When the bed height is constant mark the bed height on the column. Stop the flow and remove the packing reservoir or extra tube. Note that the bed height will increase temporarily when the flow is stopped. If needed, adjust the bed height by removing excess resin. Be careful not to remove too much resin. Gently fill the column with packing solution to its rim without disrupting the packed bed. Open the adaptor inlet if needed to let packing solution out during insertion of the adapter on top of the packed bed. Adjust the adaptor to the mark. Apply a small axial compression of less than 2% of the final bed height by lowering the adapter further below the mark.

6. Apply a flow

Apply the operating flow of 25 – 50 cm/h or higher (e.g., 50 – 250 cm/h) in a high-flow application (taking in account section 4). Check for any gap formation above the surface of the resin bed. If a gap is observed, stop the flow and adjust the adaptor to eliminate the gap.

Evaluation of the packed column

Test the column efficiency to check the quality of packing. Testing should be done after packing, at regular intervals during the usage of the column or when separation performance is seen to deteriorate.

The best method of expressing the efficiency of a packed column is in terms of the height equivalent to a theoretical plate (HETP) and the asymmetry factor (A_s). These values are easily determined by applying a sample such as 1% acetone solution to the column.

For optimal results, the sample volume should be 2.5% of the column volume (CV) and the flow rate 30 cm/h.

If an acceptance limit is defined in relation to column performance, the column plate number can be used as one of the acceptance criteria for column use.

Note: The calculated number of plates will vary according to the test conditions and should only be used as a reference value. Keep test conditions and equipment constant so that results are comparable. Changes of for example solute, solvent, eluent, sample volume, flow velocity, liquid pathway, temperature, etc. will influence the results.

Measuring HETP and A_s

Calculate HETP and A_S from the UV curve (or conductivity curve).

$$HETP = \frac{L}{N}$$

$$N = 5.54 \times \left(\frac{V_R}{W_h}\right)^2$$

L = bed height (cm)

N = number of theoretical plates

V_R = volume eluted from the start of sample application to the peak maximum

W_h = peak width measured as the width of the recorded peak at half of the peak height

V_R and W_h are in the same units

The concept of reduced plate height is often used for comparing column performance. The reduced plate height, h, is calculated:

$$h = \frac{HETP}{d_{50v}}$$

d_{50v} = Median particle size of the cumulative volume distribution (cm)

As a guideline, a value of < 3 is very good.

The peak must be symmetrical, and the asymmetry factor as close to 1 as possible. (A typical acceptable range is $0.7 < A_S < 1.3$). A change in the shape of the peak is usually the first indication of bed deterioration.

Peak asymmetry factor calculation:

$$A_s = \frac{a}{b}$$

a = ascending part of the peak width at 10% of peak height

b = descending part of the peak width at 10% of peak height

Figure 1 below shows a UV trace for acetone in a typical test chromatogram from which the HETP and A_s values are calculated.

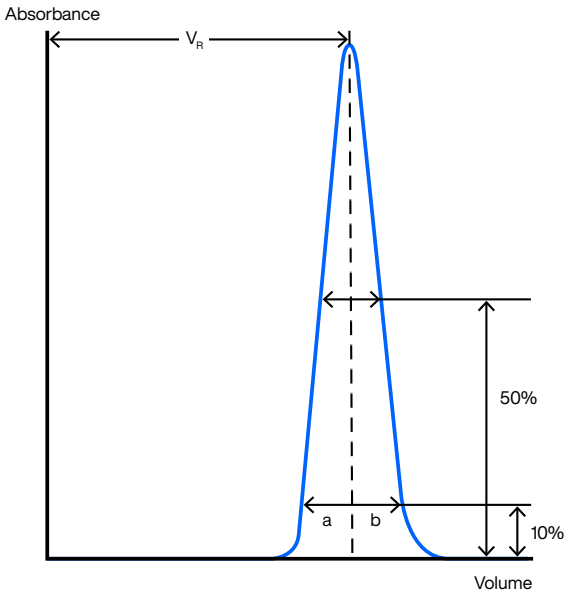

Figure 1. A typical test chromatogram showing the parameters used for HETP and A_s calculations.

Purification

SEC purifications can often be done under standard recommended conditions, see recommended flow rates in Table 1 and buffer conditions in Table 2. Although this may be a good starting point for testing, optimization may be needed to obtain desired results. One of the most important parameters that affects resolution is the flow rate (especially for large proteins). The goal for most separations is to achieve the required resolution in the shortest possible time. If the analysis shows low resolution between protein peaks, the first action should be to set a lower flow rate for the run.

Generally, a lower flow rate will allow time for molecules to diffuse in and out of the chromatography matrix and improve resolution. The effect is most pronounced for large molecules. Decreasing the flow rate can, on the other hand, negatively impact on resolution for very small molecules. Several other factors, such as sample complexity, resin separation range, column bed height, packing quality and sample volume are also influencing the resolution.

