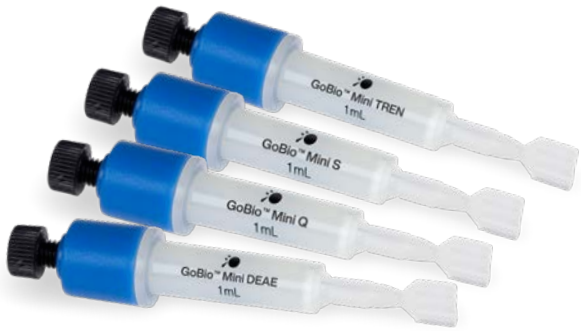
Bio-Works

INSTRUCTION

GoBio Mini IEX Screening Kit

GoBio™ Mini IEX Screening Kit includes four different ready-to-use 1 mL ion exchange chromatography columns for easy and convenient optimization and purification of proteins, peptides and oligonucleotides, by utilizing the difference in these molecules surface charge. The columns included in this kit are GoBio Mini S (strong cation exchanger), GoBio Mini Q (strong anion exchanger), GoBio Mini DEAE (weak anion exchanger) and GoBio Mini TREN (multimodal anion exchanger). The columns are prepacked with WorkBeads™ 40S, WorkBeads 40Q, WorkBeads 40 DEAE and WorkBeads 40 TREN resins.



- Prepacked ready-to-use columns for fast screening of four different IEX resins
- Easy screening for optimal running conditions
- High binding capacity even at high flow rates

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 general short protocol is for the use of GoBio Mini S, GoBio Mini Q, GoBio Mini DEAE and GoBio Mini TREN columns. Detailed instructions and recommendations for optimization are given later in this instruction. Recommended and useful buffers are listed in Table 3. GoBio Mini S is suitable for basic proteins, i.e. proteins with a high isoelectric point (pI), while GoBio Mini Q and GoBio Mini DEAE are suitable for purification of acidic proteins, i.e. proteins with low pI. GoBio Mini TREN is prepacked with an anion multimodal IEX resin and will give different selectivity compared to standard IEX resins and should therefore be checked to find optimal separations.

1. Choose a suitable pH and buffer for the binding of the target protein. One pH unit below pI (GoBio Mini S columns) or above pI (GoBio Mini Q, GoBio Mini DEAE and GoBio Mini TREN columns) is a good starting point.
2. Connect the column to the chromatography system, syringe or pump.
3. Equilibrate the column with 10 column volumes (CV) 20 – 50 mM binding buffer at the chosen pH.
4. Apply a clarified sample to the column at low ionic strength and the chosen pH (similar with the binding buffer) to allow binding of the target protein.
5. Wash the column using 10 – 20 CV binding buffer.
6. Elute the target protein.

   Alternative 1: Desorb the target protein with 5 CV elution buffer.

   Alternative 2: For increased purity, gradient elution is recommended. For example, use a gradient from 0 to 100% with 20 CV binding buffer including 1 M NaCl.
7. Clean the column using 0.5 – 1.0 M NaOH for 15 – 30 minutes (optional).
8. Wash the column with 5 CV deionized water to remove the cleaning solution.
9. Equilibrate with 10 CV 20% ethanol for storage. Close the column using the included cap and plug. For GoBio Mini S column it is recommended to include 0.2 M sodium acetate in the storage solution.

Principle

Ion exchange chromatography

Ion exchange chromatography (IEX) can be used for purification of biomolecules, such as proteins, peptides and oligonucleotides, by utilizing the difference in their surface charge. The biomolecules interact with the immobilized ion exchange groups with opposite charge on the chromatography resin. WorkBeads resins are available with sulfonate groups (WorkBeads 40S), quaternary amines (WorkBeads 40Q) and tertiary amines (WorkBeads 40 DEAE) as the ion exchange groups.

