Bio-Works

DATA SHEET

WorkBeads 40 IEX resins
GoBio Mini IEX Screening kits
GoBio prepacked columns

WorkBeads 40S, WorkBeads 40Q, WorkBeads 40 DEAE, WorkBeads 40 TREN,
GoBio Mini IEX Screening kit, GoBio Mini Peptide Purification kit, GoBio prepacked column family

WorkBeads™ 40S, WorkBeads 40Q and WorkBeads 40 DEAE resins for ion exchange chromatography (IEX) are designed for research and industrial scale purification of proteins, peptides and oligonucleotides by utilizing the difference in their surface charge.

WorkBeads 40S resin is a strong cation exchanger derivatized with sulfonate ligands. WorkBeads 40Q resin is a strong anion exchanger derivatized with quaternary amine ligands. WorkBeads 40 DEAE is a weak anion exchanger with tertiary amine ligands. These resins demonstrate the property of high-resolution separation while giving low backpressure facilitate both capture and polishing purification applications in standard bioprocess columns.

WorkBeads 40 TREN resin for multimodal ion exchange chromatography (IEX) can be used for several different applications, especially due to its higher salt tolerant properties, e.g., for alternative IEX selectivity, for sample cleanup in monoclonal antibody (mAb) purification processes to guard the protein A column from different host cell impurities, or as a polishing step in the mAb purification process.

These resins are also available in several different ready-to-use prepacked column sizes, such as GoBio™ Mini 1 mL, GoBio Mini 5 mL, GoBio Screen 7x100 (3.8 mL), GoBio Prep 16x100 (20 mL), GoBio Prep 26x100 (53 mL) and GoBio Prod columns starting from 1 L.

GoBio Mini IEX Screening kit for easy screening to find optimal running conditions includes one GoBio Mini 1 mL column of each of the four ion exchangers. GoBio Mini Peptide Purification kit is a bundle of GoBio Mini S 1 mL and GoBio Mini Q 1 mL.

- High binding capacity, throughput, and purity
- High chemical stability for easy cleaning-in-place and reproducible results
- GoBio Mini IEX Screening kits for fast and easy screening of optimal selectivity
- 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 a 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 40S is a strong cation exchange resin derivatized with sulfonates as functional groups.

WorkBeads 40Q is a strong anion exchanger derivatized with quaternary amines as functional groups.

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

WorkBeads 40 TREN resin contains ligands based on Tris(2-aminoethyl)amine (TAEA). WorkBeads 40 TREN resin can be used for the separation of biomolecules exploiting surface charge to purify proteins, peptides, and oligonucleotides. It can also be used in flow through mode to adsorb impurities while letting the target pass through the column (negative chromatography mode).

The functional groups are coupled to the resins via chemically stable linkages. The structures of the ligands used in WorkBeads 40S, WorkBeads 40Q, WorkBeads 40 DEAE and WorkBeads 40 TREN can be seen in Figure 1. More details about WorkBeads 40 TREN and GoBio Mini TREN can be found in data sheet DS 40 600 020.

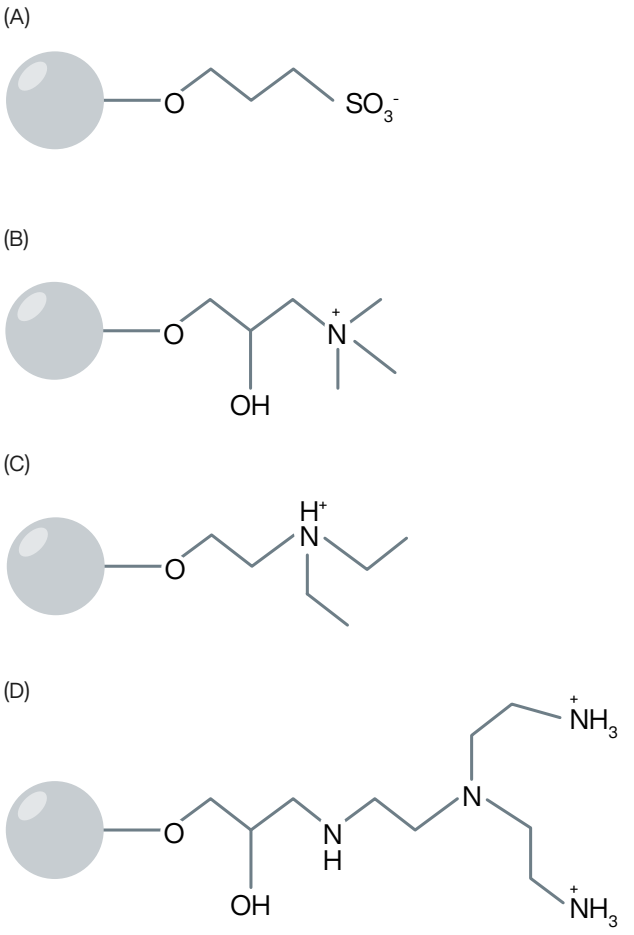


Figure 1. Structure of the ligand used in (A) WorkBeads 40S, (B) WorkBeads 40Q, (C) WorkBeads 40 DEAE and (D) WorkBeads 40 TREN.

