Bio-Works

INSTRUCTION

WorkBeads 40 Butyl SH

WorkBeads™ 40 Butyl SH is a resin for hydrophobic interaction chromatography (HIC) designed for research and industrial scale purification of proteins, peptides, plasmids, and oligonucleotides by utilizing the difference in their surface hydrophobicity. HIC is a commonly used chromatography technique that can be optimized to achieve excellent separation of different molecules. It's often used to complement other techniques that separate according to either charge or size.

The functional ligand of WorkBeads 40 Butyl SH is *n*-butyl thioether. Since butyl is a very hydrophobic linear chain, minimal mixed-mode interactions are expected. The resin is optimized to offer reliable binding performance.

The property of high-resolution separation, in combination with low backpressure, allows both capture and polishing purification applications in standard bioprocess columns.

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

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 in the process validation and submissions to regulatory authorities.

The GoBio™ prepacked column family has been developed for convenient, reproducible, and rapid results and can be used for small-scale purification, extending all the way to process development and full-scale manufacturing.

Safety

Please read the Safety Data Sheets (SDS) for WorkBeads 40 Butyl SH 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 packing columns with WorkBeads 40 Butyl SH. Detailed instructions and recommendations for optimization are given later in this document. Recommended buffers are listed in Table 1.

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 5%.
5. Equilibrate the column with a binding buffer.
6. Apply sample.
7. After sample application, remove unbound material by washing with, for example, 10 – 20 column volumes (CV) of binding/washing buffer.
8. Elute the target molecule with elution buffer.
9. Wash the column with 0.02 M NaCl before applying the cleaning-in-place solution.
10. Perform cleaning-in-place (CIP) and wash with 0.02 M NaCl solution.
11. Equilibrate the column with 20% ethanol for storage.

Principle

Hydrophobic interaction chromatography (HIC) separates molecules according to differences in their surface hydrophobicity through a reversible interaction between the molecules and the hydrophobic surface of the HIC resin. A high salt concentration enhances the interaction, and a low salt concentration weakens the interaction. The extent of the reversible interaction between the molecule and the hydrophobic surface of a HIC resin depends on the properties of the HIC resin and target molecule, and the running conditions, such as the salt concentration. The principle for molecule adsorption to HIC resins is orthogonal to ion exchange and size exclusion chromatography.

In HIC, the molecules to be separated are usually loaded onto the column under conditions of a high salt concentration, which promotes the exposure of hydrophobic regions and increased hydrophobic interactions. The more hydrophobic the molecule, the less salt is needed to promote binding. As the sample is applied, molecules with higher hydrophobicity tend to bind more strongly to the hydrophobic ligands. In contrast, less hydrophobic molecules will bind less strongly, and molecules with minor hydrophobicity will even pass through the column (or elute in the flow through).

To elute the bound molecules, a decreased salt gradient is typically applied, which reduces the hydrophobicity of the molecules and the hydrophobic ligands, allowing them to be eluted in order of decreasing hydrophobicity. Elution can also be achieved by a stepwise decrease of salt in the elution buffer.

Anti-chaotropic salts, like ammonium sulfate, enhance molecule binding to hydrophobic surfaces. Sample elution can be facilitated by adding mild organic modifiers or detergents to the elution buffer. Commonly, ammonium sulfate at a neutral pH (1-2 M) is used for binding, while sodium chloride may require higher molarity (up to 3 M). However, process optimization is crucial, considering factors from resin to running conditions, to achieve the desired purity and yield of the target molecule.

Binding conditions play a crucial role in HIC separation, impacting selectivity, resolution, and capacity. Samples should be in the same salt conditions as the binding buffer. Buffer exchange may be unnecessary, as the influence of buffer ions and pH tends to be less prominent in many cases. Adjust pH directly if needed. Given that increased salt concentrations can lead to the precipitation of many molecules, it is crucial to assess the stability range of the target molecule at various salt concentrations before optimizing binding conditions. A practical method for determining the stability range is to observe the sample in a test tube at different salt concentrations and monitor the activity of the target molecule left in the supernatant.

