DATA SHEET

WorkBeads 40 Butyl SH GoBio prepacked columns

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

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

The resin is also available in several different ready-to-use prepacked column sizes, such as GoBio™ Mini 1 mL and 5 mL, GoBio Screen 7 x 100 (3.8 mL), GoBio Prep 16 x 100 (20 mL) and 26 x 100 (53 mL) as well as GoBio Prod columns starting at 1 L.

- High throughput, binding capacity, and purity
- High chemical stability for easy cleaning-in-place and reproducible results
- Prepacked GoBio columns for convenience and reproducibility

Resin description

WorkBeads are agarose-based chromatographic resins manufactured using a proprietary method that results in porous beads with tight size distribution and exceptional mechanical stability. Agarose-based matrices have been successfully used for decades in biotechnology purification, from research to production scale, due to their exceptional compatibility with biomolecules, including proteins, peptides, nucleic acids, and carbohydrates. WorkBeads resins are designed for separations requiring optimal capacity and purity.

WorkBeads 40 Butyl SH is a hydrophobic resin derivatized with *n*-butyl thioether as a functional group. The functional group is coupled to the resin via a chemically stable covalent linkage, as illustrated in Figure 1.

The main characteristics of WorkBeads 40 Butyl SH resin are shown in Table 1. For more detailed instructions of how to use WorkBeads 40 Butyl SH, see instruction IN 40 500 010.

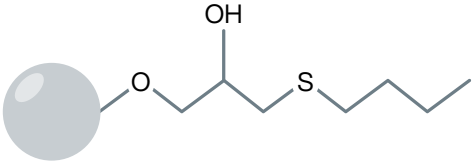

Figure 1. Structure of the ligand used in WorkBeads 40 Butyl SH.

Table 1. Main characteristics of WorkBeads 40 Butyl SH resin.

	WorkBeads 40 Butyl SH
Target substances	Proteins, peptides, plasmids, oligonucleotides
Matrix	Rigid, highly cross-linked agarose
Average particle size (D_{V50})[1]	45 µm
Ligand	*n*-butyl thioether ($CH_3 - CH_2 - CH_2 - CH_2 - S$ -)
Ligand density	46 – 62 µmol/mL resin
Dynamic binding capacity (DBC)[2]	43 mg β-lactoglobulin/mL resin
Max flow rate[3]	600 cm/h (20 cm bed height, 5 bar)
Chemical stability	Compatible with all standard aqueous buffers exhibiting some conductivity, 1 M NaOH, 30% isopropanol, 30% ethanol. Note: Sensitive to oxidants, e.g., H_2O_2.
pH stability	2 – 13
Storage	2 to 25°C in 20% ethanol

[1] The median particle size of the cumulative volume distribution.
[2] Dynamic binding capacity at 10% breakthrough determined at a residence time of 4 min (150 cm/h) in a 6.6x100 mm column. Buffer conditions: 0.1 M sodium phosphate, 2 M ammonium sulfate, pH 7.
[3] Decrease the maximum flow rate if the liquid has a higher viscosity. Higher viscosities can be caused by low temperature (use half of the maximum flow rate when operating 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.

Table 2. Main characteristics of GoBio Mini, GoBio Screen and GoBio Prep columns.

	GoBio Mini 1 mL & 5 mL	**GoBio Screen 7x100**	**GoBio Prep 16x100**	**GoBio Prep 26x100**
Column hardware	Polypropylene	Acrylic	Acrylic	Acrylic
Top and bottom filters	Polyethylene	Polyamide	Polyamide	Polyamide
Top and bottom plugs	Polypropylene	Polypropylene	Polypropylene	Polypropylene
Connections	1/16" female (top) 1/16" male (bottom)	1/16" female (both ends)	1/16" female (both ends)	1/16" female (both ends)
Column volumes	1 mL 5 mL	3.8 mL	20 mL	53 mL
Column dimensions	7 × 28 mm (1 mL) 13 × 38 mm (5 mL)	7 × 100 mm	16 × 100 mm	26 × 100 mm
Max. column hardware pressure[1]	0.3 MPa, 3 bar, 43 psi	5 bar, 0.5 MPa, 70 psi	5 bar, 0.5 MPa, 70 psi	5 bar, 0.5 MPa, 70 psi
Chemical stability	1 M NaOH, 30% isopropanol, 70% ethanol	1 M NaOH, 20% isopropanol, 20% ethanol	1 M NaOH, 20% isopropanol, 20% ethanol	1 M NaOH, 20% isopropanol, 20% ethanol

