



INSTRUCTION

WorkBeads 40S WorkBeads 40Q WorkBeads 40 DEAE

WorkBeads™ 40S, WorkBeads 40Q and WorkBeads 40 DEAE resins for ion exchange chromatography are designed for research and industrial scale purification of proteins, peptides and oligonucleotides by utilizing the difference in their surface charge. WorkBeads 40S resin is a strong cation exchanger with sulfonate ligands. WorkBeads 40Q resin is a strong anion exchanger with quaternary amine ligands. WorkBeads 40 DEAE is a weak anion exchanger with tertiary amine ligands. The property of high-resolution separation while giving low backpressure allow 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 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 and all the way up to process development and full-scale manufacturing.

Safety

Please read the Safety Data Sheets (SDS) for WorkBeads 40S, WorkBeads 40Q, WorkBeads 40 DEAE and WorkBeads 40 TREN, 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 of WorkBeads 40S, WorkBeads 40Q and WorkBeads 40 DEAE. Detailed instructions and recommendations for optimization are given later in this document. Recommended buffers are listed in Table 1. WorkBeads 40S resin is suitable for basic proteins (i.e., protein with high isoelectric point (pI)) and WorkBeads 40Q and WorkBeads 40 DEAE resins are suitable for purification of acidic proteins (i.e., proteins with low pI).

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 binding buffer.
6. Apply sample.
7. After sample application, remove unbound material by washing with, e.g., 20 – 30 CV (column volumes) washing buffer.
8. Elute the target protein with elution buffer.
9. Wash the column with deionized water.
10. Equilibrate the column with 20% ethanol for storage. For WorkBeads 40S it is recommended to also add 0.2 M sodium acetate in the storage solution

Principle

Ion exchange chromatography (IEX) can be used for the purification of biomolecules, such as proteins, peptides, viruses and oligonucleotides, by utilizing differences in surface charge. The biomolecules interact with the immobilized ion exchange groups of opposite charge on the chromatography resin.

WorkBeads 40S is a strong cation exchanger with sulfonate ligands. It will bind positively charged substances and can be used over a broad pH range (3 – 13).

WorkBeads 40Q is a strong anion exchanger with quaternary amine. It will bind negatively charged substances and can also be used over a broad pH range (3 – 13).

WorkBeads 40 DEAE is a weak anion exchanger with tertiary amine ligands (diethylaminoethyl). This resin should be used as an alternative to WorkBeads 40Q when looking for alternative selectivities. The density of positive charges in WorkBeads 40 DEAE will decrease gradually when the pH is increased above pH 6. This effect can be used to modulate the selectivity of the resin, although the binding capacity may be reduced at basic pH values.

The functional groups are coupled to the resins via chemically stable linkages. For these resins the strength of the binding will depend on the number of charges involved in the interaction, and the distribution of the charges on the surface of the biomolecule. Areas of the biomolecule with the same charge as the resin ligands may reduce the interaction by repulsion.

We recommend using WorkBeads 40Q as the first choice of anion exchanger, and to use WorkBeads 40 DEAE as an alternative when there is a need to modify the selectivity.

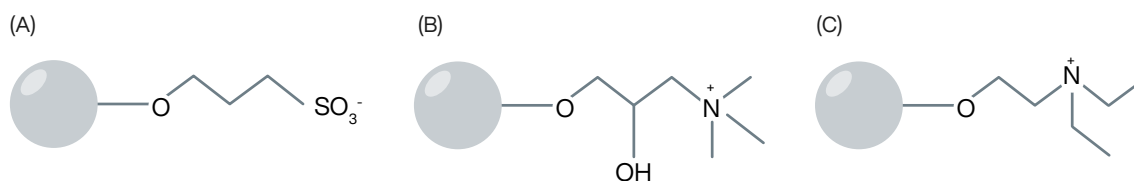


Figure 1. Structure of the ligand used in WorkBeads 40S (A), WorkBeads 40Q (B) and WorkBeads 40 DEAE (C).