Variations in ionic strength, organic solvents, temperature, and pH (especially at the isoelectric point, pI) can influence the structure and solubility of the molecule, impacting its interaction with HIC resins.

The functional ligand of WorkBeads 40 Butyl SH is *n*-butyl thioether, shown in Figure 1.

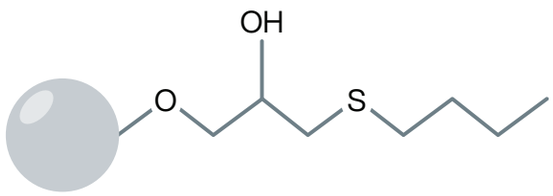

Figure 1. Structure of the ligand used in WorkBeads 40 Butyl SH.

Column packing

Columns with i.d. ≤ 10 mm

WorkBeads resins are cross-linked using a proprietary method that results in a very rigid resin that tolerates pressures of several bars and consequently can run at high flow rates. Follow both this general advice when packing a column and the column manufacturer's specific instructions. Preferably, use a column with an adjustable adaptor. In some instances, a packing reservoir or column extension may be needed.

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.

For laboratory-scale columns, we recommend a so-called single-step flow packing procedure since the contribution of the wall support is significant. A single-step flow packing is fast and easy to implement. The wall support phenomena are described in the next section.

1. Wash the resin

WorkBeads 40 Butyl SH resin is supplied in 20% ethanol. Wash the desired amount of resin with several column volumes (CV) of packing buffer before packing.

Note that a higher backpressure will be obtained when using ethanol in the packing solution due to its increased viscosity.

2. Make a slurry

Add packing solution to the washed resin to obtain a 40% to 60% 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:

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

The concentration of slurry can be determined using different methods. One such method is the centrifugation method: Transfer homogenized slurry to a graded centrifuge test tube. Centrifuge for 3 minutes at 3000 rpm and determine the relation between total volume and resin volume. If the relation is not within the desired range, modify the slurry concentration of the resin to be packed by either adding or removing the packing solution.

Note: If there is a limitation on the total slurry volume, slurry concentrations up to 70% can be used to compensate for the lower volume.

Note: Different packing solutions can be used. We recommend using a solution exhibiting some conductivity, such as 0.02 – 0.4 M NaCl in water or in 20% ethanol. Alternatively, columns can be packed using 20% ethanol alone without the need for added salt. Due to its high hydrophobicity, WorkBeads 40 Butyl SH is not completely compatible with pure water, particularly noticeable at larger scales.

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 to extend the column volume to accommodate the entire slurry volume during packing. If no packing adaptor is available packing can be done by stepwise additions and packing. Although not recommended this will give acceptable results for most applications.

4. Pack the bed

Pack the resin with a downward flow higher than the intended operational flow. We recommend 600 cm/h for columns up to 10 mm i.d. Make sure the packing flow rate does not exceed the maximum pressure of the column hardware or the resin. The operational flow should not be more than 75% of the packing flow rate.

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

$$\text{Column cross sectional area (cm}^2\text{)} = \frac{\pi \times d^2}{4}$$

d = column inner diameter in cm

After flow compression and before mechanical compression, the bed height can be adjusted (i.e., using a spatula) so that the exact bed height is achieved, but do this with care since it is important not to disturb the flow compressed bed left in the column.

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 but be careful not to remove too much. Gently fill the column with packing solution to its rim without disrupting the packed bed. Insert the adjustable adapter on top of the packed bed. Apply an axial compression of less than 5% of the final bed height by lowering the adapter into the packed bed.

6. Apply a flow

Apply a flow of 450 cm/h (taking account of section 4) and check for any gap formation above the Apply a flow corresponding to 75% of the packing flow and 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.

Columns with i.d. > 10 mm

During scale-up, the phenomena of wall support gradually decrease. Wall support means that the column wall supports the resin bed and gives it better flow properties. This mainly occurs when the column's inner diameter is smaller, i.e., ≤ 10 mm. To accommodate for the lack of wall support in wider columns, we recommend a two-step flow packing procedure. The bed should first be consolidated at a low flow rate to ensure the bed is optimally settled (60 cm/h for at least 1.5 CV). After the initial consolidation step, the bed is further compressed at a higher flow rate (300 – 450 cm/h depending on restrictions for at least 1.5 CV). When the bed has been flow packed, a mechanical compression of 10 – 20% can be performed.

