OptioBio 40S 10x100
OptioBio 40Q 10x100

The prepacked OptioBio™ glass columns are designed for small-scale purification as well as screening and optimization in bioprocess development and scale-up. OptioBio 40S 10x100 and OptioBio 40Q 10x100 columns are prepacked with WorkBeads™ 40S and WorkBeads 40Q resins for ion exchange chromatography (IEX). The resins are designed for research and industrial scale purification of proteins, peptides and nucleic acids and utilise the difference in their surface charge. WorkBeads 40S is a strong cation exchange resin with sulfonate ligands, and WorkBeads 40Q is a strong anion exchange resin with quaternary amine ligands. The property of high-resolution separation in combination with low backpressure facilitates both capture and polishing purification applications.

- Prepacked for reliable and reproducible results
- Optimal for high-performance small scale purification and method optimization in bioprocess development
- High throughput and purity

Short protocol

Use this short protocol for a quick and easy start-up. Detailed instructions and recommendations for optimization are provided later in this document. Recommended and useful buffers are listed in Table 2. OptioBio 40S 10x100 is suitable for purification of basic proteins, i.e., proteins with high isoelectric point (pI), while OptioBio 40Q 10x100 is suitable for acidic proteins, i.e., proteins with low pI.

1. Select a suitable pH and buffer for the binding of the target protein. One pH unit below pI of the protein (OptioBio 40S 10x100) or above pI (OptioBio 40Q 10x100) is a good starting point.

2. Connect the column to the pump or chromatography system.
   Note: The columns are delivered containing 20% ethanol. Make sure not to exceed 3 ml/min when removing the storage solution.

3. Equilibrate the column with 10 column volumes (CV) of 20 - 50 mM binding buffer at the selected pH.

4. Apply a clarified sample with a similar ionic strength and pH as the binding buffer to the column to allow binding of the target protein.

5. Wash the column using 10 - 30 CV of binding buffer (until the absorbance has reached the baseline).

6. Elute the target protein.
   Alternative 1, step gradient: Elute the target protein with 5 CV elution buffer.
   Alternative 2, linear gradient: For increased purity, a linear gradient elution is recommended.
   For example, use a gradient from 0 to 100% binding buffer with 1.0 M NaCl over 20 CV.

7. Clean the packed column using 0.5 - 1.0 M NaOH for 15 - 30 minutes (see *Maintenance*) and re-equilibrate the column with 10 CV of binding buffer to restore the pH.

   After the elution and cleaning, the column is ready for the next purification, otherwise proceed to point 8.

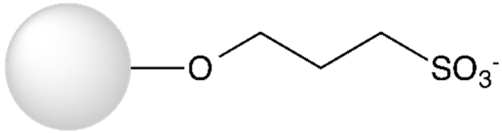
8. Wash the column with 5 CV of deionized water to remove the buffer salts.

9. Equilibrate with 10 CV of 20% ethanol for storage. Close the column using the included plugs.

Principle

Ion exchange chromatography (IEX) can be used for the purification of biomolecules, such as proteins, peptides and nucleic acids, by utilizing the difference in their surface charge. The biomolecules interact with the opposite charged immobilized ion exchange groups (ligands) of the chromatography resin. WorkBeads 40S is a strong cation exchanger and will bind positively charged substances. WorkBeads 40Q is a strong anion exchanger and will bind negatively charged substances. 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. Charges on the biomolecule that are the same as on the resin may reduce the interaction by repulsion. The structure of the ligands used in WorkBeads 40S and WorkBeads 40Q are shown in Figure 1.

(A)



(B)

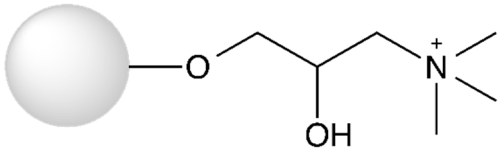


Figure 1. Structure of the ligand used in WorkBeads 40S (A) and WorkBeads 40Q (B).

The charges available on the surface of a protein depend on the pH of its environment. When the pH is equal to the 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 protein pI.