[1] 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.

Table 3. Main characteristics of GoBio Prod columns.

	GoBio Prod 80x200, GoBio Prod 130x200, GoBio Prod 200x200, GoBio Prod 280x200, GoBio Prod 330x250
Column hardware	Acrylic
Top and bottom filters	Polyamide
Top and bottom plugs	Polypropylene
Connections	TC-connections
Column volumes	1 L, 2.7 L, 6 L, 9 L, 21.4 L
Column dimensions	80 × 200 mm (1 L), 130 × 200 mm (2.7 L), 200 × 200 mm (6 L), 280 × 200 mm (9 L), 330 × 250 mm (21.4 L)
Max. column hardware pressure[1]	5 bar, 0.5 MPa, 70 psi
Chemical stability	1 M NaOH, 20% isopropanol, 20% ethanol

[1] 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.

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.

Selectivity

The WorkBeads 40 Butyl SH resin exhibits a unique selectivity, characterized by its high density of *n*-butyl thioether ligands.

Figure 2 illustrates the separation of proteins with different hydrophobicities – cytochrome C, ribonuclease A, lysozyme, and α-chymotrypsinogen A – achieved using a GoBio Mini Butyl SH 1 mL column.

Column:	GoBio Mini Butyl SH 1 mL
Binding buffer:	1.5 M $(NH_4)_2SO_4$, 0.1 M $NaHPO_4$, pH 7.0
Elution buffer:	0.1 M $NaHPO_4$, pH 7.0
Sample load:	1.25 mg/mL resin of ribonuclease A and 0.625 mg/mL resin of lysozyme, cytochrome C, and α-chymotrypsinogen A in binding buffer
Flow rate:	39 cm/h (0.25 mL/min)
Linear gradient:	0 – 100% elution buffer in 20 CV

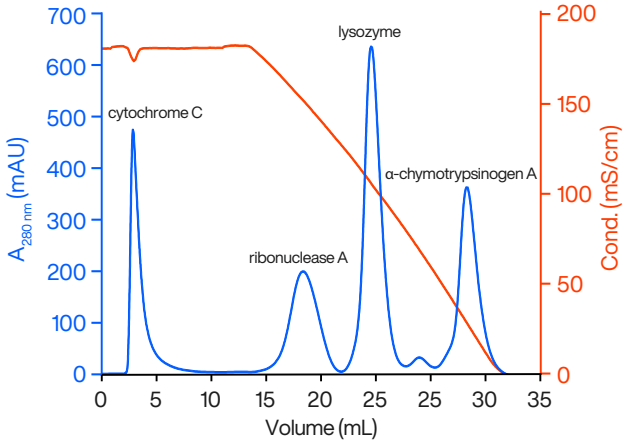

Figure 2. Separation using WorkBeads 40 Butyl SH. Peaks from left to right, cytochrome C, ribonuclease A, lysozyme, and α-chymotrypsinogen A. 0.5 mL sample applied on GoBio Mini 1 mL. The blue trace corresponds to absorbance at 280 nm and the red line to conductivity.

Dynamic binding capacity

Different HIC resins may be optimal to use during purification, depending on the nature and chemical properties, especially the hydrophobicity of the molecules. To reach the required results, optimization of purification conditions must be undertaken. Figure 3 shows the dynamic binding capacity (DBC) for GoBio Mini Butyl SH in comparison with a butyl agarose resin (low sub), a phenyl agarose resin (low sub), and a hydroxylated methacrylate butyl resin. All resins are packed in the same format and run under identical conditions.