The ideal mechanical compression to use will vary based on the column dimensions and type of resin. Therefore, it's crucial to assess the packed column before use (asymmetry and plate number tests as described below).

We recommend using a solution exhibiting some conductivity, such as 0.02 – 0.4 M NaCl in water or in 20% ethanol. Alternatively, columns can be packed using 20% ethanol alone without the need for added salt. When packing larger scale columns, the backpressure may be a limiting factor, making 0.02 – 0.4 M NaCl in water a more suitable packing solution.

Note: After flow compression and before mechanical compression, the bed height can be adjusted (i.e., using a spatula) so that the exact bed height is achieved, although this isn't recommended at larger scales. There is a guide available for large-scale packing at www.bio-works.com/packing-guide-download

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 for WorkBeads 40 resins, the flow rate should be 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 2 below shows a UV trace for acetone in a typical test chromatogram from which the HETP and A_s values are calculated.

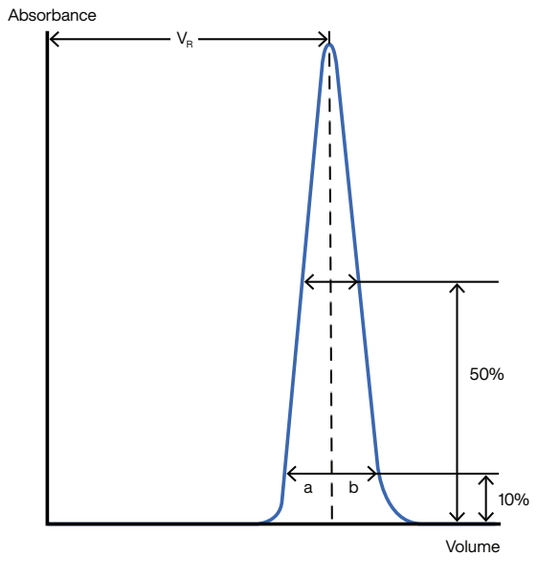

Figure 2. A typical test chromatogram showing the parameters used for HETP and A_s calculations.

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 also recommended to pass the sample through a 0.22 – 0.45 µm filter to avoid transferring any remaining contaminating particles onto the column. The application of a sample that has not been properly clarified may reduce the performance and lifetime of the column.

Adjust the sample to the salt concentration of the binding buffer to promote hydrophobic interaction. If possible, use a high-concentration stock solution for this adjustment to avoid precipitation. Adjust the pH of the sample directly to pH 5.0 – 8.5, depending on the sample. Performing a buffer exchange might not be necessary, as the influence of buffer ions and pH tends to be less prominent in certain cases.

Purification

As temperature is a parameter that can affect separations using HIC, keep sample, binding and elution buffers, columns, and chromatographic equipment at the same, constant temperature throughout the purification to ensure consistent and reproducible results.

1. Prepare binding and elution buffers, see Table 1 for typical buffers.
2. Wash the column with 3 – 5 column volumes (CV) low-salt elution buffer.
3. Equilibrate with 5 CV binding buffer.
4. Apply the sample.
5. Wash with 10 – 20 CV binding buffer or until no material appears in the eluent. Note: Do not have a too short wash step since some molecules elute late in the wash step since they are partially retained on the resin.

6. Carry out intermediate wash steps with 5 – 10 CV with partially reduced salt concentration if needed.
7. Elute bound material, preferably with a linear gradient for 10 – 20 CV.
8. A cleaning-in-place (CIP) step using 3 – 5 CV 1 M NaOH in upflow direction is recommended between all runs and should be followed by a careful re-equilibration with binding buffer before the next run. To prevent any precipitation, wash with 3 – 5 CV 0.02 M NaCl before re-equilibration with 3 – 5 CV binding buffer. If the column is going to be stored, apply instead 3 – 5 CV 20% ethanol as the storage solution after the wash.