The main characteristics of WorkBeads 40S, WorkBeads 40Q and WorkBeads 40 DEAE are described in Table 1. For more details, see IN 40 100 010. The main characteristics of WorkBeads 40 TREN is shown in Table 2.

For the main characteristics of all different prepacked columns formats, see Tables 3 and 4.

Table 1. Main characteristics of WorkBeads 40S, WorkBeads 40Q and WorkBeads 40 DEAE resins.

	WorkBeads 40S	WorkBeads 40Q	WorkBeads 40 DEAE
Target substance	Proteins, peptides	Protein, peptides, viruses, oligonucleotides	Protein, peptides, oligonucleotides
Matrix	Rigid, highly cross-linked agarose	Rigid, highly cross-linked agarose	Rigid, highly cross-linked agarose
Average particle size (D_{V50})[1]	45 µm	45 µm	45 µm
Ionic group (ligand)	Sulfonate ($-SO_3^-$)	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]
Max. flow rate[4] (20 cm bed height, 5 bar)	600 cm/h	600 cm/h	600 cm/h
Chemical stability	Compatible with all standard aqueous buffers used for protein purification, 1 M NaOH, 30% isopropanol and 70% ethanol. Should not be stored at low pH for prolonged time.		
Operational pH[5]	3 – 12	2 – 13	2 – 13 3 – 9 (recommended pH)
CIP and screening pH range[5]	2 – 14	2 – 14	2 – 14
Storage	2 to 25 °C in 20% ethanol with 0.2 M sodium acetate	2 to 25 °C in 20% ethanol	2 to 25 °C in 20% ethanol

1 The median particle size of the cumulative volume distribution.
2 Dynamic binding capacity determined at 4-minutes residence time in 20 mM sodium citrate, pH 4.0.
3 Dynamic binding capacity determined at 2.5-minutes residence time in 50 mM Tris-HCl, 50 mM NaCl, pH 8.0.
4 Optimal flow rate during binding is depending on the sample.
5 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.

Table 2. Main characteristics of WorkBeads 40 TREN resin.

	WorkBeads 40 TREN
Target substances	Proteins, peptides, oligonucleotides, viruses
Matrix	Rigid, highly cross-linked agarose
Average particle size (D_{V50})[1]	45 µm
Ligand	Tris(2-ethylaminoethyl) (TAEA)
Ionic capacity	130 – 200 µmol Cl^-/mL resin
Dynamic binding capacity	50 mg BSA/mL resin[2]
Max. flow rate[3] (20 cm bed height and 5 bar)	600 cm/h
Chemical stability	Compatible with all standard aqueous buffers used for protein purification. Should not be stored at low pH for prolonged time.
Operational pH[4]	2 – 13
CIP and screening pH range4	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.
4 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.

Table 3. 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.

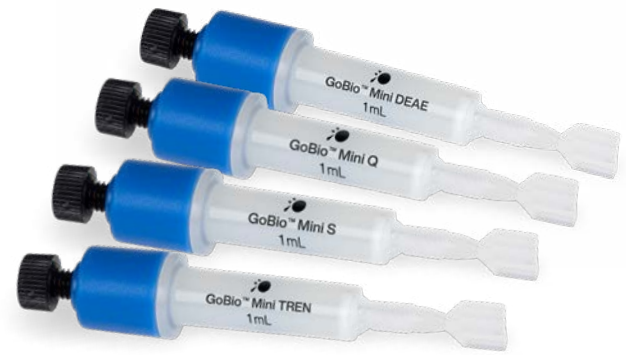
Table 4. Main characteristics of and GoBio Prod columns.

	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.

GoBio Mini IEX Screening kit

GoBio Mini IEX Screening kit includes four GoBio Mini 1 mL columns, one of each ion exchanger (S, Q, DEAE, and TREN). This kit enables easy and fast optimization of specific purification protocols using the best ion exchanger ligand and optimization of running conditions.



GoBio Mini Peptide Purification kit

GoBio Mini Peptide Purification kit is a bundle of one GoBio Mini S 1 mL × 1 and one GoBio Mini Q 1 mL × 1 for easy and fast testing for optimal peptide purifications.

Principles

Ion exchange chromatography

Ion exchange chromatography separates biomolecules according to their surface charge. For example, proteins interact with different affinities with oppositely charged groups on the resin. This depends on the number of charges involved in the interaction and on the distribution of the charges on the protein. 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 between the protein and the resin surface is dependent on the charge distribution on the surface on the protein.

A protein may therefore also interact with an ion exchange resin at the isoelectric point. The likelihood of binding to either the cation or the anion exchange resin will increase as the pH moves away from the pI. A strong ion exchanger, such as WorkBeads 40Q is ionized over a broad pH range (2-13), while a weak ion exchanger, such as WorkBeads 40 DEAE is ionized over a more limited pH range (3-9).