WorkBeads 40S is a strong cation exchanger and will bind positively charged molecules. WorkBeads 40Q and WorkBeads 40 DEAE are strong and weak anion exchangers respectively and will bind negatively charged molecules. WorkBeads 40 TREN is a multimodal anion exchanger that is positively charged below pH 9. The structure of the ligands used in WorkBeads 40S, WorkBeads 40Q and WorkBeads 40 DEAE are shown in Figure 1.

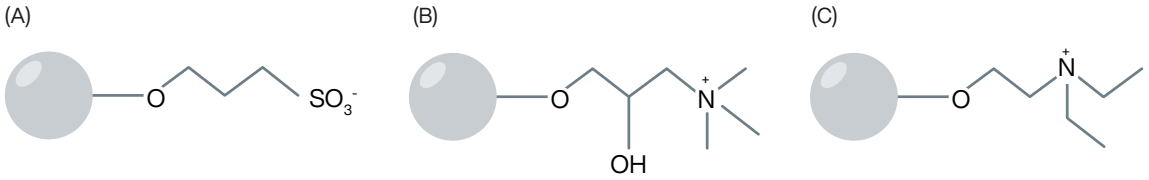


Figure 1. Structure of the ligand used in WorkBeads 40S (A), WorkBeads 40Q (B) and WorkBeads 40 DEAE (C).

The surface charge of proteins depends on the pH of their 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 above 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 (net charge). A protein may therefore interact with an ion exchange resin also at the pI. The likelihood of binding to either the cation or the anion exchange resin will increase when moving away from the pI.

Ion exchange chromatography begins with equilibration of the column to establish the desired pH and charging the resin with suitable counter ions to the charged ligands on the resin (e.g., the negative sulfonate groups can interact with Na+ ions and the positive trimethyl amine groups 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. When the sample is applied, proteins with suitable charge will bind to the charged groups of the resin while displacing the counter ions. Desorption of the proteins (elution) is carried out by increasing the concentration of counter ion (salt gradient elution). The counter ions will displace the proteins as the salt concentration increases. Various additives (e.g., enzyme inhibitors, non-ionic detergents, urea and low concentrations of organic solvent) can be used in samples and buffers for IEX as long as they do not strongly interact strongly with the charged groups on the resin or on the protein which could interfere with the protein binding to the resin.

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. It is also one of the more cost-effective chromatography techniques and is therefore excellent for scale-up.

Multimodal chromatography

Multimodal chromatography separates protein, peptides and other biomolecules via a ligand acting with more than one interaction sites. The interaction utilizes two, or more, different properties, for example charge and hydrophobicity. Depending on the chromatographic conditions the interactions differ, and work either together or separate in the purification procedure.

Using multimodal chromatography can be an excellent alternative, when the common techniques (e.g., ion exchange chromatography, size exclusion chromatography or affinity chromatography) are insufficient. However, to reach the optimal purification using multimodal chromatography, the purification process needs to be optimized according to the target molecule properties.

GoBio Mini TREN columns are prepacked WorkBeads 40 TREN resin. WorkBeads 40 TREN resins contain ligands based on Tris(2-aminoethyl)amine (TAEA). The structure of the ligand used in WorkBeads 40 TREN is shown in Figure 2.

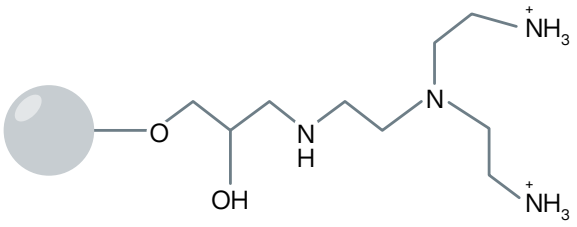


Figure 2. Structure of the ligand used in WorkBeads 40 TREN.

