Bio-Works

INSTRUCTION

GoBio Mini Butyl SH

The ready-to-use GoBio™ Mini Butyl SH columns are prepacked with WorkBeads™ 40 Butyl SH resin and are available in two column sizes, 1 mL and 5 mL. The functional ligand of WorkBeads 40 Butyl SH is *n*-butyl thioether. Since butyl is a very hydrophobic linear chain, minimal mixed-mode interactions are expected. This resin can be used for several different applications, such as purifications of proteins, peptides, plasmids and oligonuclotides.

- Prepacked and ready-to-use columns for fast and reliable results
- High throughput, binding capacity, and purity
- Reliable and reproducible results
- High chemical stability for easy cleaning-in-place

Intended use

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

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

Safety

Please read the Safety Data Sheet (SDS) for WorkBeads 40 Butyl SH and the safety instructions for any equipment to be used.

Unpacking and inspection

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

Short protocol

This is a general short protocol for the use of GoBio Mini columns. Detailed instructions and recommendations for optimization are given later in this instruction.

1. Connect the column to the chromatography system, syringe or pump.
2. Equilibrate with high-salt binding buffer.
3. Apply the sample.
4. After sample application, remove unbound material by washing the column with e.g., 20 – 30 columns volumes (CV) binding buffer.
5. Elute with a low-salt elution buffer.
6. Wash the column with 5 CV 0.02 M NaCl to remove the elution buffer.
7. Equilibrate the column with 5 CV 20% ethanol for storage. Close the column using the included cap and plug.

Principle

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

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

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

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

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

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

The functional ligand of WorkBeads 40 Butyl SH is n-butyl thioether, shown in Figure 1. Table 1 offers guidance on selecting appropriate buffers to initiate the purification design process.

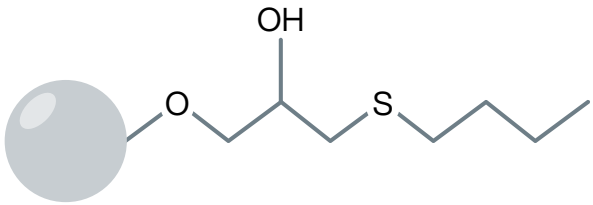

Figure 1. Structure of the ligand used in WorkBeads 40 Butyl SH.

Table 1. Typical buffer composition for purification using GoBio Mini Butyl SH

Target molecule	Binding buffer	Elution buffer
Proteins	50 mM phosphate, 1.2 – 2 M ammonium sulphate, pH 7	50 mM phosphate, pH 7.0
Plasmids	50 mM phosphate, 1.5 – 2 M ammonium sulphate, pH 7.0	50 mM phosphate, pH 7.0
PO ON[1] (DMT-on[2])	50 mM Tris-HCl, 0.7 – 1.3 M ammonium sulphate, pH 8.5	50 mM Tris-HCl, 0 – 20 mM ammonium sulphate, pH 8.5

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

Purification

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

1. Prepare 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 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.

Adjust the sample to match the salt concentration of the binding buffer in order to promote hydrophobic interaction. To avoid precipitation caused by the high salt concentrations that can occur locally when adding salt as a solid, salt can be added from a high-concentration stock solution. Adjust the pH of the sample directly. Performing a buffer exchange might not be necessary, as the influence of buffer ions and pH tends to be less prominent in certain cases.

The sample should be applied at 0.5 – 1 mL/min for the GoBio Mini 1 mL or 2 – 5 mL/min for the GoBio Mini 5 mL columns. A too high flow rate may reduce the yield.

2. Connect the column

Cut off or twist off the end at the outlet of the column, see Figure 2.

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 2. Fill the equipment with 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) or 5 mL/min (GoBio Mini 5 mL column).

Figure 2. Removal of the cut-off end at the column outlet should be done by cutting or by twisting (A) not bending (B).

Table 2. 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 low-salt elution buffer. Avoid flow rates higher than 2 mL/min for GoBio Mini 1 mL columns or 10 mL/min for GoBio Mini 5 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 with 5 – 10 CV high-salt binding buffer.