Ion exchange chromatography begins with equilibration of the column in order 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 interfering effects on the separation. Upon loading the resin the sample proteins with suitable charge will bind to the charged groups of the resin displacing the counter ions. Desorption of the proteins (elution) is achieved by increasing the concentration of counter ion (salt gradient elution). Various additives, e.g., enzyme inhibitors, non-ionic detergents, urea and low concentrations of organic solvent, may be used in samples and buffers for IEX. It is important that they do not interact strongly 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, if required, be done in the presence of up to 30% acetonitrile or ethanol to keep the peptide soluble and to facilitate a good separation.

Instructions

Purification can be carried out at room temperature or at temperatures down to 4°C depending if the target is unstable at room temperature. Operation at a lower temperature may require a reduced flow rate due to the increased viscosity of the buffer. The recommended flow rates are listed in Table 1. If the chromatography system has a pressure limit function, set the maximum pressure over the column to 2.1 MPa (21 bar, 305 psi) (remember to take the system fluidics contribution to the pressure into account).

Note: The column is delivered containing 20% ethanol. Make sure to not exceed 3 ml/min when removing the storage solution.

Note: It is recommended to make a blank run (no sample) to make sure that the column is fully equilibrated.

1. Sample preparations

After cell disruption or extraction, clarify the sample by centrifugation at 10 000 - 20 000 × *g* for 15 - 30 minutes. It is generally recommended to also pass the sample through a 0.22 - 0.45 µm filter to avoid applying any remaining particles onto the column. If the sample contains only small amounts of particles, centrifugation may be omitted and it is often enough only to carry out filtration. Application of a sample that has not been properly clarified may reduce the performance and lifetime of the column. The sample should be applied under conditions similar to those of the binding buffer. Exchange of buffer can be carried out using a BabyBio Dsalt column, see *Related products*, or by ultrafiltration. Reduction of ionic strength can sometimes be achieved by dilution, especially if no change in pH is required.

2. Column preparation

Connect the column to the pump or chromatography system using finger tight connectors (coned 10-32 threads) for 1/16” o.d. tubing. Fill the equipment with deionized water or buffer and make a drop-to-drop connection, to avoid getting air into the column.

Note: The column contains a storage solution of 20% ethanol on delivery. This storage solution should be washed out before use. Wash the column with 5 CV of deionized water or buffer. Avoid flow rates above 3 ml/min (225 cm/h) until the storage solution has been completely washed out to avoid overpressure and column damage.

3. Equilibrate the packed column

Choose a suitable pH and buffer for the binding of the target protein. The buffer should be selected to give a good buffering capacity, with pK_a within 0.5 units from the intended pH for binding the target protein on the selected IEX column. Examples of buffers to be used are listed in Table 2. Equilibrate the column with 10 CV binding buffer at the chosen pH.

Note: To avoid bacterial growth and poor column performance, use only freshly prepared and filtered buffers.

4. Apply the sample

Apply a clarified sample to the column at low ionic strength and the chosen pH to allow binding of the target protein. Samples should have a pH that confers the target protein with charges that are opposite the charge of the column resin. The pH together with the ionic strength in the sample solution might need adjustment for optimal binding. Apply the sample at recommended flow rates; a too high flow rate may reduce yield.

5. Wash

After sample application, remove unbound impurities by washing the column with 10 - 30 CV of binding buffer or until desired absorbance at 280 nm of the wash fractions (e.g., 0.01 - 0.02) is obtained.

6. Elute the target protein

Alternative 1, step gradient: Elute the target protein with 5 CV elution buffer.
Alternative 2, linear gradient: For increased purity, a linear gradient elution is recommended. For example, use a linear gradient from 0 to 100% binding buffer with 1 M NaCl over 20 CV.

Note: A wash with 2 M NaCl can be included after elution, to ensure desorption of tightly bound substances.

7. Clean and re-equilibrate

Clean the column with 0.5 - 1 M NaOH for 15 - 30 minutes and re-equilibrate the column with 10 CV of binding buffer to restore the pH. If new purification will be carried out, restart at step 4.

8. Column storage

Wash the packed column with 5 CV deionized water to remove the remaining buffer. Equilibrate the column with 10 CV 20% ethanol for storage. Close the column using the including plugs.

Table 1. Recommended and maximum flow rates[1] for OptioBio 40S 10x100 and OptioBio 40Q 10x100 columns.

OptioBio 10x100	Volumetric flow rate (cm/h)	Linear flow rate (ml/min)
Recommended flow rate	150 - 300	2 - 4
Maximum flow rate	450	6

1. Water or aqueous buffers. Eluents with higher viscosity may require reduced flow rate.

Optimization

The following paragraphs give indications on some parameters that can be tuned to provide the optimal conditions for purification of proteins, peptides and nucleic acids using OptioBio 40S 10x100 and OptioBio 40Q 10x100 columns.