The charges available on the surface of a protein depend on the pH of its 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 greater than 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 interact with an ion exchange resin also at its isoelectric point. The likelihood of binding to either the cation or the anion exchange resin will increase the further the pH moves away from the pI.

Ion exchange chromatography begins with equilibration of the column to establish the correct pH and to charge the resin with suitable counter ions to the charged ligands on the resin. The negative sulfonate groups can interact with Na⁺-ions, and the positive trimethyl amine groups (quaternary amine) can interact with Cl⁻ ions. It is common to use an equilibration buffer composed of a buffer substance to control the pH, and NaCl to include suitable counter ions. Avoid using buffer substances that have a charge opposite the charge of the resin to avoid uncontrollable negative effects on the separation.

On applying the sample proteins with suitable charge will bind to the charged groups of the resin in the process displacing the counter ions. Desorption of the proteins (elution) is done by increasing the concentration of counter ion (salt gradient elution). A high enough concentration of the counter ion will displace the proteins. Various additives, e.g., enzyme inhibitors, non-ionic detergents, urea and low concentrations of organic solvent, can usefully be used in samples and buffers for IEX. It is important that they do not strongly interact with the charged groups on the resin or the protein and interfere with the protein binding to the resin. As an example, purification of peptides can be done in the presence of up to 30 % acetonitrile or ethanol if required to keep the peptide soluble and to facilitate a good separation.

Ion exchange chromatography 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 because the eluted protein is usually concentrated. It is also one of the more cost-effective chromatography techniques and is therefore excellent for scale-up.

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 tolerate pressures of several bars and consequently can run at high flow rates. Follow both these general advices 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

Most WorkBeads resins are supplied in 20% ethanol. To avoid undue backpressure when packing, wash the desired amount of resin with several column volumes (CV) of deionized water or packing buffer before packing.

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 desired range, modify the slurry concentration of the resin to be packed by either adding or removing packing solution.

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

Note: Different packing buffers can be used, such as 0.4 M NaCl or 20% ethanol. Some resins are better packed in packing buffer with higher conductivity.

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.

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

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

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 1 – 2% of the final bed height by lowering the adapter into the packed bed.

6. Apply a flow (conditioning of bed)

Apply a flow of 450 cm/h 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 is decreased/diminished. Wall support means that the column wall supports the resin bed and gives it better flow properties. This mainly occurs when the column 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 optimal mechanical compression to apply will depend on the dimensions and resin type, therefore it is essential to evaluate the packed column prior to use (asymmetry and plate number tests as described below).

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 but this is not to recommend at larger scales.

There is a guide available for large-scale packing,
<https://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 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).

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

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

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:

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

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.

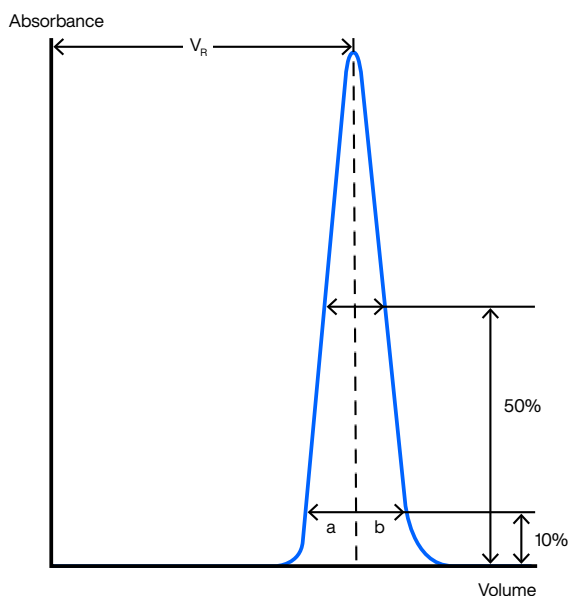


Figure 2. A typical test chromatogram showing the parameters used for HETP and A_s calculations.