Collect eluates in all steps, especially when a new sample is separated, and it's not known when the target will elute.

Note: When working with samples that tend to aggregate, start with a lower concentration of ammonium sulphate to avoid protein precipitation, or dilute your sample.

Table 1. Typical buffer composition for purifications using WorkBeads 40 Butyl SH.

Target molecule	Binding buffer	Elution buffer
Proteins	50 mM phosphate, 1.2 – 2 M ammonium sulphate, pH 7.0	50 mM phosphate, pH 7.0
Plasmids	50 mM phosphate, 1.5 – 2 M ammonium sulphate, pH 7.0	50 mM phosphate, pH 7.0
PO ON[1] (DMT-on[2])	50 mM Tris-HCl, 0.7 – 1.3 M ammonium sulphate, pH 8.5	50 mM Tris-HCl, 0 – 20 mM ammonium sulphate, pH 8.5

[1] PO ON: phosphorothioate oligonucleotides
[2] DMT-on: Dimethoxytrityl is a 5' protective group used in oligonucleotide synthesis. "on" means that the group is left on the oligonucleotide.

Optimization

The goal when optimizing a purification is to identify the parameters that promote the binding of the highest amount of the target molecule in the shortest possible time with the greatest possible recovery of the target at the lowest cost.

Conditions should be selected to achieve the binding of the target, while avoiding the binding of impurities, to maximize the purity and yield of the target molecule. If the target molecule is a protein, conditions must also be selected that preserve its native state.

Optimization of salt and salt concentration

In HIC, the binding process is more selective than the elution process, and it is essential to optimize the conditions of the binding buffer. The correct salt and salt concentration are the most important parameters that influence capacity and final selectivity.

Sulphate salts, in the order of ammonium, potassium, and sodium sulphates effectively promote ligand-protein interactions in HIC and have a stabilizing influence on protein structure. These so-called kosmotropic (anti-chaotropic) salt ions have higher polarity and bind water strongly and therefore exhibit a higher 'salting-out' effect on the molecules, see Hofmeister series in Figure 2. Commonly used salts are $(NH_4)_2SO_4$, Na_2SO_4, NaCl, KCl, and CH_3COONH_4. The salts that increase surface tension in aqueous solutions tend to promote hydrophobic interactions. The choice of salt for a HIC separation is a matter of trial and error since each salt differs in its ability to promote hydrophobic interactions. As the salt concentration increases, the bond between the target and the resin will become stronger leading to more target being bound to the resin.

Ammonium sulphate often gives a good resolution compared to other salts, and it can be used in concentrations up to 2 M. Sodium chloride can be used up to concentrations of 3 M. Sodium sulphate is a good salting-out agent but can cause problems with sample solubility at high concentrations. See Table 1 for recommended buffer compositions to start with for different molecules.

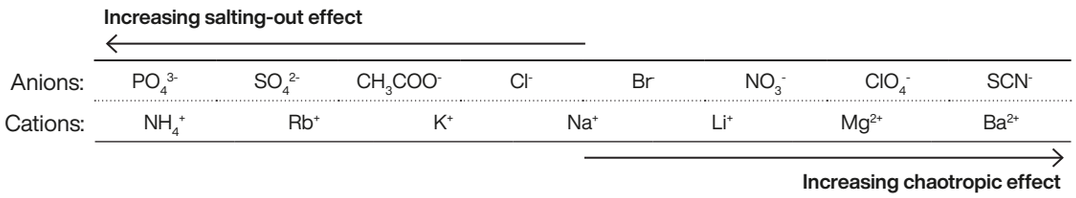

Figure 2. The Hofmeister series.

Optimization of buffer ions and pH

The choice of buffering ions is not critical to hydrophobic interaction. Phosphate buffers and tris buffers are most commonly used. The pH should be compatible with the target's stability and activity. A neutral pH is recommended as a starting point, but it ultimately depends on the properties of the target molecule.

Use a buffer concentration of 20 to 100 mM to maintain buffering capacity pH during sample loading and changes in salt concentration.