Selection of buffer

Selecting a buffer with optimal binding and elution conditions for the target protein will improve the result of the purification. The buffer should be selected with a high buffering capacity and with a pK_a-value within 0.5 units from the intended pH. Table 2 shows examples of buffers which can be used for ion exchange chromatography, however the buffer choice will be depending on the target molecule and aim of the purification procedure. For other useful buffers and their pK_a-values at 25 °C see: Methods in Enzymology, Volume 463, pp 46-47, Burgess, R.R and Deutcher M.P.

Table 2. Example of buffers using OptioBio 40S 10x100 and OptioBio 40Q 10x100 columns.

Product	Buffer	Buffer composition
OptioBio 40S 10x100	Binding buffer	50 mM Na-phosphate, pH 7.0
	Elution buffer	50 mM Na-phosphate, 1 M NaCl, pH 7.0
OptioBio 40Q 10x100	Binding buffer	50 mM Tris-HCl, pH 7.4
	Elution buffer	50 mM Tris-HCl, 1 M NaCl, pH 7.4

The buffer chemistry should be selected to have the same charge as the resin. A buffer with opposite charge will interact with the charged groups in the resin and may cause local pH disturbances that produces unwanted effects on the separation. Usually, a low conductivity of the binding buffer is preferred but optimization with regards to pH and conductivity can improve binding capacity and purity. An increase in ionic strength may decrease binding of impurities while the target protein remains bound. However, chromatographic conditions must be selected keeping the protein stable during purification.

Optimization of binding conditions

The key conditions to optimize 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 protein. The conditions must also be selected to keep the protein in its native active state.

The flow rate during sample loading affects the dynamic binding capacity (DBC) and resolution during elution. A low flow rate during sample application promotes binding capacity since more time is allowed for mass transport of the target substance molecule into the pores of the resin. Increasing the flow reduces the DBC. A small molecule, e.g., a peptide that has a high diffusion rate will have fast mass transport into the pores. The DBC is therefore less dependent on flow for these substances. A larger target substance (e.g., a large protein) has a lower diffusion rate and is more hindered by the walls in the pores, and will therefore have a slower mass transport. A high binding capacity of this substance may require a reduced flow rate. In small-scale purifications it may be convenient to apply a moderate sample load to avoid having to optimize the sample size. In process development it is recommended to determine the DBC, and adjust the feed volume load not more than 75-80% of DBC to avoid loss of target substance during sample application. This is an insurance against reduced yield due to a decline in DBC following multiple purification cycles. If only a part of the capacity is used, it may possible to increase the flow during the sample application without reduction of yield.

The residence time can be defined as the time between entering and exiting the column of a specific part of the sample or buffer. The residence time depends 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 in columns with 100 - 200 mm bed heights. See further discussion about flow in the section *Scale-up*.

In addition to the described method, the IEX columns can be used in a flow through mode (negative chromatography). The binding conditions are optimized to allow impurities to bind to the column, while allowing the target molecule to pass through the column.

Optimization of washing

A continuously decreasing UV signal is an indication of unbound material still being washed out. The washing step should continue until the UV signal is stable and the same as for the washing buffer, or at least not more than 0.01 to 0.02. 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, with a slightly higher salt concentration or different pH compared to the binding buffer, to improve purification.

Optimization of elution conditions

Elution can be carried out using a high salt concentration to displace the target, or by altering the pH to change the charge of the adsorbed protein. A stronger binding requires higher salt concentration for elution. The optimal salt concentration and pH depend the properties of the target protein and the impurities. There is usually a need for a trade-off between purity and DBC (or yield). Applying linear gradient elution gives increased purity than step elution, but step elution may be necessary to obtain the highest possible concentration of the target protein.

In order to optimize the salt concentration for step elution an initial linear gradient test run should be carried out to obtain suitable step elution conditions, see Figure 2. Step gradient elution is often used in larger scale purifications due to its simplicity and because it confers stability of the process.