Purification

Strong ion exchangers, such as WorkBeads S and WorkBeads Q, can be used with a broad pH range. A weak ion exchanger such as WorkBeads 40 DEAE will gradually have lower charge density when the pH is increasing. This allows modulation of its selectivity. When decreasing the charge density, the binding capacity may decrease. Because of the mentioned reason, WorkBeads 40 DEAE can be used in a pH range of 3 – 9.

The limitations in pH that can be used with an ion exchanger will also depend on the protein stability. It is often possible to use either an anion exchange column or a cation exchange column to purify the same target protein. This can be carried out by moving the pH of the buffers below or above the protein pI to change its overall charge.

The buffer species and buffer concentration are important for robust and reproducible methods. Choose a suitable pH and buffer for the binding of the target protein. Good starting points are one pH unit below pI for WorkBeads 40S and one pH unit above pI for WorkBeads 40Q, WorkBeads 40 DEAE and WorkBeads 40 TREN.

The binding conditions should be optimized to achieve binding of the target molecule, while minimizing the binding of impurities (vice versa if operated in “negative mode”). When scouting for the best binding conditions it is important to start with sufficiently low ionic strength. See examples of buffers to use for samples with unknown charge properties in Table 1.

Table 1. Typical buffer compositions for purification using WorkBeads 40S, WorkBeads 40Q and WorkBeads 40 DEAE.

Resin	Buffer composition
WorkBeads 40S	Binding buffer: 20 mM phosphate, pH 7.0 Elution buffer: 20 mM phosphate, 1 M NaCl, pH 7.0 or Binding buffer: 50 mM sodium acetate, pH 5.0 Elution buffer: 50 mM sodium acetate, 1 M NaCl, pH 5.0
WorkBeads 40Q	Binding buffer: 20 mM Tris-buffer, pH 8.0 Elution buffer: 20 mM Tris-buffer, 1 M NaCl, pH 8.0
WorkBeads 40 DEAE	Binding buffer: 20 mM Tris-buffer, pH 8.0 Elution buffer: 20 mM Tris-buffer, 1 M NaCl, pH 8.0

Note: Peptides may require a lower ionic strength of the buffers to achieve optimal binding capacities. The buffers used for oligonucleotide and peptide purifications may require addition of organic solvents, such as 5-30% acetonitrile, for optimal performance.

Elution can be carried out by applying a linear gradient of increasing concentration of NaCl, by gradually increasing the proportion of elution buffer (high salt). See Table 2 for buffer examples. A short step gradient to 1 or 2 M NaCl for 5 column volumes (CV) can be included after elution to ensure desorption of all interacting proteins. When suitable elution conditions are known it is common to apply step gradient elution. This will reduce process time and is generally recommended for capture step purification using high-flow large particle resins. A cleaning-in-place (CIP) step using 5 CV 1 M NaOH is recommended between all runs and should be followed by a careful re-equilibration before the next run.

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, e.g., a syringe filter, to avoid transferring any remaining contaminating particles onto the column. Application of a sample that has not been properly clarified may reduce the performance and lifetime of the column.

The sample should have a pH that confers a net charge to the target protein that is opposite to the charge of the column resin. The ionic strength should be low. Optimal binding conditions are a result of the combination of the pH and the ionic strength. The sample solution may therefore be adjusted before being applied to the column. It is generally recommended that the sample should have a pH and conductivity similar to the binding buffer. Sample adjustments can be done by dilution using for example the binding buffer, by fast chromatographic desalting or diafiltration, or through adjusting the pH by addition of an acid or base.

Optimization

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

The key conditions to be optimized are usually pH and conductivity (by addition of NaCl or other salts, or dilution). Conditions should be selected to achieve binding of the target while avoiding the binding of impurities to maximize purity and yield of the target molecule. If the target molecule is a protein, conditions must also be selected that preserve its native state.

The following paragraphs will give indications of some parameters that can be tuned to get the optimal conditions. For other useful buffers and their pK_a -values at 25 °C see reference: Methods in Enzymology, Volume 463, pp 46-47, Burgess, R.R and Deutcher, M.P.