IEX is one of the most frequently used chromatography techniques because of its versatility and ability to separate proteins even with small differences in charge and it is also used as a concentration step. It is also one of the more cost-efficient chromatography techniques and is therefore excellent for scale-up.

The resins can be used for research- and industrial-scale purification of proteins, peptides and oligonucleotides. The particle size has been selected to enable high-resolution separations at moderate backpressure. The resins can therefore be used in a capture step during purification, as well as for polishing. For purifications requiring exceptionally high flow rates to handle large sample volumes, WorkBeads 100S and WorkBeads 100Q should be considered for the capture step.

Multimodal ion exchange chromatography

Multimodal ion exchange chromatography separates protein, peptides and other biomolecules via a ligand acting with more than one interaction site. 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 separately in the purification procedure. The TREN ligand is positively charged below pH 9. The use of WorkBeads 40 TREN in binding or flowthrough mode will efficiently facilitate removal of nucleic acids, viruses, host cell proteins and other cell-derived impurities. WorkBeads 40 TREN has a higher salt tolerance compared to “standard” IEX resins. For more information, see DS 40 600 020.

Applications

Protein selectivity

GoBio Mini

In Figure 2 below the basic proteins, concanavalin A, ribonuclease A, lysozyme and α-chymotrypsinogen A are separated on GoBio Mini S columns.

In Figure 3, the acidic proteins apo-transferrin, α-lactalbumin and soybean trypsin inhibitor are separated on GoBio Mini Q 1 mL, GoBio Mini DEAE 1 mL and GoBio Mini TREN 1 mL columns respectively. The selectivity differences are visible.

Columns:	(A) GoBio Mini S 1 mL (B) GoBio Mini S 5 mL
Binding buffer:	50 mM MES, pH 6.0
Elution buffer:	50 mM MES, 1 M NaCl, pH 6.0
Sample:	1.5 mg/mL concanavalin A, 1.5 mg/mL ribonuclease A 0.5 mg/mL α-chymotrypsinogen A 0.5 mg/mL lysozyme in binding buffer
Flow rate:	150 cm/h (1 mL/min GoBio Mini S 1 mL) (3.5 mL/min GoBio Mini S 5 mL)
Sample volumes:	0.25 mL (GoBio Mini S 1 mL) 1.25 mL (GoBio Mini S 5 mL)
Linear gradient:	0 – 50% elution buffer in 20 column volumes (CV)

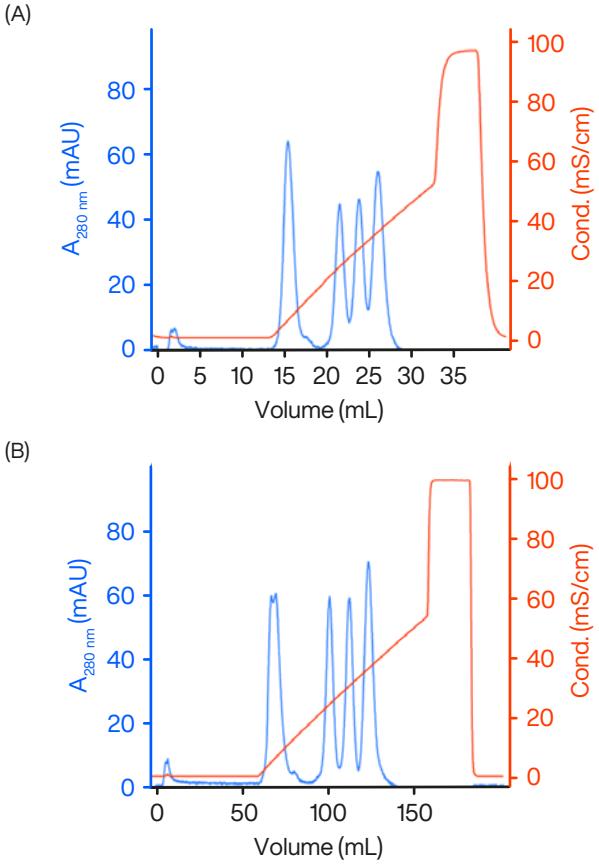


Figure 2. Separation using cation exchange chromatography. Peaks from left to right, concanavalin A, α-chymotrypsinogen A, ribonuclease A and lysozyme. (A) 0.25 mL sample applied onto GoBio Mini S 1 mL and (B) 1.25 mL sample applied onto GoBio Mini S 5 mL. The blue line corresponds to absorbance at 280 nm and the red line to conductivity.