There is a difference in loading capacities for the β-lactoglobulin, where WorkBeads 40 Butyl SH stands out. Additionally, all agarose-based resins display better mass transport compared to the methacrylate resin. The DBC will vary depending on the specific molecule being studied.

Column:	6.6 x 100 mm (3.4 mL)
Binding buffer:	2 M $(NH_4)_2SO_4$, 0.1 M $NaHPO_4$, pH 7.0
Elution buffer:	0.1 M $NaHPO_4$, pH 7.0
Sample:	1 mg β-lactoglobulin/mL in binding buffer
Flow rate:	150 cm/h (0.86 mL/min)

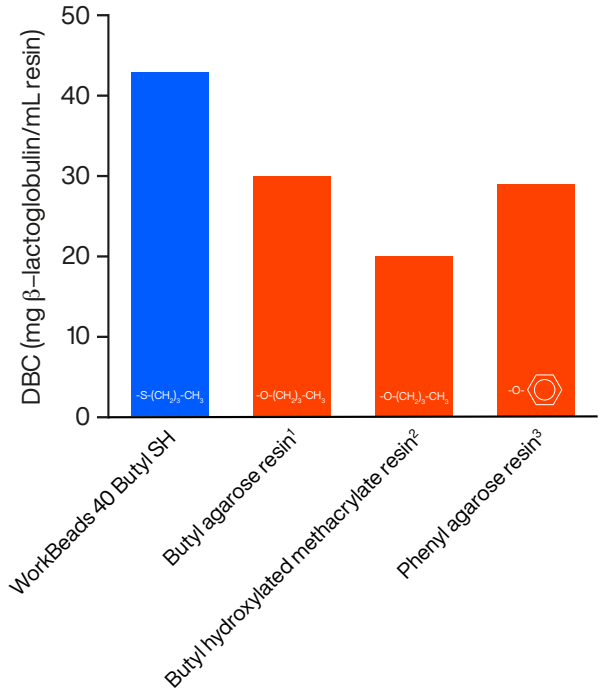

Figure 3. Dynamic binding capacity determined at 10% breakthrough for β-lactoglobulin at a residence time of 4 min.

[1] Capto™ Butyl ImpRes
[2] Toyopearl™ Butyl 650-S
[3] Capto Phenyl ImpRes.

Applications

Protein selectivity

The optimal running conditions vary based on the molecule to be purified and if it should be a capture, intermediate, or polishing step. In Figure 4A-C, the difference in selectivity is shown for three HIC resins utilizing different ligands or base matrices (green line) compared to WorkBeads 40 Butyl SH (blue line). All resins are packed in the GoBio Mini 1 mL format and run under identical conditions. The GoBio Mini format is optimal for this kind of screening studies.

As can be seen in Figure 4, the resins display different selectivity patterns. While the more hydrophilic protein cytochrome C does not bind to any of the resins, ribonuclease A elutes in the gradient on WorkBeads 40 Butyl SH but in the flow through for the other resins. All proteins elute in the same order on all resins except lysozyme, which elutes earlier on WorkBeads 40 Butyl SH than on the methacrylate butyl resin, as shown in Figure 4B. According to these results, WorkBeads 40 Butyl SH demonstrates the highest hydrophobicity among the resins in this collection. This characteristic is most often beneficial for molecules like plasmids, insulin, DMT-on oligonucleotides (PO), etc.