WorkBeads 40 TREN resin is positively charged below approx. pH 9. Choose a suitable pH and buffer for the binding of the target protein or the impurities (if the purification is done in “negative mode”). One pH unit above pI, for WorkBeads 40 TREN is a good starting point. The binding conditions should be optimized to achieve binding of the target protein or the impurities. When scouting for the best conditions it is important to start with sufficiently low ionic strength. Guideline for a starting point for designing the experiment is given in Table 3.

Instructions

Purification can be carried out at room temperature or at temperatures down to 4°C. Operation at a low temperature may require a reduced flow rate due to the increased viscosity of the buffer. All steps can be carried out with a syringe, a peristaltic pump or a chromatography system. If the chromatography system has a pressure limit functionality, set the maximum pressure over the column (resin bed) to 3 bar (remember to take the system fluidics contribution to the pressure into account).

1. Prepare the sample

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 pass the sample through a 0.22 – 0.45 µm filter (e.g. a syringe filter) to avoid inadvertently applying any remaining particles onto the column. If the sample contains only small amounts of particles it may be 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 with those of the binding buffer.

2. Connect the column

Cut off or twist off the end at the outlet of the column, see Figure 3.

Note: It is of high importance to cut off the tip at the very end of the cone, preferable using a scalpel. Incorrect removal of the end piece will affect the performance of the column.

Connect the column to your equipment using the recommended connectors shown in Table 1. Fill the equipment with deionized water or buffer and make drop-to-drop connection with the column to avoid getting air into the column. Carry out all steps, except for sample application, at 1 mL/min (GoBio Mini 1 mL column).

Figure 3. Removal of the cut-off end at the column outlet should be done by cutting or by twisting (A) not bending (B).

Table 1. Recommended connectors for coupling GoBio Mini columns to the equipment of choice.

Equipment	Accessories for connection
Syringe	Female luer/male coned 10 – 32 threads
Chromatography system	Fingertight connectors (coned 10 – 32 threads) for 1/16" o.d. tubing

3. Remove the storage solution

The column contains 20% ethanol on delivery. This storage solution should be washed out before use. Wash the column with 5 CV deionized water or buffer. Avoid flow rates higher than 2 mL/min for GoBio Mini 1 mL columns before the storage solution has been removed to avoid overpressure due to the relatively high viscosity of the 20% ethanol solution.

4. Equilibrate the column

Equilibrate the column with 10 CV of binding buffer. The buffer should be selected to provide a good buffering capacity, with pK_a within 0.5 units from the intended pH for capturing the target protein on the selected ion exchange chromatography column. Examples of buffers to be used are listed in Table 3.

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

5. Apply the sample

Apply the sample at 0.5 – 1 mL/min for the GoBio Mini 1 mL. A too high flow rate may reduce the yield.

Applied samples should have a pH that gives the target protein a charge that is opposite the charge of the column resin. The pH together with the ionic strength in the sample solution might need adjustment for optimal binding.

6. Wash

After sample application, remove unbound impurities by washing the column with 20 – 30 CV washing buffer or until desired A_{280} nm absorbance of the wash fractions (e.g., 0.01 – 0.02) is obtained.

7. Elute

Alternative 1: Desorb the target protein with 5 CV elution buffer.

Alternative 2: For high purity, gradient elution is recommended. For example, use a linear gradient from 0 to 100% with 20 CV binding buffer including 1 M NaCl.

A stringent wash step with, for instance, 2 M NaCl can be included after elution, to ensure desorption of all interacting proteins.

8. Re-equilibrate

Clean the column with 0.5 – 1 M NaOH for 15 – 30 minutes and re-equilibrate the column with 10 CV binding buffer to restore the pH. If additional separation will be carried out, restart at step 5.

Some resins become yellowish during CIP with NaOH (0.5 M or 1 M), and they will become white again when washing with deionized water (recommended 3 – 5 column volumes) and after this apply the preferred equilibration buffer.

From the product stability point of view our thorough work shows that, for example, WorkBeads 40S is tolerant to up to 1 M NaOH (one week at room temperature) without significantly decrease in ionic capacity and dynamic binding capacity or any significant change of pattern of selectivity. We conclude that this phenomenon is intrinsic to agarose products to a lesser or larger degree and will not change the functionality of the resin based on our measurements and analysis.