Column:	(A) GoBio Mini Q 1 mL (B) GoBio Mini DEAE 1 mL (C) GoBio Mini TREN 1 mL
Binding buffer:	50 mM Tris-HCl, pH 7.4
Elution buffer:	50 mM Tris-HCl, 1 M NaCl, pH 7.4
Sample:	0.3 mg/mL apo-transferrin, 0.2 mg/mL α-lactalbumin, 0.6 mg/mL soybean trypsin inhibitor in binding buffer
Sample volumes:	2 mL (GoBio Mini Q 1 mL) 2.5 mL (GoBio Mini DEAE 1 mL) 2.5 mL (GoBio Mini TREN 1 mL)
Flow rate:	150 cm/h (1 mL/min)
Linear gradient:	0 – 40% elution buffer in 20 CV

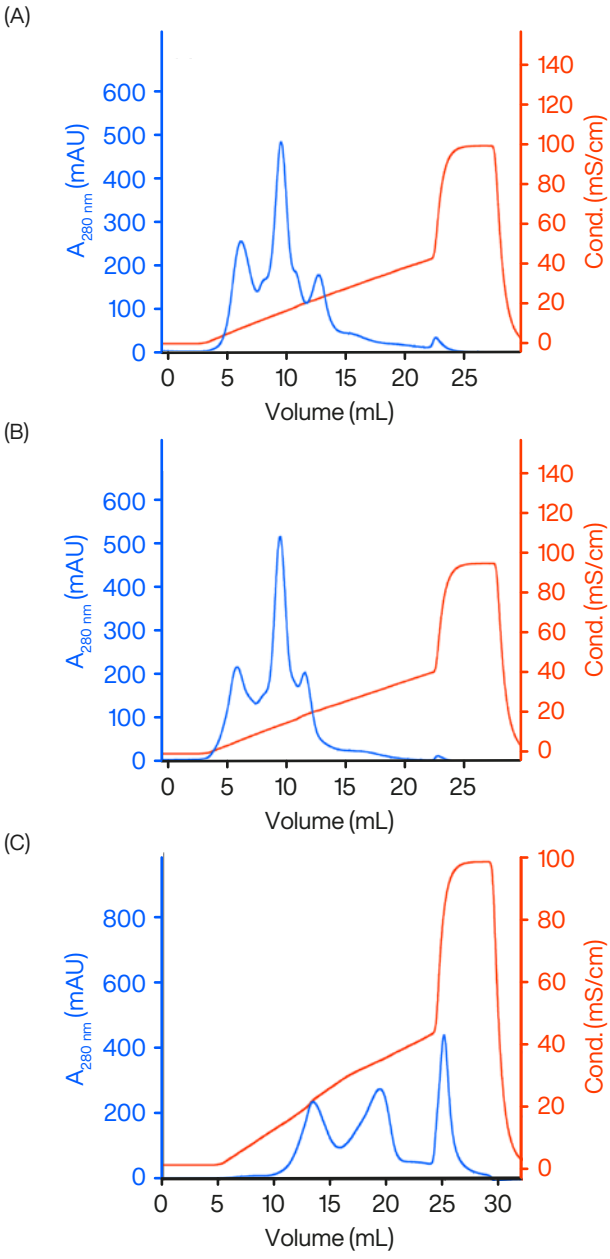
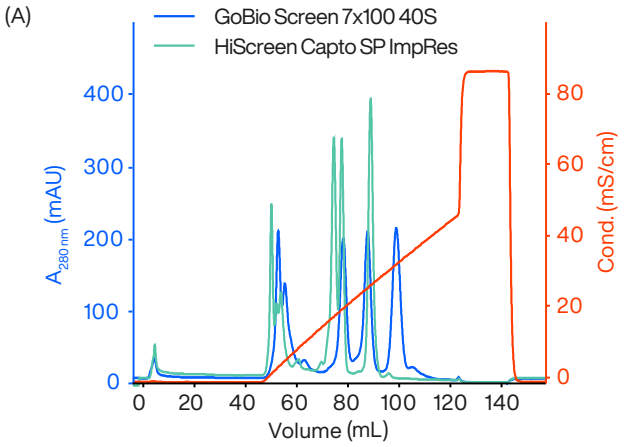


Figure 3. Separation using anion exchange chromatography. Peaks from left to right, apo-transferrin, α-lactalbumin and soybean trypsin inhibitor. (A) 2 mL sample on GoBio Mini Q 1 mL, (B) 2.5 mL sample on GoBio Mini DEAE 1 mL, (C) 2.5 mL sample on GoBio Mini TREN 1 mL. The blue line corresponds to absorbance at 280 nm and the red line to conductivity.

GoBio Screen 7x100

In Figure 4 examples of separation of the basic proteins, concanavalin A, ribonuclease A, lysozyme and α-chymotrypsinogen A are presented using the prepacked columns GoBio Screen 7x100 40S, HiScreen™ Capto™ SP ImpRes (Cytiva) and HiScreen Capto S ImpAct (Cytiva).