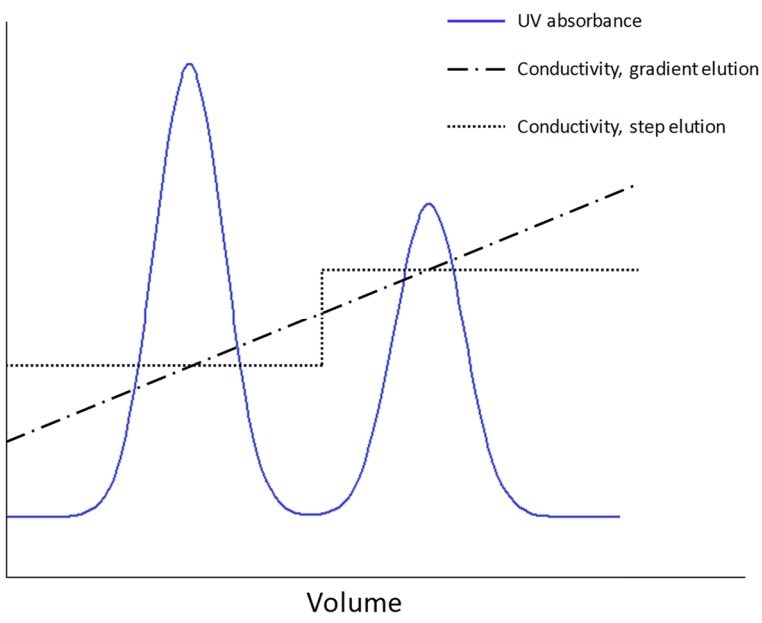


Figure 2. Optimization of step elution with salt. A test run with linear gradient elution gives information about suitable salt concentrations to be used in the step elution. Note: Remember to take the system dead volume into account when comparing the print out of the gradient and the trace.

Scale-up

The OptioBio columns are packed with WorkBeads resins. After the development of a purification procedure using an OptioBio 10x100 column, WorkBeads resins can be packed in larger columns for scale-up. Large-scale purification is often carried out in columns with bed heights of 100 - 350 mm. Scale-up from a 10x100 mm to a 25x200 mm column (approximate 12 times) may be a first step.

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 constant. The linear flow rate should remain the same while the volumetric flow rate increases. The volumetric flow rate 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 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 are:

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

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

In initial process development projects it is common to use a small column to save sample, buffers and time. Such a column often has a shorter bed height than the final column which may have a bed height of 200 mm or more. The flow rate to be used for the larger column can be calculated from the flow rate that was established on the small column, using the equation above, by keeping the residence time from the small column the same for the larger column. This allows 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 heights are kept constant during scale-up the linear flow rate should be kept constant (as well as the residence time).

Table 3. Examples of scale-up.

Column dimension	Residence time (minutes)	Linear flow rate (cm/h)	Volumetric flow rate (ml/min)
10 × 100	4	150	1.96
25 × 200	4	300	24.5
50 × 200	4	300	98.2

Additional purification steps

Optimization of the purification process by tuning the binding, washing and/or elution conditions of the IEX purification step may still not be enough to obtain the required purity. Combining two or more purification steps based on additional chromatography techniques is then recommended. For example, cation exchange chromatography and anion exchange chromatography are useful combinations in a purification process. Other techniques, such as affinity chromatography (AC), size exclusion chromatography (SEC) (gel filtration) and hydrophobic interaction chromatography (HIC) are commonly used alternatives. Each purification step should be thoroughly optimized, preferably in the context of the other steps, in the overall process.

Desalting and buffer exchange

Buffer exchange or desalting of a sample is very useful before analysis and/or before/after ion exchange chromatography. This can be carried out quickly and easily in lab-scale, using BabyBio Dsalt 1 ml or BabyBio Dsalt 5 ml columns (see *Related products*). BabyBio Dsalt columns are also a quick and convenient alternative to dialysis for larger sample volumes or when samples need to be processed rapidly to avoid degradation. For larger processes, diafiltration is recommended.

To find out more about chromatography resins for additional purification, please visit www.bio-works.com

Maintenance

Unpacking and inspection

Unpack the shipment as soon as it arrives and inspect it for damage. Please, promptly report any damage or discrepancies to your local supplier.

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. Fouling is typical even for well clarified samples. The severity of this process depends on the composition of the sample applied to the column. The impurities bound to the resin 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. CIP of the packed column can be carried out as followed:

1. Wash the column with 5 CV deionized water.
2. Apply 3 - 10 CV of 0.5 - 1 M NaOH for 15 - 30 minutes.

   Note: The contact time is the important factor, overnight treatment with NaOH can be necessary if severely fouled.