One explanation for the resin sometimes becoming yellowish during CIP with 0.5 – 1 M NaOH is that during the CIP we get a dehydration of the resin reducing the water content which makes the resin more compact resulting in a darker (yellowish) color of the resin.

9. Column storage

Wash the column with 5 CV deionized water to remove the remaining buffer. Equilibrate the column with 10 CV 20% ethanol for storage. For GoBio Mini S column it is recommended to include 0.2 M sodium acetate in the storage solution. Close the column using the cap and plug (included).

Scale-up

GoBio Mini S 1 mL, GoBio Mini Q 1 mL, GoBio Mini DEAE 1 mL and GoBio Mini TREN 1 mL columns can be used for purification of up to 50 – 100 mg of protein. Scale-up from a GoBio Mini 1 mL column can be carried out by using a GoBio Mini 5 mL column and applying a five times larger sample volume. GoBio Mini columns can be connected in series with a maximum of five columns (column stacking). This will increase the total capacity of the run accordingly. By connecting GoBio Mini columns in series, any column volumes from 1 mL to 25 mL can be obtained. This means a binding capacity of 1000 – 2000 mg of protein can be achieved. Column size selection should be based on estimated amount of target protein in each run.

GoBio Mini columns are easily connected without accessories. Up to five columns may be connected in series (column stacking). The pressure drop across each column bed will be the same as for a single column, but the upstream columns will be subjected to a higher internal pressure from the added pressure drops from downstream columns. It may therefore be necessary to decrease the flow rate accordingly to avoid exceeding the maximum pressure limit onto the first column. If possible, the maximum pressure of the chromatography system should be set according to Table 2. Remember always to take the system fluidics contribution to the pressure into account.

Table 2. Recommended maximum pressure settings for GoBio Mini columns connected in series. Notice that the maximum pressure over each column is always 3 bar.

No. of columns in series	Max pressure GoBio Mini 1 mL (bar)	Max pressure GoBio Mini 5 mL (bar)
1	3.0	3.0
2	6.0	6.0
3	9.0	9.0
4	12	10[1]
5	15	10[1]

[1] The maximum pressure is defined by the column hardware maximum pressure.

Column size selection should be based on the estimated amount of protein to be purified. A test run with a defined small volume of sample on a GoBio Mini 1 mL column should be used to estimate the concentration of the target protein in the sample. A general recommendation is to use 70 – 80% of the column binding capacity. For large sample volumes with low concentrations of the target protein, it may be suitable to use a larger column than the one calculated to allow higher sample flow rates, and consequently shorter application time. For example, using a 5 mL column instead of a 1 mL column allows a flow rate five times higher due to the larger cross-section of the column. Have in mind that too high flow rate may reduce binding capacity.

For columns larger than 20 mL, it is recommended to pack a single column using bulk resin or for convenience and reproducibility use one of the prepacked columns from the GoBio column family, as the limitations of column stacking will then impact chromatographic performance, see “Related products”.

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Optimization

The following paragraphs will give indications on some parameters that can be tuned to find the optimal conditions for the purification.

Buffer selection

Selecting a buffer with optimal conditions for the target protein will improve the result of the purification. The buffer should be chosen with a pK_a-value within 0.5 units from the intended pH

to obtain a high enough buffering capacity. Table 3 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 reference: Methods in Enzymology, Volume 463, pp 46-47, Burgess, R.R and Deutcher M.P.

Table 3. Example of buffers for model protein purification using GoBio Mini S, GoBio Mini Q, GoBio Mini DEAE and GoBio Mini TREN. Other buffers can be used.