Table 1. Recommended operating flow rates.

Resin	Recommended flow rate (cm/h)
WorkBeads 40/100 SEC	15 – 150
WorkBeads 40/1000 SEC	15 – 150
WorkBeads 40/10 000 SEC	15 – 150
WorkBeads Macro SEC	15 – 150

Table 2. Recommended buffer compositions.

Buffer
20 mM sodium phosphate, 150 mM NaCl, pH 7.4 (PBS)
50 mM sodium phosphate buffer, pH 7.0
20 mM Tris-HCl, 100 mM NaCl, pH 8.0

Equilibration

Before purification the column must be equilibrated with a suitable buffer (eluent). First the storage solution is removed by applying 0.2 column volumes (CV) of water, then with 1 – 2 CV of running buffer. It is recommended to monitor absorbance at 280 nm (A_{280}), conductivity and pH of the column outlet. Stable signals are a strong indication of completed equilibration. Equilibration can be done at elevated flow, although may then require a slightly bigger volume to establish equilibrium.

Sample preparation

After cell disruption or extraction, clarify the sample by centrifugation at 10 000 – 20 000 × g for 15 – 30 minutes. It is generally recommended also to pass the sample through a 0.22 – 0.45 µm filter (e.g., a syringe filter) to avoid inadvertently applying any remaining particles onto the column. If the sample contains only small amounts of particles, centrifugation may be omitted, and it is enough to only carry out filtration. Application of a sample that has not been properly clarified may reduce the performance and lifetime of the column.

Note: We recommend not to use Blue Dextran as a molecular weight marker due to it may give unspecific binding to the resin.

Sample application

For preparative purifications it is recommended to apply sample volumes of 1 – 4 % of the column volume, e.g., up to 4.6 mL on a 120 mL column.

Elution

The elution is done with the same buffer as used for equilibration. Normally, all sample components are eluted within 1 CV. If interactions between the sample components and the resin occurs, the elution volume can be larger than 1 CV. It is therefore recommended to use 1.3 CV for elution. If an additional purification will be done using the same sample and separation conditions, the column can be re-equilibrated with a small volume, e.g., 0.5 CV, before application of the next sample.

Preparation for storage

Wash the column with 0.5 CV water, then with 1.5 CV 20% ethanol or other desired storage solution.

Cleaning-in-place, CIP

After elution, apply 1 CV 1 M NaOH over 1 – 2 hours.

Optimization

The conditions used for SEC purifications may have to be optimized to give the optimal results. Below follow some recommendations.

Sample

Most sample compositions can be applied to the equilibrated column. The components of the sample will quickly move into the buffer that was used for equilibration, since the components to be separated move much faster than the buffer through the column. Very high viscosity of the sample may give zone broadening, thus reduced separation, e.g., protein concentrations of more than approx. 70 mg may negatively affect the separation. A recommendation is to slightly dilute the sample before loading but keep track of the sample volume.

Sample volume

Resolution, i.e., separation between the peaks, depends on the sample volume loaded on the column. Sample volumes of 1% – 4% of the total column volume is recommended. Smaller sample volumes 0.25% – 1 % of CV may be used to further improve resolution.

Notice that application of very small amounts of sample may compromise the sample yield due to inevitable adsorption of minute amounts of material to the resin during separation. In special applications where there is a large difference in elution volume between the sample components to be separated, the sample volumes can be as much as 25% of the column volume.

Resin bed height

A longer bed height will increase the resolution. Normally a bed height of 600 – 900 mm is recommended for preparative size exclusion chromatography. For small-scale purifications a 10 × 300 mm column may be enough for the required separation.

Flow rate

Optimization of the resolution can be carried out by lowering the flow rate. Optimum flow rate for proteins is often approx. 15 – 150 cm/h. A higher flow rate decreases the resolution. The selection of flow rate in SEC is often a trade-off between resolution and purification time. For purifications of large substances that are eluted in the void volume, a higher flow rate can often be used.

Buffer composition

It is recommended to use buffers with slightly elevated ionic strength (or conductivity, salt concentration) to mask electrostatic interactions between the resin and the substances to be separated. Addition of 150 mM NaCl to any buffer is a general recommendation.

Separation can generally be done over a broad range of pH from 3 to 10. However, extreme pH and high salt concentrations may affect the structure of the proteins, or target molecules. Selection

of buffer should be done within the stability window of the target substance, and to avoid any aggregation during separation.

Additives such as 1 – 10% glycerol, detergents, 0.5% arginine, or denaturants such as urea and guanidine-HCl are sometimes added to the buffer for stabilisation or to keep the target soluble during separation.