Column:	GoBio Mini 1 mL
Resins:	WorkBeads 40 Butyl SH, Capto Butyl ImpRes, Capto Phenyl ImpRes and Toyopearl Butyl 650-S
Binding buffer:	1.5 M $(NH_4)_2SO_4$, 0.1 M $NaHPO_4$, pH 7.0
Elution buffer:	0.1 M $NaHPO_4$, pH 7.0
Sample load:	1.25 mg/mL resin of ribonuclease A & 0.625 mg/mL resin of lysozyme, cytochrome C, and α-chymotrypsinogen A in binding buffer 39 cm/h (0.25 mL/min)
Flow rate:	39 cm/h (0.25 mL/min)
Linear gradient:	0 – 100% elution buffer in 20 CV

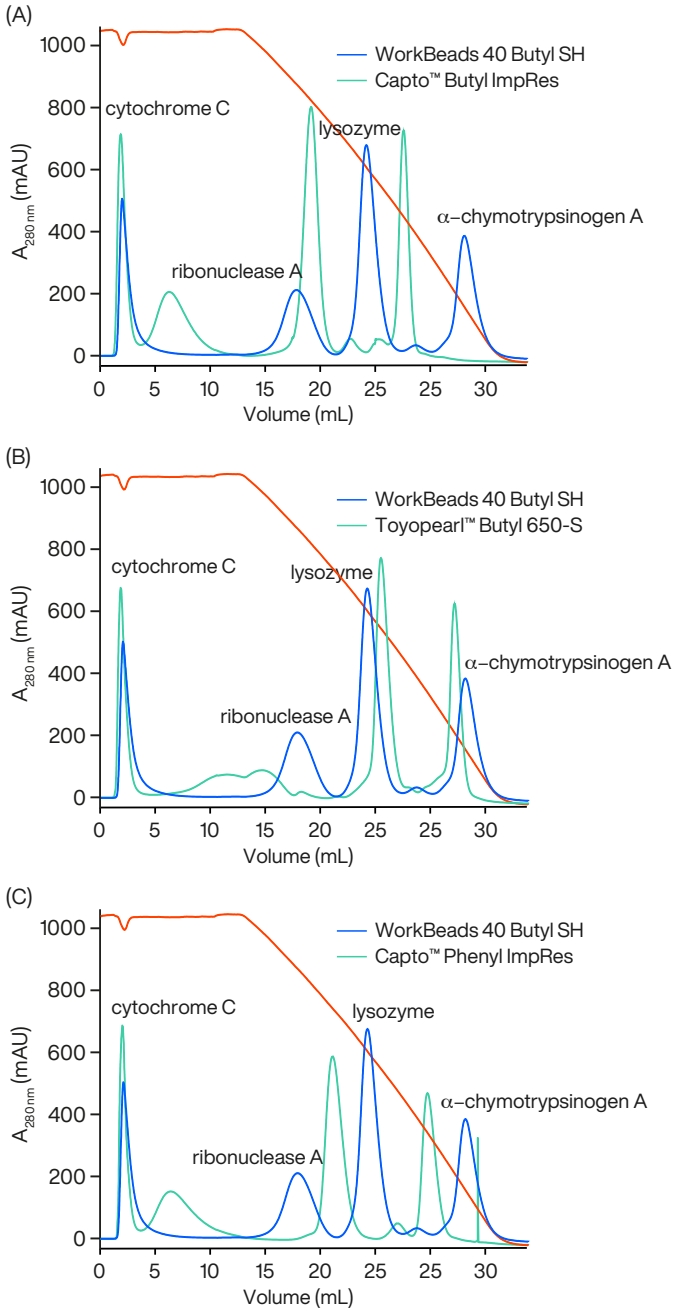

Figure 4. Separations are performed using four different resins utilizing different ligands and/or base matrices. Peaks from left to right, cytochrome C, ribonuclease A, lysozyme, and α-chymotrypsinogen A (peaks labeled for WorkBeads 40 Butyl SH). The blue and green lines correspond to absorbance at 280 nm, and the red line to conductivity. (A) WorkBeads 40 Butyl SH (blue) vs. Capto Butyl ImpRes (green), (B) WorkBeads 40 Butyl SH (blue) vs. Toyopearl Butyl 650-S (green), (C) WorkBeads 40 Butyl SH (blue) vs. Capto Phenyl ImpRes (green).