Buffer	Product	Buffer composition
Binding buffer	GoBio Mini S	50 mM sodium phosphate, pH 7.0
Binding buffer	GoBio Mini Q	50 mM Tris-HCl, pH 7.4
Binding buffer	GoBio Mini DEAE	50 mM Tris-HCl, pH 7.4
Binding buffer	GoBio Mini TREN	50 mM Tris-HCl, pH 7.4
Elution buffer	GoBio Mini S	50 mM sodium phosphate, 1 M NaCl, pH 7.0
Elution buffer	GoBio Mini Q	50 mM Tris-HCl, 1 M NaCl, pH 7.4
Elution buffer	GoBio Mini DEAE	50 mM Tris-HCl, 1 M NaCl, pH 7.4
Elution buffer	GoBio Mini TREN	50 mM Tris-HCl, 1 M NaCl, pH 7.4

The buffer substance 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 destroys the separation. Usually, low conductivity in the binding buffer is preferred but optimization with regards to pH and conductivity can improve binding capacity. An increase in ionic strength may decrease the ability of contaminants to bind while the target protein remains bound. However, chromatographic conditions should be chosen so that the protein is stable during purification.

Optimization of binding

The key conditions to be optimized is usually pH and conductivity (by addition of NaCl, other salts, or dilution). Conditions selection should be to maximize purity and/or yield of the target protein, while keeping it in a native/active state.

pH and salt optimization

Strong ion exchangers (S and Q) are used over a broad pH range. The useful pH range is limited by the target protein pH stability and solubility window. Weak ion exchangers have a narrower pH range for usage. The weak DEAE anion exchanger must be used at a pH below its pK_a value of 9.0 – 9.5 to retain its positive charge. The difference between the Q and DEAE ligands may give desired differences in selectivity. Purification is often done by combining an anion exchange column and a cation exchange column with or without changing the pH.

It should be noted that the binding capacity and purity depends on the combination of pH and counter ion concentration (i.e., salt concentration or ionic strength). Therefore, it is recommended to investigate the combination effects of pH and salt concentration during optimization. A low salt concentration is considered to give strong binding with high capacity, but it should be noted that it is often observed that an intermediate concentration of salt gives a better binding capacity. For example, a concentration of 50 mM NaCl in the buffer may give a better capacity than 20 mM NaCl. This may be attributed to improved mass transport of target substance into the pores of the resin, obtained by reduced pore exclusion. Pore exclusion can be explained as a hindrance of diffusion caused by strong interaction of substance on the walls of the outer pores, causing, in effect, a “traffic jam” and thus reduced diffusion rate into the pores. A slightly elevated salt concentration reduces, but does not eliminate, the interactions with the resin by creating a dynamic adsorption-desorption equilibrium that allows further diffusion into the resin, thus increasing the binding capacity.

Tuning the flow rate

Flow rate is another factor that can be optimized to improve the binding capacity during sample application or the 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 into the pores of the resin. A small substance (e.g., a peptide) has a high diffusion rate and is not hindered by the walls in the pores and will have fast mass transport into the resin and thus be adsorbed at a high flow rate. A large target substance (e.g., a large protein) has a lower diffusion rate and is held back by the walls of the pores slowing its mass transport. A high binding capacity of this substance may require a lower flow rate. If only a part of the binding capacity of the resin is used the sample application can be done at a higher flow rate without loss of the target substance.

For scale-up planning it is useful to use the expression residence time instead of flow rate. The residence time can be defined as the time between entering and exiting the column of a specific part of the sample or buffer. It can be calculated as column volume divided by the volumetric flow (e.g., the residence time for 1 mL column at 0.5 mL/min is 1 mL and at 0.5 mL/min is 2 minutes). The residence time is typically 1 to 5 minutes in IEX. When a suitable residence time has been selected using GoBio Mini Q, GoBio Mini S, GoBio Mini DEAE or GoBio Mini TREN columns, this value can be used for calculation of a suitable flow rate on a larger column with higher bed. The linear flow rate can be increased if the bed height is increased while keeping the residence time constant.