Column:	GoBio Screen 7x100 40S (3.8 mL) HiScreen Capto SP ImpRes (7.7 × 100 mm, 4.7 mL)
Flow rate:	124 cm/h (1.0 mL/min)
Binding buffer:	50 mM MES, pH 6.0
Elution buffer:	50 mM MES, 1 M NaCl, pH 6.0
Sample:	1.5 mg/mL concanavalin A, 1.5 mg/mL ribonuclease A, 0.5 mg/mL α-chymotrypsinogen A, 0.5 mg/mL lysozyme in binding buffer
Sample volume:	1 mL
Linear gradient:	0 – 50% elution buffer in 20 CV



Column:	GoBio Screen 7x100 40S (3.8 mL) HiScreen Capto S ImpAct (7.7 × 100 mm, 4.7 mL)
Flow rate:	124 cm/h (1.0 mL/min)
Binding buffer:	50 mM MES, pH 6.0
Elution buffer:	50 mM MES, 1 M NaCl, pH 6.0
Sample:	1.5 mg/mL concanavalin A, 1.5 mg/mL ribonuclease A, 0.5 mg/mL α-chymotrypsinogen A, 0.5 mg/mL lysozyme in binding buffer
Sample volume:	1 mL
Linear gradient:	0 – 50% elution buffer in 20 CV

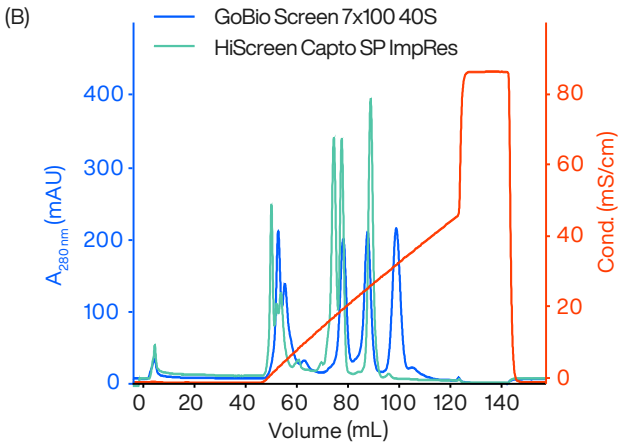


Figure 4. Separation of basic proteins using cation exchange chromatography. Peaks from left to right, concanavalin A, α-chymotrypsinogen A, ribonuclease A and lysozyme. (A) 1 mL sample applied onto GoBio Screen 7x100 40S and HiScreen Capto SP ImpRes. (B) 1 mL sample applied onto GoBio Screen 7x100 40S and HiScreen Capto S ImpAct. The blue and green traces correspond to absorbance at 280 nm and the red line to conductivity

GoBio Screen 7x100 40S shows improved separation compared to HiScreen Capto SP ImpRes prepacked with Capto SP ImpRes (average bead size 34 µm) and HiScreen Capto S ImpAct prepacked with Capto S ImpAct (average bead size 50 µm).

In Figure 5, the acidic proteins apo-transferrin, α-lactalbumin and soybean trypsin inhibitor are separated on the prepacked columns GoBio Screen 7x100 40Q and HiScreen Capto Q ImpRes ImpRes prepacked with Capto Q ImpRes (average bead size 34 µm) (Cytiva).

Column:	GoBio Screen 7x100 40Q (3.8 mL) HiScreen Capto Q ImpRes (7.7 × 100 mm, 4.7 ml)
Flow rate:	124 cm/h (1.0 mL/min)
Binding buffer:	50 mM Tris-HCl, pH 7.4
Elution buffer:	50 mM Tris-HCl, 1 M NaCl, pH 7.4
Sample:	0.3 mg/mL apo-transferrin, 0.2 mg/mL α-lactalbumin, 0.6 mg/mL soybean trypsin inhibitor in binding buffer
Sample volume:	10 mL
Linear gradient:	0 – 40% elution buffer in 20 CV

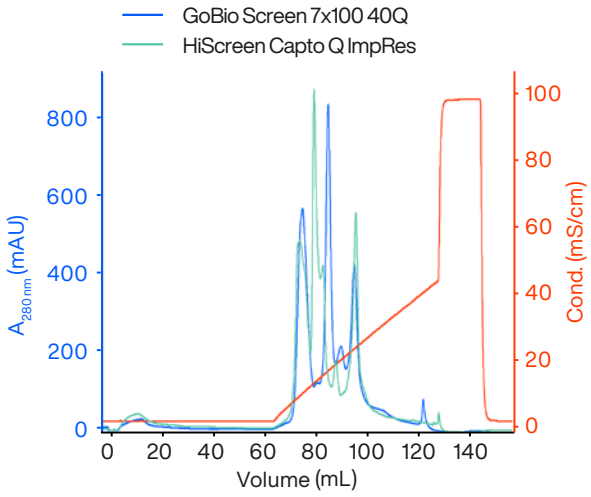


Figure 5. Separation of acidic proteins using anion exchange chromatography. Peaks from left to right, apo-transferrin, α-lactalbumin and soybean trypsin inhibitor. 10 mL sample onto GoBio Screen 7x100 40Q and HiScreen Capto Q ImpRes. The blue and green traces correspond to absorbance at 280 nm and the red line to conductivity.

GoBio Prep 16x100 mm

Figure 6 shows the separation of the basic proteins, concanavalin A, ribonuclease A, lysozyme and α-chymotrypsinogen A on the prepacked GoBio Prep 16x100 40S column. The result gives an almost base line separations between the different protein peaks.