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 or 2 – 5 mL/min for the GoBio Mini 5 mL columns. A too high flow rate may reduce the yield.

6. Wash

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

7. Elute

Elute bound material with preferably a linear gradient, 0% – 100 % elution buffer in 10 – 20 CV.

8. Re-equilibrate

To prevent any precipitation, wash with 3 – 5 CV 20 – 150 mM NaCl before cleaning the column with 1 M NaOH for 15 – 30 minutes . Wash with 5 – 10 CV 20 – 150 mM NaCl and re-equilibrate the column with 10 CV binding buffer to restore the pH.

9. Column storage

Wash the column with 5 CV 0.02 M NaCl. Equilibrate the column with 10 CV 20% ethanol for storage. Close the column using the cap and plug (included).

Scale-up

Scale-up from a GoBio Mini 1 mL column can easily be done 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. This will increase the capacity accordingly. By connecting GoBio Mini columns in series, column volumes from 1 mL to 25 mL can be obtained.

Note: Many different columns sizes in the prepacked GoBio column family are available with WorkBeads 40 Butyl SH, from 1 mL to 21.4 L, see “Related products”.

GoBio Mini columns are easily connected without accessories. The pressure drop across each column bed will be the same as for a single column, but the upstream columns will be exposed to a higher internal pressure since it is affected by the added pressure drops across the downstream columns. It may therefore be necessary to decrease the flow rate accordingly to avoid reaching the maximum pressure limit in the first column. If possible, the maximum pressure of the chromatography system should be set according to Table 3. Remember to take the system fluidics contribution to the pressure into account.

Table 3. 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.

The column size should be selected based on the estimated amount of target molecule to be purified or impurities to be bound. A test run with a defined small volume of sample on a GoBio Mini 1 mL column can be used to estimate the concentration of the target molecule or impurities 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 molecule, it may be suitable to use a larger column than the calculated one to allow higher sample flow rates, and consequently shorter application time. For example, a 5 mL column allows a flow rate five times higher than a 1 mL column due to the larger cross-section of the column. Have in mind that too high flow rate may reduce the binding capacity.

Optimization

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

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

Optimization of salt and salt concentration

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

Sulphate salts, in the order of ammonium, potassium and sodium sulphates effectively promote ligand-protein interactions in HIC and have a stabilizing influence on protein structure. These so-called kosmotropic (anti-chaotropic) salt ions have higher polarity and bind water strongly and therefore exhibit a higher 'salting-out' effect on the molecules, see Hofmeister series in Figure 3. Commonly used salts are $(NH_4)_2SO_4$, Na_2SO_4, NaCl, KCl, and CH_3COONH_4. The salts that increase surface tension in aqueous solutions tend to promote hydrophobic interactions. The choice of salt for a HIC separation is a matter of trial and error since each salt differs in its ability to promote hydrophobic interactions. As the salt concentration increases, the bond between the target and the resin will become stronger leading to more target being bound to the resin.

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

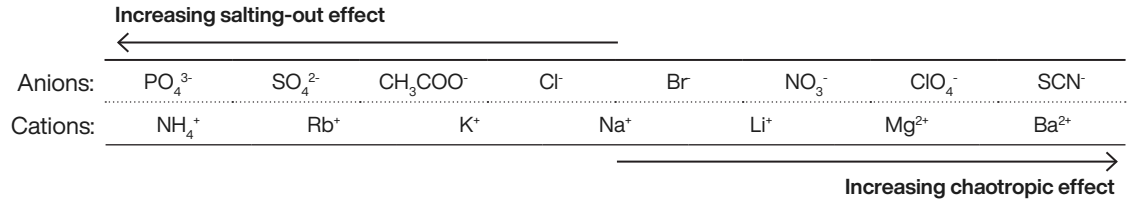

Figure 3. The Hofmeister series.