Optimization of washing and elution

Prolonged wash

A continuously decreasing UV signal during washing is an indication of unbound material being washed out (it may be target substance if it is weakly bound). Washing should be continued until the UV absorbance signal has reached 0.01-0.02 (“10-20 mAU”). A GoBio Mini column should be washed with at least 10 CV buffer.

Optimizing elution conditions

Elution can be carried out using a high salt concentration or by altering the pH to change the charge of the adsorbed protein. A stronger binding may require higher salt concentration for elution.

Step elution

The optimal salt concentration is dependent on purity and recovery requirements as well as properties of the target protein and the sample. Using a gradient elution gives increased purity compared to step elution, but step elution may be necessary to obtain the highest possible concentration of the target protein. To optimize the salt concentration for step elution an initial gradient test run can be carried out to identify suitable step elution conditions for purification of the target protein, see Figure 4.

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

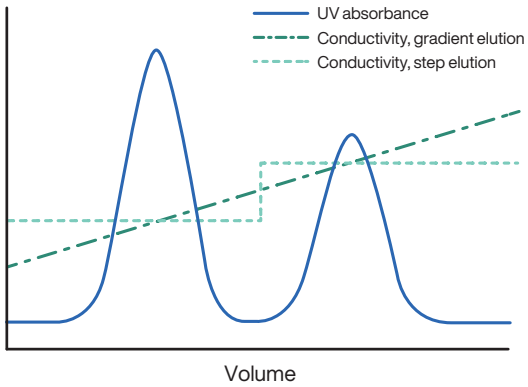

Figure 4. Optimization of step gradient elution with salt. A test run with linear gradient elution gives information about suitable salt concentrations to be used during the step elution.

Extra purification step

Optimization of the overall purification process by tuning the binding, washing and/or elution steps is an option. However, an additional purification step based on another chromatography technique is recommended, see “Additional purification”.

Desalting and buffer exchange

Buffer exchange or desalting of a sample can be used before analysis and/or after purification with for example ion exchange chromatography. 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) columns depending on sample volumes, see “Related products”. These columns are also very useful alternatives to dialysis or when samples need to be processed rapidly to avoid degradation. For even larger sample volumes prepacked GoBio Prod columns starting from 1 L are available or diafiltration can be used.

Additional purification

Ion exchange chromatography and multimodal chromatography are powerful single protein purification steps or combined with other chromatography techniques. The overall process needs to be optimized for each purification step.

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Maintenance of the column

Cleaning

During purification impurities such as cell debris, lipids, nucleic acids and protein precipitates from the samples may gradually build up in the resin. The severity of this process depends on the type of sample applied to the column, and the pre-treatment of the sample. The impurities may reduce the performance of the column over time. Regular cleaning (Cleaning-in-place, CIP) keeps the resin clean, reduces the rate of further contamination, and prolongs the capacity, resolution and flow properties of the column. Cleaning of a resin using 1 M NaOH applied by a low reversed flow for 2 hours or overnight is often sufficient. Do not use elevated temperature during the CIP treatment, since this may reduce the lifetime of the resin.

CIP of the 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, treatment with NaOH overnight can be necessary if severely fouled.
3. Wash the column with 5 – 10 CV deionized water or binding buffer (until the column is neutral after CIP).
4. Equilibrate the column with 10 CV 20% ethanol (for storage). For GoBio Mini S column it is recommended to include 0.2 M sodium acetate in the storage solution.

Some resins turn more yellow 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 done using combinations of NaOH and ethanol (e.g., incubation with a mixture of 0.5 M NaOH and 40% ethanol for 3 hours). The sanitization procedure and its effectiveness will depend on the microorganisms to be removed and needs to be evaluated for each case.

Storage

Equilibrate the column with 20% ethanol and close it securely using the included plug and cap.

For GoBio Mini S column it is recommended to include 0.2 M sodium acetate in the storage solution. Store the column at 2 to 25 °C.