In Figure 7, the acidic proteins apo-transferrin, α-lactalbumin and soybean trypsin inhibitor are separated on a prepacked GoBio Prep 16x100 40Q column.

Dynamic binding capacity

Table 5 shows the dynamic binding capacity (DBC) for GoBio Screen 7x100 40S and GoBio Screen 7x100 40Q in comparison with HiScreen Capto SP ImpRes, HiScreen Capto S ImpAct and HiScreen Capto Q ImpRes.

Column:	GoBio Prep 16x100 40S (20 mL)
Flow rate:	150 cm/h (5.0 mL/min)
Binding buffer:	50 mM MES, pH 6.0
Elution buffer:	50 mM MES, 1 M NaCl, pH 6.0
Sample:	1.5 mg/mL concanavalin A, 1.5 mg/mL ribonuclease A, 0.5 mg/mL α-chymotrypsinogen A, 0.5 mg/mL lysozyme in binding buffer
Sample volume:	4.6 mL
Linear gradient:	0-50% elution buffer in 20 CV

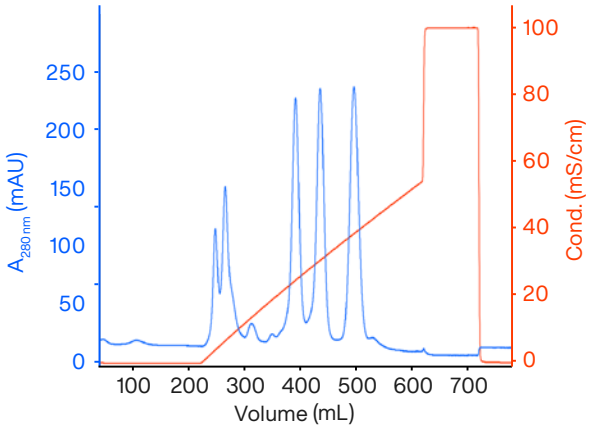


Figure 6. Separation using cation exchange chromatography. Peaks from left to right, concanavalin A, α-chymotrypsinogen A, ribonuclease A and lysozyme. 4.6 mL sample applied on GoBio Prep 16x100 40S. The blue trace corresponds to absorbance at 280 nm and the red line to conductivity.

Column:	GoBio Prep 16x100 40Q (20 mL)
Flow rate:	150 cm/h (5.0 mL/min)
Binding buffer:	50 mM Tris-HCl, pH 7.4
Elution buffer:	50 mM Tris-HCl, 1 M NaCl, pH 7.4
Sample:	0.3 mg/mL apo-transferrin, 0.2 mg/mL α-lactalbumin, 0.6 mg/mL soybean trypsin inhibitor in binding buffer
Sample volume:	46 mL
Linear gradient:	0 – 40% elution buffer in 20 CV

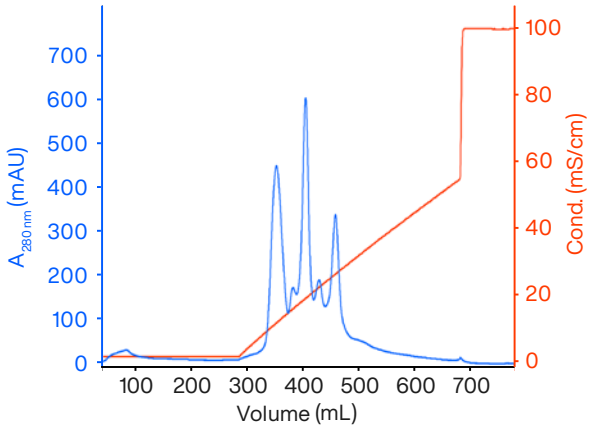


Figure 7. Separation using anion exchange chromatography. Peaks from left to right, apo-transferrin, α-lactalbumin and soybean trypsin inhibitor. 46 mL sample applied on GoBio Prep 16x100 40Q. The blue trace corresponds to absorbance at 280 nm and the red line to conductivity.

Table 4. Main characteristics of and GoBio Prod columns.

Prepacked column	DBC at 4 min residence time (150 cm/h), mg BSA/mL resin	DBC at 2.5 min residence time (240 cm/h), mg BSA/mL resin
GoBio Screen 7x100 40S	113[1]	99[1]
HiScreen Capto SP ImpRes	108[1]	99[1]
HiScreen Capto S ImpAct	88[1]	84[1]
GoBio Screen 7x100 40Q	54[2]	52[2]
HiScreen Capto Q ImpRes	40[2]	37[2]

[1] Dynamic binding capacity determined at 4 min (150 cm/h) and 2.5 min (240 cm/h) residence time in 20 mM sodium citrate, pH 4.0.
[2] Dynamic binding capacity determined at 4 min (150 cm/h) and 2.5 min (240 cm/h) residence time in 50 mM Tris-HCl, 50 mM NaCl, pH 8.0.