Optimization of flow rate

The flow rate during sample loading affects the binding capacity and resolution during the elution.

A low flow rate during sample application promotes binding capacity since more time is allowed for the mass transport of the target substance into the pores of the resin. A small substance, e.g., a peptide, that has a high diffusion rate will have rapid mass transport into the resin and can thus be adsorbed efficiently at higher flow rates as it has fast binding kinetics. A large target substance has a lower diffusion rate and is more hindered by the walls in the pores, resulting in slower mass transport. Achieving a high binding capacity of this substance may require lowering the flow rate. If only a part of the binding capacity of the column is used, sample application can be done at a higher flow rate without loss of the target substance.

Typical linear flow rates are 150 – 300 cm/h. Figure 3 shows how the resolution is affected by the flow velocity for three different protein pairs.

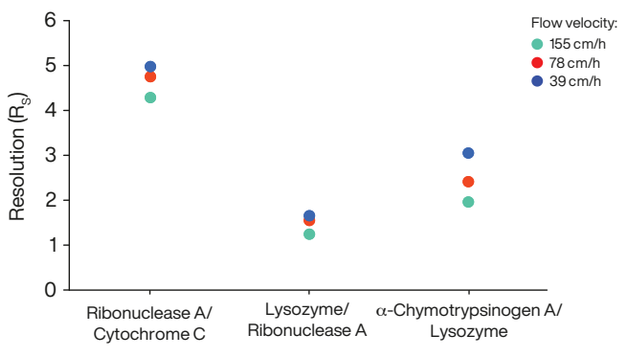

Figure 3. The relationship between resolution and flow velocity achieved on a GoBio Mini Butyl SH 1 mL column. The flow velocities used: 39 cm/h (blue), 78 cm/h (red), and 155 cm/h (green).

Optimization of washing

A continuously decreasing UV signal is an indication that unbound material is still being washed out. The washing should continue until the UV signal is stable and is the same as in the buffer used for washing, or at least not more than 20 mAU. The washing buffer can be the same as the binding buffer, but in some cases, it may be useful to add an additional step, such as one or two intermediate wash steps, to improve purification.

Optimization of elution conditions

For optimizing the elution condition, it is recommended to use a linear gradient to decrease the ionic strength, see Figure 4.

The results will then be used to determine the optimal elution buffer when moving to a step elution method, which is often the preferred method in process scale as the target substance is eluted in a more concentrated form. Buffer consumption can be reduced, and the purification cycle times can be shortened.

Elution is, in most cases, carried out using zero or low salt concentration. The optimal salt concentration is dependent on the purity and recovery requirements, as well as the properties of the target substance.

Applying gradient elution gives higher purity than step elution in most cases, but step elution may be preferred if the target needs to be more concentrated in the eluate. To optimize the salt concentration for step elution, an initial linear gradient test run should be carried out to determine suitable step elution conditions.

Note: Remember to take the system dead volume into account when comparing the gradient and the trace.

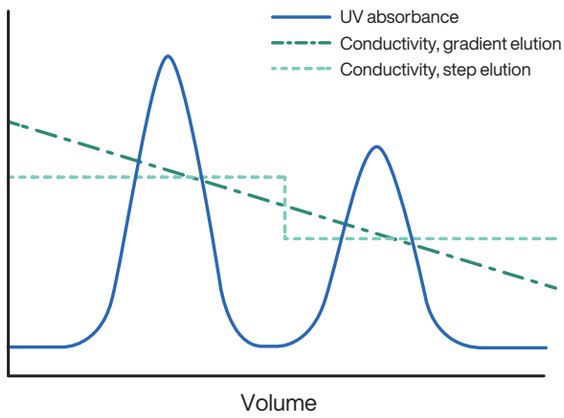

Figure 4. Optimization of step elution with low-salt buffer. A test run with linear gradient elution gives information about suitable salt concentrations to be used in step elution.