Strong ion exchangers, such as WorkBeads S and WorkBeads Q, can be used with a broad pH range. A weak ion exchanger such as WorkBeads 40 DEAE will gradually have lower charge density as the pH increases. This allows modulation of its selectivity. When decreasing the charge density, the binding capacity may decrease and because of this, WorkBeads 40 DEAE can be used in a pH range of 3 – 9.5. The limitations in pH that can be used with an ion exchanger will also depend on the target molecule stability. It is often possible to use either an anion exchange column or a cation exchange column to purify the same target. This can be carried out by altering the pH of the buffers to below or above the molecule's pI to change its overall charge.

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 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. A large target substance (e.g., a large protein or a pegylated molecule) 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.

The residence time can be defined as the time between a molecule entering and exiting the column. The residence time depend on the flow rate and the dimensions of the column and is typically 1 to 5 minutes in IEX. Typical linear flow rates are 150 – 300 cm/h. See further discussion about flow in the section “Scale-up.”

Optimization of binding conditions

Selecting a buffer with optimal binding conditions for the target protein will improve the result of the purification. The buffer should have a good buffering capacity and preferably a pK_a -value within 0.5 units from the intended pH. The buffer substance selected should have the same charge as the resin.

Screen for optimal binding conditions by testing a range of pH values in which the target molecule is known to be stable. In some cases, sample conductivity is as important as the pH when screening for optimal binding conditions. Usually, binding buffer with low conductivity is preferred but optimization of the conductivity can improve binding capacity. An increase in ionic strength may decrease the ability of contaminants to bind while the target protein remains bound. As described above, the flow rate is also important for achieving optimal binding conditions. However, it is important that chromatographic conditions are chosen so that the target molecule is stable during the purification. Screen at the temperature at which the process is planned to be run.

Optimization of washing

A continuously decreasing UV signal is an indication of unbound material still being washed out. The washing should continue until the UV signal is stable and 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 it may be useful to add an additional step with a dedicated washing buffer to improve purification.

Optimization of elution conditions

For optimizing the elution condition, it is recommended to always use a linear ionic strength gradient. The results will then be used for the optimal elution buffer when moving to a step elution method which is often the preferred method in process scale as the target molecule is eluted in a more concentrated form. Buffer consumption can be reduced and purification cycle times shortened.

Elution is in most cases carried out using a high salt concentration but altering the pH to change the charge of the adsorbed target molecule is an alternative. A stronger binding may require higher salt concentration for elution. The optimal salt concentration is dependent on the purity and recovery requirements, as well as the properties of the target molecule.

Applying gradient elution gives higher purity than step elution, but step elution may be preferred to obtain the highest possible concentration of the target. To optimize the salt concentration for step elution an initial linear gradient test run should be carried out to determine suitable step elution conditions, see Figure 3.

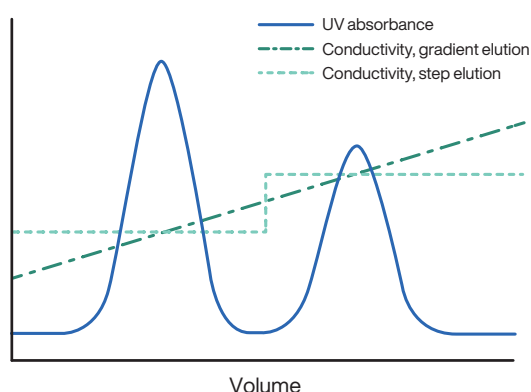


Figure 3. Optimization of step elution with salt. A test run with linear gradient elution gives information about suitable salt concentrations to be used in step elution. Note: Remember to take the system dead volume into account when comparing the gradient and the trace.

Scale-up

After developing a chromatographic procedure in 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.

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 is common to use a small column, e.g., 7 × 100 mm, to save sample, 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 3 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 3. 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 IEX purification step may not be enough to obtain the required purity. Combining two or more purification step based on additional chromatography techniques is then recommended. For example, cation exchange chromatography and anion exchange chromatography can be combined in a purification process. Other techniques, such as size exclusion chromatography (gel filtration) and hydrophobic interaction chromatography (HIC) are commonly used alternatives. Each purification step should be thoroughly optimized, and preferably in the context of the other steps applied on the overall process.