The binding may be too strong for certain molecules, making useful elution conditions hard to identify. In these cases, we recommend using additives or a less hydrophobic resin. If a new resin is desired, a custom resin project can be initiated. Learn more at www.bio-works.com/custom-resins

Resolution

To achieve high productivity, it's beneficial to use a high flow rate without being limited by the binding kinetics. Figure 5 illustrates the relationship between resolution and flow velocity. The highest resolution is obtained at the lowest flow velocity, but the resolution is not severely affected by the flow rate. To achieve optimal productivity, a compromise must be made between resolution and time.

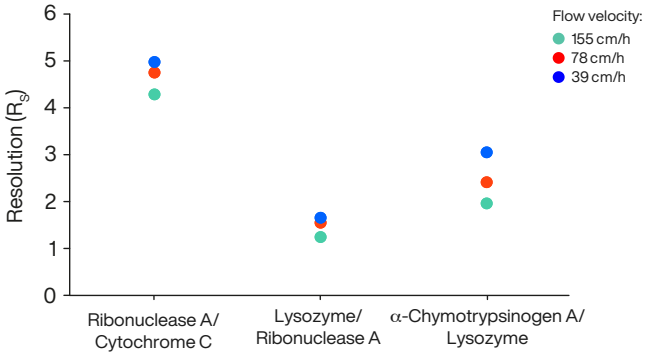

Figure 5. The relationship between resolution and flow velocity acheived using a GoBio Mini Butyl SH 1 mL column. The flow velocities used: 39 cm/h (blue), 78 cm/h (red), and 155 cm/h (green).

The resolution (R_S) between two peaks depends on the separation (retention volume) and the peak width. If $R_S > 1.5$, the peaks are considered to be baseline resolved.

Cleaning and sanitation

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 resin.

CIP of WorkBeads 40 Butyl SH can be performed using NaOH at concentrations up to 1 M for a contact time of 15 minutes or more.

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.

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

Scale-up

Scale-up can conveniently be carried out from a 1 mL GoBio Mini column to a 21.4 L GoBio Prod column. Bulk packages of WorkBeads resins can also be packed into other column formats of choice. Prepacked columns can be used with most standard liquid chromatography equipment. Purification using GoBio Mini columns can also be carried out using a syringe connected to the column by a Luer or a standard HPLC connector.

Storage

Store at 2 to 25°C in 20% ethanol.

For prolonged storage of the prepacked GoBio Screen and GoBio Prep columns, connect the included transport syringe filled with storage solution to the bottom end of the column.

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 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 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
Bulk resins		
WorkBeads 40S	25 mL	40 200 001
WorkBeads 40Q	25 mL	40 100 001
WorkBeads 40 DEAE	25 mL	40 150 001
WorkBeads Dsalt	300 mL	40 360 003

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
Prepacked columns		
GoBio Mini Butyl SH 1 mL	1 mL x 1 1 mL x 5 1 mL x 10	45 500 101 45 500 103 45 500 104
GoBio Mini Butyl SH 5 mL	5 mL x 1 5 mL x 5 5 mL x 10	45 500 105 45 500 107 45 500 108
GoBio Screen 7x100 Butyl SH	3.8 mL × 1	55 500 001
GoBio Prep 16x100 Butyl SH	20 mL × 1	55 500 021
GoBio Prep 26x100 Buty SH	53 mL × 1	55 500 031
GoBio Prod 80x200 Butyl SH[1]	1 L x 1	55 500 042
GoBio Prod 130x200 Butyl SH[1]	2.7 L x 1	55 500 062
GoBio Prod 200x200 Butyl SH[1]	6 L x 1	55 500 072
GoBio Prod 240x200 Butyl SH[1]	9 L x 1	55 500 082
GoBio Prod 330x250 Butyl SH[1]	21.4 L x 1	55 500 093
Bulk resin		
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

[1] Packed on request.

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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