Other elution possibilities are:

- Increasing the concentration of chaotropic ions in the buffer with an increasing gradient (linear or step)
- Eluting with a polarity-reducing organic solvent (e.g., ethylene glycol) added to the buffer
- Eluting with detergent added to the buffer
- Isocratic elution is an option for some feeds due to a partial retention
- Changing the temperature to alter the elution pattern

Increasing the salting-out effect strengthens hydrophobic interactions; increasing the chaotropic effect weakens hydrophobic interactions, see Figure 2.

Scale-up

After developing a chromatographic procedure in a small-scale column, e.g., 7 (i.d.) × 100 mm (bed height), WorkBeads resins can be packed into larger columns for scale-up. Large-scale purification is often carried out in columns with bed heights of 200 – 300 mm. In large-scale resin packing, back-pressure can be a limiting factor. It's advisable to reduce the flow rate when using 20% ethanol. For WorkBeads 40 Butyl SH, packing solutions exhibiting some conductivity (0.02 – 0.4 M NaCl) are recommended due to its incompatibility with pure water, most noticeable at larger scales.

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., 200 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 are important when scaling 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:

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

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

In the initial process development work, it's common to use a small column, e.g., 7 × 100 mm, to save samples, buffers and time. This column has a shorter bed height than the final column, which may have a bed height of 200 mm or more. 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 as for the larger column. This will allow an increase of the linear flow in proportion to the increase in bed height between the columns, see Table 2 for examples. If the column bed height is kept constant during scale-up, the linear flow rate should be kept constant (as well as the residence time).

Table 2. Example of scale-up parameters

Column dimension	Residence time (minutes)	Linear flow rate (cm/h)	Volumetric flow rate (mL/min)
16x100	4	150	5.0
26x100	4	150	13.3
80x200	8	150	126
130x200	8	150	332
200x200	8	150	785
240x200	8	150	1131
330x250	10	150	2138

Additional purification steps

Optimization of the purification process by tuning the binding, washing and/or elution conditions of the HIC purification step may not be enough to obtain the required purity. Combining two or more purification steps based on additional chromatography techniques is then recommended. For example, before a HIC step, an ion exchange chromatography step can be added. If you include an IEX step before the HIC, rather than after, it reduces the required sample handling. Another technique, such as size exclusion chromatography (gel filtration), is a commonly used alternative and often used as a polishing step. Each purification step should be thoroughly optimized, and preferably in the context of the other steps applied in the overall process.

Desalting and buffer exchange

Buffer exchange or desalting of a sample can be performed before analysis and/or after purification. This can be carried out quickly and easily in lab-scale using GoBio Mini Dsalt 1 mL, GoBio Mini Dsalt 5 mL, GoBio Prep 16x100 Dsalt (20 mL), and GoBio Prep 26x100 Dsalt (53 mL) prepacked columns depending on sample volumes. GoBio Prod prepacked columns starting from 1 L are available for larger sample volumes, see “Related products”. These columns are very useful alternatives to dialysis for larger sample volumes or when samples need to be processed rapidly to avoid degradation.

Pre-swollen WorkBeads Dsalt is also available in bulk for packing into the column format of choice. To find out more about Bio-Works chromatography products, visit www.bio-works.com.

Maintenance

Cleaning and sanitization

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 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.

CIP of the column can be carried out as followed:

1. Wash the column with 5 CV 0.02 M NaCl.
2. Apply 3 – 10 CV of 0.5 – 1 M NaOH for 15 – 30 minutes.

   Note: The contact time is the important factor. Treatment with NaOH overnight can be necessary if severely fouled.
3. Wash the column with 5 – 10 CV 0.02 M NaCl (until the pH is neutral after CIP).
4. Equilibrate the column with 10 CV 20% ethanol (for storage).

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. It is important to note that concentrations exceeding 20% ethanol should not be used for GoBio Screen, GoBio Prep, or GoBio Prod columns.

Storage

Store the resin at 2 to 25°C in 20% ethanol.