Desalting and buffer exchange

Buffer exchange or desalting of a sample can be used 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 column format of choice.

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

Maintenance

Cleaning and sanitization

During purification, cell-derived impurities such as cell debris, lipids, nucleic acids and protein precipitates, or synthesis-derived impurities such as failure sequences and counter ions from the samples may gradually build-up in the resin and cause fouling. The severity of this process depends on the type of sample applied to the column, and the pre-treatment of the sample. The impurities coating the resin may reduce the performance of the column over time. Regular cleaning (cleaning-in-place, CIP) reduces the rate of further fouling and prolongs the capacity, resolution and flow properties of the column. Cleaning using 1 M NaOH applied at a low flow for 15 – 30 min is often sufficient. Do not use elevated temperature during the CIP treatment, since this may reduce the lifetime of the resin.

Some resins become yellowish during CIP with NaOH (0.5 M or 1 M) due to dehydration which makes the resin more compact, but they will become white again after washing with deionized water (recommended 3 – 5 column volumes, CV) followed by applying the preferred equilibration buffer.

Our studies show that for example WorkBeads 40S can tolerate up to 1 M NaOH (one week at room temperature) without significant decrease in ionic capacity and dynamic binding capacity or any significant change in pattern of selectivity.

Sanitization (reduction of microorganisms) can be carried out using combinations of NaOH and ethanol. The sanitization procedure and its effectiveness will depend on the microorganisms to be removed and needs to be evaluated for each case.

Storage

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

For WorkBeads 40S it is recommended to include 0.2 M sodium acetate in the storage solution.

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

Product information

	WorkBeads 40S	WorkBeads 40Q	WorkBeads 40 DEAE
Target substance	Proteins, peptides	Protein, peptides, viruses, oligonucleotides	Protein, peptides, oligonucleotides
Matrix	Rigid, highly cross-linked agarose	Rigid, highly cross-linked agarose	Rigid, highly cross-linked agarose
Average particle size (D_{v50}) ¹	45 μm	45 μm	45 μm
Ionic group (ligand)	Sulfonate ($-\text{SO}_3^-$)	Quaternary amine ($-\text{N}^+(\text{CH}_3)_3$)	Diethylaminoethyl ($-\text{CH}_2\text{CH}_2\text{N}^+\text{H}(\text{CH}_2\text{CH}_3)_2$)
Ion capacity	180 – 250 $\mu\text{mol H}^+/\text{mL resin}$	180 – 250 $\mu\text{mol Cl}^-/\text{mL resin}$	110 – 160 $\mu\text{mol Cl}^-/\text{mL resin}$
Dynamic binding capacity	130 mg BSA/mL resin ²	50 mg BSA/mL resin ³	40 mg BSA/mL resin ³
Max. flow rate ⁴ (20 cm bed height and 5 bar)	600 cm/h	600 cm/h	600 cm/h
Chemical stability	Compatible with all standard aqueous buffers used for protein purification, 1 M NaOH ⁵ , 30% isopropanol and 70% ethanol. Should not be stored at low pH for prolonged time.		
Operational pH range ⁶	3 – 12	2 – 13	2 – 13 3 – 9 (recommended pH)
CIP and screening pH range ⁶	2 – 14	2 – 14	2 – 14
Storage	2 to 25 °C in 20% ethanol with 0.2 M sodium acetate	2 to 25 °C in 20% ethanol	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 20 mM sodium citrate, pH 4.0.

³ Dynamic binding capacity determined at 2.5 minutes residence time in 50 mM Tris-HCl, 50 mM NaCl, pH 8.0.

⁴ Optimal flow rate during binding is depending on the sample.

⁵ For more information, see *Maintenance*.

⁶ Within the operational pH range, the resin can be operated without significant change in function. Within the CIP (Cleaning-in-place) and screening pH range the resin can be subjected to the denoted pH range without significant change in function.