Product information

	GoBio Mini S	**GoBio Mini Q**	**GoBio Mini DEAE**
Target substance	Proteins, peptides	Protein, peptides, oligonucleotides, viruses	Protein, peptides, oligonucleotides
Resin	WorkBeads 40S	WorkBeads 40Q	WorkBeads 40 DEAE
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
Ligand	Sulfonate ($-SO_3^-$)	Quaternary amine ($-N^+(CH_3)_3$)	Diethylaminoethyl ($-CH_2CH_2N^+H(CH_2CH_3)_2$)
Ion capacity	180 – 250 µmol H^+/mL resin	180 – 250 µmol Cl^-/mL resin	110 – 160 µmol Cl^-/mL resin
Dynamic binding capacity	130 mg BSA/mL resin[2]	50 mg BSA/mL resin[3]	40 mg BSA/mL resin[3]
Column volume	1 mL 5 mL	1 mL 5 mL	1 mL 5 mL
Column dimension	7 × 28 mm (1 mL) 13 × 38 mm (5 mL)	7 × 28 mm (1 mL) 13 × 38 mm (5 mL)	7 × 28 mm (1 mL) 13 × 38 mm (5 mL)
Recommended flow rate[4]			
GoBio Mini 1 mL	0.25 – 1 mL/min (37 – 150 cm/h)	0.25 – 1 mL/min (37 – 150 cm/h)	0.25 – 1 mL/min (37 – 150 cm/h)
GoBio Mini 5 mL	1.25 – 5 mL/min (56 – 225 cm/h)	1.25 – 5 mL/min (56 – 225 cm/h)	1.25 – 5 mL/min (56 – 225 cm/h)
Maximum flow rate[5]			
GoBio Mini 1 mL	5 mL/min (780 cm/h)	5 mL/min (780 cm/h)	5 mL/min (780 cm/h)
GoBio Mini 5 mL	20 mL/min (900 cm/h)	20 mL/min (900 cm/h)	20 mL/min (900 cm/h)
Maximum back pressure	0.3 MPa, 3 bar, 43 psi	0.3 MPa, 3 bar, 43 psi	0.3 MPa, 3 bar, 43 psi
Chemical stability	Compatible with all standard aqueous buffers used for protein purification 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 (0.25 mL/min in 1 mL column) in 20 mM Na-citrate, 60 mM NaCl, pH 4.0.
[3] Dynamic binding capacity determined at 4 minutes residence time (0.25 mL/min in 1 mL column) in 50 mM Tris-HCl, 50 mM NaCl, pH 8.0.
[4] Optimal flow rate during binding is depending on the sample. During column wash and elution, a flow rate of 1 mL/min and 5 mL/min can be used for 1 mL and 5 mL columns, respectively. Note: The maximum pressure the packed bed can withstand depends on the sample/liquid viscosity and chromatography resin characteristics. The pressure also depends on the tubing used to connect the column and the system restrictions after the column outlet.
[5] 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).
[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.