3. Wash the column with 5 - 10 CV deionized water or binding buffer (until the column is neutralized 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.

Storage

Store the prepacked column at 2 to 25°C in 20% ethanol. Make sure that the columns is securely closed.

Additional information

Product description

	OptioBio 40S 10x100	OptioBio 40Q 10x100
Target substance	Proteins and peptides	Protein, peptides and oligonucleotides
Resin	WorkBeads 40S	WorkBeads 40Q
Matrix	Rigid, highly cross-linked agarose	Rigid, highly cross-linked agarose
Average particle size (DV_{50})[1]	45 µm	45 µm
Ionic group (ligand)	Sulfonate ($-SO_3^-$)	Quarternary amine ($-N^+(CH_3)_3$)
Ionic capacity	0.18 - 0.25 mmol Na^+/ml resin	0.18 - 0.25 mmol Cl^-/ml resin
Dynamic binding capacity (DBC)	150 mg BSA/ml resin[2]	47 mg BSA/ml resin[3]
Column volume (CV)	7.9 ml	7.9 ml
Column dimension	10 x 100 mm	10 x 100 mm
Recommended flow rate	2 - 4 ml/min (150 - 300 cm/h)	2 - 4 ml/min (150 - 300 cm/h)
Maximum flow rate[4]	6 ml/min (450 cm/h)	6 ml/min (450 cm/h)
Column hardware pressure limit	2.1 MPa, 21 bar, 305 psi	2.1 MPa, 21 bar, 305 psi
Chemical stability	Compatible with all standard buffers used for protein purification, 1 M NaOH, 30 % isopropanol or 70 % ethanol. Should not be stored at < pH 3 for prolonged time.	
pH stability	3 - 12 (working range) 2 - 13 (cleaning)	3 - 12 (working range) 2 - 13 (cleaning)
Storage	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 in 20 mM Na-citrate, pH 4.0, at a flow of 2 ml/min (150 cm/h; 4 minutes residence time).
3. Dynamic binding capacity determined in 50 mM Tris-HCl, 50 mM NaCl, pH 8.0, at a flow of 2 ml/min (150 cm/h; 4 minutes residence time).
4. Maximum flow rate for aqueous buffers at 20 °C. 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 for 20% ethanol.

Intended use

OptioBio 40S 10x100 and OptioBio 40Q 10x100 columns are intended for research, process development and bioprocesses only.

Safety

Please, read the Safety Data Sheets (SDS) for the product and the safety instructions for any equipment to be used. Make precautions to avoid exceeding the maximum column hardware pressure. If available, use the max pressure-function of the chromatography system. Use safety glasses when handling glassware.

Notes:

Related products

Product name	Pack size[1]	Article number
Prepacked columns		
BabyBio S 1 ml	1 ml x 5	45 200 103
BabyBio S 5 ml	5 ml x 5	45 200 107
BabyBio Q 1 ml	1 ml x 5	45 100 103
BabyBio Q 5 ml	5 ml x 5	45 100 107
BabyBio DEAE 1 ml	1 ml x 5	45 150 103
BabyBio DEAE 5 ml	5 ml x 5	45 150 107
BabyBio Dsalt 1 ml	1 ml x 5	45 360 103
BabyBio Dsalt 5 ml	5 ml x 5	45 360 107
Bulk resins		
WorkBeads 40S	25 ml	40 200 001
	200 ml	40 200 002
	1 L	40 200 010
	5 L	40 200 050
WorkBeads 40Q	25 ml	40 100 001
	200 ml	40 100 002
	1 L	40 100 010
	5 L	40 100 050
WorkBeads 40 DEAE	25 ml	40 150 001
WorkBeads 100S	25 ml	10 200 001
WorkBeads 100Q	25 ml	10 210 001

1. Other pack sizes can be found in the complete product list on our website www.bio-works.com

Ordering information

Product name	Pack size	Article number
OptioBio 40S 10x100	7.9 ml x 1	55 420 011
OptioBio 40Q 10x100	7.9 ml x 1	55 410 011

Orders: sales@bio-works.com or contact your local distributor.

For more information about local distributor and products please visit www.bio-works.com or contact us at info@bio-works.com

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