Optimization of buffer ions and pH

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

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

Optimization of flow rate

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

A low flow rate during sample application promotes binding capacity since more time is allowed for the mass transport of the target substance into the pores of the resin. A small substance, e.g., a peptide, that has a high diffusion rate will have rapid mass transport into the resin and can thus be adsorbed efficiently at higher flow rates as it has fast binding kinetics. A large target substance has a lower diffusion rate and is more hindered by the walls in the pores, resulting in slower mass transport. Achieving a high binding capacity of this substance may require lowering the flow rate.

If only a part of the binding capacity of the column is used, sample application can be done at a higher flow rate without loss of the target substance.

Typical linear flow rates are 150 – 300 cm/h. Figure 4 shows how the resolution is affected by the flow velocity for three different protein pairs.

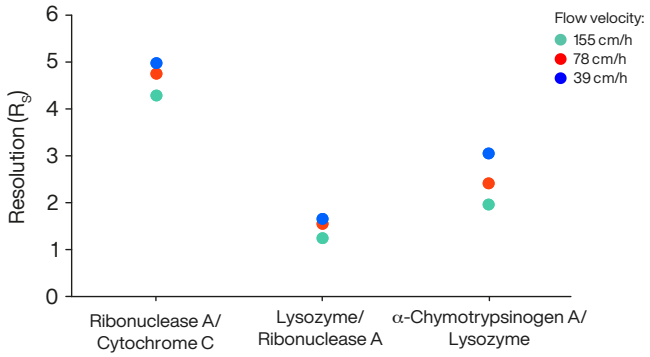

Figure 4. The relationship between resolution and flow velocity achieved on a GoBio Mini Butyl SH 1 mL column. The flow velocities used: 39 cm/h (blue), 78 cm/h (red), and 155 cm/h (green).

Optimization of washing

A continuously decreasing UV signal is an indication that unbound material is still being washed out.

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

Optimization of elution conditions

For optimizing the elution condition, it is recommended to use a linear gradient to decrease the ionic strength, see Figure 5.

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 substance is eluted in a more concentrated form. Buffer consumption can be reduced, and the purification cycle times can be shortened.

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

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

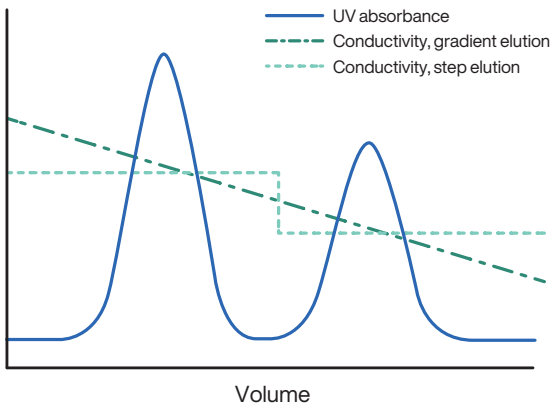

Figure 5. Optimization of step gradient elution with low-salt elution buffer. A test run with linear gradient elution gives information about suitable salt concentrations to be used during the step elution.

Other elution possibilities are:

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

Increasing the salting-out effect strengthens hydrophobic interactions; increasing the chaotropic effect weakens hydrophobic interactions, see Figure 3.

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".

Additional purification

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

Desalting and buffer exchange

Buffer exchange or desalting of a sample can be performed before analysis and/or after purification with for example hydrophobic interaction 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. 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, see "Related products".

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

Maintenance of the column

Cleaning and sanitization

During purification, impurities such as cell debris, lipids, nucleic acids and protein precipitates from the samples may gradually build up in the resin. The severity of this process depends on the type of sample applied to the column, and the pre-treatment of the sample. The impurities covering the resin may reduce the performance of the column over time. Regular cleaning (Cleaning-in-place, CIP) keeps the resin clean, reduces the rate of further fouling, and prolongs the capacity, resolution and flow properties of the column.

CIP of the column can be carried out as follows:

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

   Note: The contact time is the important factor. Treatment with NaOH overnight can be necessary if severely fouled.
3. Wash the column with 5 – 10 CV 0.2 M NaCl (until the pH is neutral after CIP).
4. Equilibrate the column with 10 CV 20% ethanol (for storage).