Product information

	GoBio Mini TREN
Target substance	Proteins, peptides, oligonucleotides, viruses
Resin	WorkBeads 40 TREN
Matrix	Rigid, highly cross-linked agarose
Average particle size (D_{v50})[1]	45 µm
Ligand	Tris(2-aminoethyl)amine (TAEA)
Dynamic binding capacity	50 mg BSA/mL resin[2]
Column volume	1 mL 5 mL
Column dimension	7 × 28 mm (1 mL) 13 × 38 mm (5 mL)
Recommended flow rate[3] GoBio Mini 1 mL GoBio Mini 5 mL	 0.25 – 1 mL/min (37 – 150 cm/h) 1.25 – 5 mL/min (56 – 225 cm/h)
Maximum flow rate[4] GoBio Mini 1 mL GoBio Mini 5 mL	 5 mL/min (780 cm/h) 20 mL/min (900 cm/h)
Maximum back pressure	0.3 MPa, 3 bar, 43 psi
Chemical stability	Compatible with all standard aqueous buffers used for protein purification. Do not keep the column at low pH for prolonged time.
Operational pH range[5]	2 – 13
CIP and screening pH range[6]	2 – 14
Storage	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 (0.25 mL/min in 1 mL column) in 50 mM Tris-HCl, 50 mM NaCl, pH 8.0.
3 Optimal flow rate during binding is depending on the sample. During column wash and elution, a flow rate of 1 mL/min and 5 mL/min can be used for 1 mL and 5 mL columns, respectively. Note: The maximum pressure the packed bed can withstand depends on the sample/liquid viscosity and chromatography resin characteristics. The pressure also depends on the tubing used to connect the column and the system restrictions after the column outlet.
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).
5 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 S 1 mL	1 mL × 1 1 mL × 5 1 mL × 10	45 200 101 45 200 103 45 200 104
GoBio Mini S 5 mL	5 mL × 1 5 mL × 5 5 mL × 10	45 200 105 45 200 107 45 200 108
GoBio Mini Q 1 mL	1 mL × 1 1 mL × 5 1 mL × 10	45 100 101 45 100 103 45 100 104
GoBio Mini Q 5 mL	5 mL × 1 5 mL × 5 5 mL × 10	45 100 105 45 100 107 45 100 108
GoBio Mini DEAE 1 mL	1 mL × 1 1 mL × 5 1 mL × 10	45 150 101 45 150 103 45 150 104
GoBio Mini DEAE 5 mL	5 mL × 1 5 mL × 5 5 mL × 10	45 150 105 45 150 107 45 150 108
GoBio Mini TREN 1 mL	1 mL × 1 1 mL × 5 1 mL × 10	45 655 211 45 655 213 45 655 214
GoBio Mini TREN 5 mL	5 mL × 1 5 mL × 5 5 mL × 10	45 655 215 45 655 217 45 655 218
GoBio Mini Peptide Purification Kit[2]	1 mL × 2	45 300 102
GoBio Mini Dsalt 1 mL	1 mL × 1 1 mL × 5 1 mL × 10	45 360 101 45 360 103 45 360 104
GoBio Mini Dsalt 5 mL	5 mL × 1 5 mL × 5 5 mL × 10	45 360 105 45 360 107 45 360 108
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[2]	3.8 mL × 1	55 415 001
GoBio Screen 7x100 40 TREN	3.8 mL × 1	55 463 001
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[2]	20 mL × 1	55 415 021
GoBio Prep 16x100 40 TREN	20 mL × 1	55 463 021
GoBio Prep 16x100 Dsalt[2]	20 mL × 1	55 700 021
GoBio Prep 26x100 40S	53 mL × 1	55 420 031
GoBio Prep 26x100 40Q	53 mL × 1	55 410 031
GoBio Prep 26x100 40 DEAE[2]	53 mL × 1	55 415 031
GoBio Prep 26x100 40 TREN[2]	53 mL × 1	55 463 031
GoBio Prep 26x100 Dsalt	53 mL × 1	55 700 031

Bulk resin		
WorkBeads 40S	25 mL 200 mL	40 200 001 40 200 002
WokBeads 40Q	25 mL 200 mL	40 100 001 40 100 001
WorkBeads 40 DEAE	25 mL 200 mL	40 150 001 40 150 002
WorkBeads 40 TREN	25 mL 150 mL	40 603 001 40 603 003
WorkBeads Dsalt	300 mL	40 360 003
Accessories		
Column plug male 1/16”	10	70 100 010
Column cap female 1/16”	10	70 100 020

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

Ordering information

Product name	Pack size	Article number
GoBio Mini IEX Screening Kit[1]	1 mL × 4	45 900 001

[1] 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.

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

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

bio-works.com

Bio-Works, Virdings allé 18, 754 50 Uppsala, Sweden. For local office contact information, visit bio-works.com/contact.

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