Peptides

Peptides, constituted by a chain of amino acids, are commonly synthesized by solid-phase synthesis. Custom-made peptides, up to 50-60 amino acids in length, are routinely synthesized but the longer the peptide sequence, the higher the frequency of abortive and erroneous sequences that must be removed in downstream purification processes. Therapeutic peptides represent a specialized niche between protein-based biopharmaceuticals and traditional small molecule therapies. The purity requirements are very high for these molecules. IEX is commonly used as a capture step to remove impurities and contaminants from a crude peptide batch. Both cation and anion exchangers have been used with success for peptide purifications. Cation exchange chromatography (CIEX) is more common than anion exchange chromatography (AIEX), but which technique to choose ultimately depends on the peptide sequence.

WorkBeads IEX resins are exceptionally compatible with peptide purifications due the small pore size and homogenous pore size distribution. These properties facilitate the mass transport of small molecules, such as peptides, resulting in improved selectivity and loading capacity.

Dynamic binding capacity

Depending on the nature and chemical properties of the peptide, such as isoelectric point, molecular weight, presence of disulfide bridges, modifications etc., different IEX resins may be optimal to use during purification. To reach the required parameters, optimizations of purification conditions must be undertaken (pH, elution conditions, ionic strength, organic modifiers and resins). In Figures 4-6, the same peptide (pI 4.5, 39 aa) was loaded onto the GoBio Mini IEX columns prepacked with four different IEX resins using different running conditions.

Figure 8 shows the difference in loading capacities for the peptide, where GoBio Mini S stands out. Figures 9-10 show the difference in binding kinetics for GoBio Mini (Q/DEAE/TREN), the anion exchangers, compared to GoBio Mini S, the cation exchanger. Whereas all the AIEX resins are insensitive to the flow rate/residence time, GoBio Mini S shows a more exceptional relationship between the flow rate and the dynamic binding capacity (DBC) value obtained, where a longer residence time/lower flow rate result in a higher DBC value. This peptide had extremely fast binding kinetics when using the AIEX resins.

Columns:	GoBio Mini S 1 mL GoBio Mini Q 1 mL GoBio Mini DEAE 1 mL GoBio Mini TREN 1 mL
Sample:	1 mg 39 aa-long peptide (pure)
Binding buffers:	5 mM NH_4Ac, 15% ACN, pH 4.1 (S) 20 mM Tris-HCl, 15% ACN, pH 8 (Q/DEAE/ TREN)
Elution buffers:	250 mM NH4Ac, 15% ACN, pH 5.6 (S)
Flow rates:	0.3, 0.6, 1 and 2 mL/min
Residence times:	4, 2, 1 and 0.5 minutes

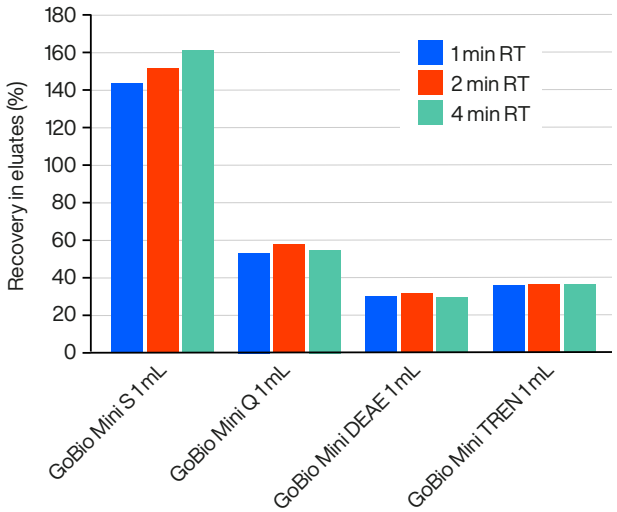


Figure 8. Dynamic binding capacity determined at 10% breakthrough for a 39 aa-long peptide at different residence times. The residence times used are specified in the graph.

Column:	GoBio Mini S 1 mL
Sample:	1 mg 39 aa-long peptide (pure)
Binding buffer:	5 mM NH_4Ac, 15% ACN, pH 4.1
Elution buffer:	250 mM NH_4Ac, 15% ACN, pH 5.6
Flow rates:	0.3, 0.6, 1 and 2 mL/min
Residence times:	4, 2, 1 and 0.5 minutes

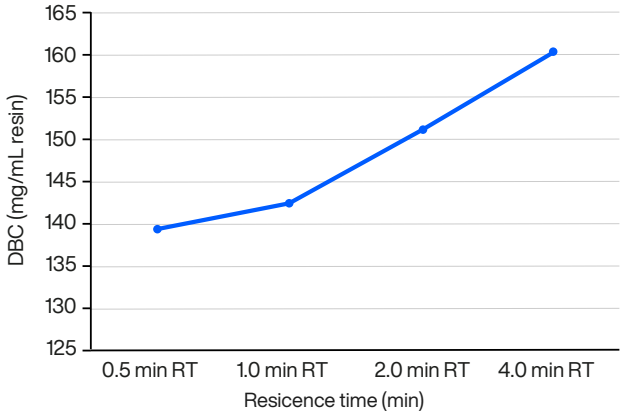


Figure 9. Relationship between DBC and residence time for a 39 aa-long peptide on GoBio Mini S (cation exchanger).