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

Product descriptions

	WorkBeads 40 Butyl SH
Target substances	Proteins, peptides, plasmids, oligonucleotides
Matrix	Rigid, highly cross-linked agarose
Average particle size (D_{V50})[1]	45 µm
Ligand	*n*-butyl thioether (CH_3 - CH_2 - CH_2 - CH_2 - S -)
Ligand density	46 – 62 µmol/mL resin
Dynamic binding capacity (DBC)[2]	43 mg β-lactoglobulin/mL resin
Max flow rate[3]	600 cm/h (20 cm bed height and 5 bar)
Chemical stability	Compatible with all standard aqueous buffers exhibiting some conductivity, 1 M NaOH, 30% isopropanol, 30% ethanol. Note: Sensitive to oxidants, e.g., H_2O_2.
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 at 10% breakthrough determined at a residence time of 4 min (150 cm/h) in a 6.6x100 mm column. Buffer conditions: 0.1 M sodium phosphate, 2 M ammonium sulfate, pH 7.
3 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 operation at +4 ºC) or by additives (e.g., use half of the maximum flow rate for 20% ethanol).

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 are used for small-scale purification and screening using a shorter packed bed.

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

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

GoBio Prep 16x600 (120 mL) and GoBio Prep 26x600 (320 mL) are used 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) are used for production-scale purifications.

Related products

Product name	Pack size[1]	Article number
Prepacked columns		
GoBio Mini Butyl SH 1 mL	1 mL × 5	45 500 103
GoBio Mini Butyl SH 5 mL	5 mL × 5	45 500 107
GoBio Screen 7x100 Butyl SH	3.8 mL × 1	55 500 001
GoBio Prep 16x100 Butyl SH	20 mL × 1	55 500 021
GoBio Prep 26x100 Butyl SH[2]	53 mL × 1	55 500 031
GoBio Prod 80x200 Butyl SH[2]	1 L × 1	55 500 042
GoBio Prod 130x200 Butyl SH[2]	2.7 L × 1	55 500 062
GoBio Prod 200x200 Butyl SH[2]	6 L × 1	55 500 072
GoBio Prod 240x200 Butyl SH[2]	9 L × 1	55 500 082
GoBio Prod 330x250 Butyl SH[2]	21.4 L × 1	55 500 093
GoBio Mini IEX Screening kit[3]	1 mL × 4	45 900 001
GoBio Mini S 1 mL	1 mL × 5	45 200 103
GoBio Mini S 5 mL	5 mL × 5	45 200 107
GoBio Mini Q 1 mL	1 mL × 5	45 100 103
GoBio Mini Q 5 mL	5 mL × 5	45 100 107
GoBio Mini Dsalt 1 mL	1 mL × 5	45 360 103
GoBio Mini Dsalt 5 mL	5 mL × 5	45 360 107
GoBio Screen 7x100 40S	3.8 mL × 1	55 420 001
GoBio Screen 7x100 40Q	3.8 mL × 1	55 410 001
GoBio Prep 16x100 40S	20 mL × 1	55 420 021
GoBio Prep 16x100 40Q	20 mL × 1	55 410 021
GoBio Prep 16x100 Dsalt[2]	20 mL × 1	55 700 021
GoBio Prep 26x100 40S[2]	53 mL × 1	55 420 031
GoBio Prep 26x100 40Q[2]	53 mL × 1	55 410 031
GoBio Prep 26x100 Dsalt	53 mL × 1	55 700 031
GoBio Prod 80x200 40S[2]	1 L × 1	55 420 042
GoBio Prod 80x200 40Q[2]	1 L × 1	55 410 042
GoBio Prod 80x200 Dsalt[2]	1 L × 1	55 700 042
Bulk resins		
WorkBeads 40S	25 mL	40 200 001
WorkBeads 40Q	25 mL	40 100 001
WorkBeads Dsalt	300 mL	40 360 003

[1] All different pack sizes and column dimensions are available on www.bio-works.com
[2] Packed on request.
[3] GoBio Mini IEX Screening Kit includes one of each: GoBio Mini S 1 mL, GoBio Mini Q 1 mL, GoBio Mini DEAE 1 mL and GoBio Mini TREN 1 mL.

Ordering information

Product name	Pack size	Article number
WorkBeads 40 Butyl SH	25 mL 200 mL 1 L 5 L 10 L	40 500 001 40 500 002 40 500 010 40 500 050 40 500 060

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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