Scale-up

Scale-up principles

During scale-up the ratio between sample volume and column volume should be kept constant. The column volume is scaled up by increasing the column diameter while keeping the bed height the same (e.g., 600 mm). The linear flow rate should remain the same while the volumetric flow rate increases. The volumetric flow rate for each column can be calculated according to:

$$\text{Volumetric flow rate (mL/min)} = \frac{\text{Linear flow rate (cm/h)} \times \text{Column cross sectional area (cm}^2\text{)}}{60}$$

Flow

The concepts of volumetric flow, linear flow rate and residence time is important when doing scale-up in chromatography. Volumetric flow is measured in mL/min or L/min, linear flow in cm/h and residence time in minutes. The relationship between these metrics is:

$$\text{Linear flow rate (cm/h)} = \frac{\text{Volumetric flow (mL/min)} \times 60}{\text{Column cross sectional area (cm}^2\text{)}}$$

$$\text{Residence time (minutes)} = \frac{\text{Column bed height (cm)} \times 60}{\text{Linear flow rate (cm/h)}}$$

If a smaller column has been used, the flow rate for the larger column can be calculated from the flow that was established on the small column, using the equation above by keeping the residence time of the small column the same for the larger column. This will allow an increase of the linear flow in proportion to the increase in bed height between the columns. If the column bed heights are kept constant during scale-up the linear flow rate should be kept constant (as well as the residence time).

Combining chromatography techniques for optimized purification

We recommend a purification strategy based on three phases: Capture, Enhance and Polish. In the capture phase, usually one purification step, it is common to use affinity chromatography or ion exchange chromatography, to remove bulk impurities and to concentrate and stabilize the target substance. The Enhance phase (one or several purifications steps) aims at further removing impurities. The Polish phase aims at removing any final impurities and adjusting the conditions of the product to be suitable for subsequent use. If the capture is performed using a selective enough method, the enhance phase may be omitted and it may be enough with a polish step.

The SEC technique is usually best applied as a polish step since it is limited in sample volume, and since it in addition to removal of impurities allows removal of co-purified aggregates of the target, and since it also may allow modification of the conditions (e.g., buffer exchange or salt removal). The technique can also be useful earlier in the purification scheme in certain applications, depending on sample and purification goals.

To find out more about Bio-Works' chromatography products visit www.bio-works.com

Maintenance of the resin

Cleaning

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 been 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.

Storage

Store at 2 to 25°C in 20% ethanol.

Note: Use a reduced flow rate during equilibration with 20% ethanol, maximum 50% of the maximum flow rate.

Product description

	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 (D_{V50})[2]	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.

GoBio prepacked column family

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

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

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

GoBio Prep 16x100 (20 mL) and GoBio Prep 26x100 (53 mL) for lab-scale purifications and scaling up.

GoBio Prep 16x600 (120 mL) and GoBio Prep 26x600 (320 mL) for preparative lab-scale size exclusion chromatography.

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

Related products

Product name	Pack size[1]	Article number
Prepacked columns		
GoBio Mini Dsalt 1 mL	1 mL × 5	45 360 103
GoBio Mini Dsalt 5 mL	5 mL × 5	45 360 107
GoBio Prep 16x600 40/100 SEC	120 mL × 1	55 434 026
GoBio Prep 26x600 40/100 SEC	320 mL × 1	55 434 036
GoBio Prep 16x600 40/1000 SEC	120 mL × 1	55 430 026
GoBio Prep 26x600 40/1000 SEC	320 mL × 1	55 430 036
GoBio Prep 16x600 40/10 000 SEC[2]	120 mL × 1	55 435 026
GoBio Prep 26x600 40/10 000 SEC[2]	320 mL × 1	55 435 036
GoBio Prep 16x600 Macro SEC[2]	120 mL × 1	55 437 026
GoBio Prep 26x600 Macro SEC[2]	320 mL × 1	55 437 036
GoBio Prep 16x600 200 SEC[2]	120 mL × 1	55 230 027
GoBio Prep 26x600 200 SEC[2]	320 mL × 1	55 230 037
Bulk resins		
WorkBeads 200 SEC	300 mL 1 L 5 L 10 L	20 300 003 20 300 010 20 300 050 20 300 060
WorkBeads Dsalt	300 mL 1 L 5 L 10 L	40 360 003 40 360 010 40 360 050 40 360 060

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

Ordering information

Product name	Pack size	Article number
WorkBeads 40/100 SEC	25 mL 300 mL 1 L 5 L 10 L	40 340 001 40 340 003 40 340 010 40 340 050 40 340 060
WorkBeads 40/1000 SEC	25 mL 300 mL 1 L 5 L 10 L	40 300 001 40 300 003 40 300 010 40 300 050 40 300 060
WorkBeads 40/10 000 SEC	25 mL 300 mL 1 L 5 L 10 L	40 350 001 40 350 003 40 350 010 40 350 050 40 350 060
WorkBeads Macro SEC	25 mL 300 mL 1 L 5 L 10 L	40 370 001 40 370 003 40 370 010 40 370 050 40 370 060

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.com

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