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 IEX Screening kit ²	1 mL × 4	45 900 001
GoBio Mini Peptide Purification kit ³	1 mL × 2	45 300 102
GoBio Mini S 1 mL	1 mL × 5	45 200 103
GoBio Mini Q 1 mL	1 mL × 5	45 100 103
GoBio Mini DEAE 1 mL	1 mL × 5	45 150 103
GoBio Mini Dsalt 1 mL	1 mL × 5	45 360 103
GoBio Mini S 5 mL	5 mL × 5	45 200 107
GoBio Mini Q 5 mL	5 mL × 5	45 100 107
GoBio Mini DEAE 5 mL	5 mL × 5	45 150 107
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 Screen 7x100 40 DEAE ⁴	3.8 mL × 1	55 415 001
GoBio Prep 16x100 40S	20 mL × 1	55 420 021
GoBio Prep 16x100 40Q	20 mL × 1	55 410 021
GoBio Prep 16x100 40 DEAE ⁴	20 mL × 1	55 415 021
GoBio Prep 16x100 Dsalt ⁴	20 mL × 1	55 700 021
GoBio Prep 26x100 40S ⁴	53 mL × 1	55 420 031
GoBio Prep 26x100 40Q ⁴	53 mL × 1	55 410 031
GoBio Prep 26x100 40 DEAE ⁴	53 mL × 1	55 415 031
GoBio Prep 26x100 Dsalt	53 mL × 1	55 700 031
GoBio Prod 80x200 40S ⁴	1 L × 1	55 420 042
GoBio Prod 80x200 40Q ⁴	1 L × 1	55 410 042
GoBio Prod 80x200 40 DEAE ⁴	1 L × 1	55 415 042
GoBio Prod 80x200 Dsalt ⁴	1 L × 1	55 700 042
GoBio Prod 130x200 40S ⁴	2.7 L × 1	55 420 062
GoBio Prod 130x200 40Q ⁴	2.7 L × 1	55 410 062
GoBio Prod 130x200 40 DEAE ⁴	2.7 L × 1	55 415 062
GoBio Prod 130x200 Dsalt ⁴	2.7 L × 1	55 700 062
GoBio Prod 200x200 40S ⁴	6 L × 1	55 420 072
GoBio Prod 200x200 40Q ⁴	6 L × 1	55 410 072
GoBio Prod 200x200 40 DEAE ⁴	6 L × 1	55 415 072
GoBio Prod 200x200 Dsalt ⁴	6 L × 1	55 700 072
GoBio Prod 240x200 40S ⁴	9 L × 1	55 420 082
GoBio Prod 240x200 40Q ⁴	9 L × 1	55 410 082
GoBio Prod 240x200 40 DEAE ⁴	9 L × 1	55 415 082

GoBio Prod 240x200 Dsalt ⁴	9 L × 1	55 700 082
GoBio Prod 330x250 40S ⁴	21.4 L × 1	55 420 093
GoBio Prod 330x250 40Q ⁴	21.4 L × 1	55 410 093
GoBio Prod 330x250 40 DEAE ⁴	21.4 L × 1	55 415 093
GoBio Prod 330x250 Dsalt ⁴	21.4 L × 1	55 700 093

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 All different pack sizes are available on www.bio-works.com

² 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.

³ GoBio Mini Peptide Purification Kit is a bundle of: GoBio Mini S 1 mL × 1 and GoBio Mini Q 1 mL × 1.

⁴ Packed on request.

Ordering information

Product name	Pack size	Article number
WorkBeads 40S	25 mL	40 200 001
	200 mL	40 200 002
	1 L	40 200 010
	5 L	40 200 050
	10 L	40 200 060
WorkBeads 40Q	25 mL	40 100 001
	200 mL	40 100 002
	1 L	40 100 010
	5 L	40 100 050
	10 L	40 100 060
WorkBeads 40 DEAE	25 mL	40 150 001
	200 mL	40 150 002
	1 L	40 150 010
	5 L	40 150 050
	10 L	40 150 060

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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