Sanitization (reduction of microorganisms) can be carried out using combinations of NaOH and ethanol, e.g., incubation with a mixture of 0.5 M NaOH and 40% ethanol for 3 hours. The sanitization procedure and its effectiveness will depend on the microorganisms to be removed, but it needs to be evaluated for each case.

Storage

Store at 2 to 25 °C in 20% ethanol.

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

Product information

	GoBio Mini Butyl SH
Target substance	Proteins, peptides, plasmids, oligonucleotides
Resin	WorkBeads 40 Butyl SH
Matrix	Rigid, highly cross-linked agarose
Average particle size (D_{V50})[1]	45 µm
Ligand	*n*-butyl thioether (CH_3 - CH_2 - CH_2 - CH_2 - S -)
Ligand density	46 – 62 µmol/mL resin
Dynamic binding capacity (DBC)[2]	43 mg β-lactoglobulin/mL resin
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 exhibiting some conductivity, 1 M NaOH, 30% isopropanol, 30% ethanol. Note: Sensitive to oxidants, e.g., H_2O_2.
pH stability	2 – 13
Storage	2 to 25°C in 20% ethanol

1 The median particle size of the cumulative volume distribution.
2 Dynamic binding capacity at 10% breakthrough determined at a residence time of 4 min (150 cm/h) in a 6.6x100 mm column. Buffer conditions: 0.1 M sodium phosphate, 2 M ammonium sulfate, pH 7.
3 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 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 at 4°C), or by additives (e.g., use half of the maximum flow rate for 20% ethanol).

GoBio prepacked column family

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

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

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

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

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

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

Related products

Product name	Pack size[1]	Article number
Prepacked columns		
GoBio Mini IEX Screening Kit[2]	1 mL × 4	45 900 001
GoBio Mini S 1 mL	1 mL × 5	45 200 103
GoBio Mini S 5 mL	5 mL × 5	45 200 107
GoBio Mini Q 1 mL	1 mL × 5	45 100 103
GoBio Mini Q 5 mL	5 mL × 5	45 100 107
GoBio Mini DEAE 1 mL	1 mL × 5	45 150 103
GoBio Mini DEAE 5 mL	5 mL × 5	45 150 107
GoBio Mini Dsalt 1 mL	1 mL × 5	45 360 103
GoBio Mini Dsalt 5 mL	5 mL × 5	45 360 107
GoBio Screen 7x100 Butyl SH	3.8 mL × 1	55 500 001
GoBio Prep 16x100 Butyl SH	20 mL × 1	55 500 021
GoBio Prep 16x100 Dsalt[3]	20 mL × 1	55 700 021
GoBio Prep 26x100 Butyl SH[3]	53 mL × 1	55 500 031
GoBio Prep 26x100 Dsalt	53 mL × 1	55 700 031
GoBio Prod 80x200 Butyl SH[3]	1 L × 1	55 500 042
GoBio Prod 130x200 Butyl SH[3]	2.7 L × 1	55 500 062
GoBio Prod 200x200 Butyl SH[3]	6 L × 1	55 500 072
GoBio Prod 240x200 Butyl SH[3]	9 L × 1	55 500 082
GoBio Prod 330x250 Butyl SH[3]	21.4 L × 1	55 500 093
Bulk resins		
WorkBeads 40 Butyl SH	25 mL 200 mL 1 L 5 L 10 L	40 500 001 40 500 002 40 500 010 40 500 050 40 500 060
WorkBeads Dsalt	300 mL 1 L	40 360 003 40 360 010
Accessories		
Column plug male 1/16”	10	70 100 010
Column cap female 1/16”	10	70 100 020

[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] Packed on request.

Ordering information

Product name	Pack size	Article number
GoBio Mini Butyl SH 1 mL	1 mL × 1 1 mL × 5 1 mL × 10	45 500 101 45 500 103 45 500 104
GoBio Mini Butyl SH 5 mL	5 mL × 1 5 mL × 5 5 mL × 10	45 500 105 45 500 107 45 500 108

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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