Columns:	GoBio Mini Q 1 mL GoBio Mini DEAE 1 mL GoBio Mini TREN 1 mL
Sample:	1 mg 39 aa-long peptide (pure)
Binding buffer:	20 mM Tris-HCl, 15% ACN, pH 8
Elution buffer:	20 mM Tris-HCl, 15% ACN, 1 M NaCl, pH 8
Flow rates:	0.3, 0.6 and 1 mL/min
Residence times:	4, 2 and 1 minutes

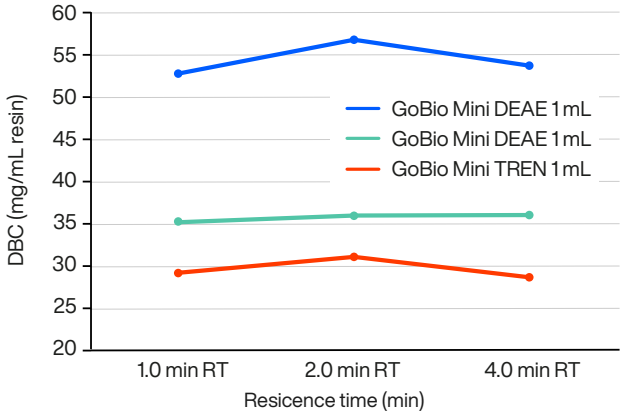


Figure 10 Relationship between DBC and residence time for a 39 aa-long peptide on GoBio Mini Q, GoBio Mini DEAE and GoBio Mini TREN (anion exchangers).

Flow properties

WorkBeads 40S and WorkBeads 40Q ion exchange chromatography resins are designed for high throughput protein separations under a variety of conditions. The high resolution that can be obtained even at high protein loadings and process applications. The pressure/flow properties for WorkBeads 40S is shown in Figure 11. The measurements were carried out with an open bed (column adaptor not pushed against the bed).

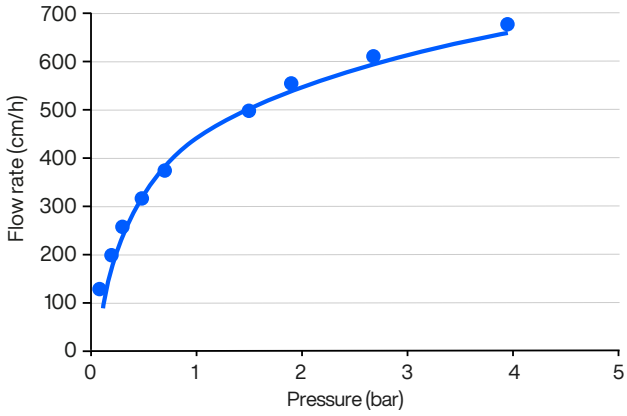


Figure 11. Pressure to flow rate properties of WorkBeads 40S determined with deionized water, 25 × 200 mm glass column.

Choice of resin

Which resin to choose ultimately depends on the target sequence and the nature of the impurities and contaminants to be removed. The choice between a cation exchanger or an anion exchanger is more straightforward than the choice between the different anion exchangers. A strong anion exchanger is easiest to optimize since it can be used at a broader pH range, but the selectivity may differ between the target molecule and the contaminants. Sometimes a weak exchanger is better at separating some contaminants from the target molecules. A weak and a strong anion exchanger are sometimes used as two orthogonal steps in purifications due to the difference in selectivity. The same principle is true for the multimodal IEX resin WorkBeads 40 TREN. The ligand in this resin has two amines that are positively charged below pH 9, which means that it can bind molecules relatively firmly even in the presence of high ionic concentrations, i.e. it is salt tolerant.

For example, some supernatants with higher ionic strength may interfere with the binding abilities of the target molecules to a standard ion exchanger, but it can still interact with WorkBeads 40 TREN. To conclude, many parameters determine which ion exchanger is most optimal to use and it all depends on the properties of the target molecule, the impurities and the surrounding conditions/buffers.

Cleaning-in-place

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 adsorbed 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 column. Cleaning using 1 M NaOH applied by a low flow for 15-30 min is often sufficient.

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 must be evaluated for each case.

Scale-up

Scale-up can conveniently be carried out from a 1 mL GoBio Mini column to a 25 L mL GoBio Prod column or by packing bulk resin of WorkBeads 40S, WorkBeads 40Q, WorkBeads 40 DEAE and WorkBeads 40 TREN into larger columns.

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

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 Dsalt 1 mL	1 mL × 5	45 360 103
GoBio Mini Dsalt 5 mL	5 mL × 5	45 360 107
GoBio Prep Dsalt[2]	20 mL × 1	55 700 021
GoBio Prep Dsalt	53 mL × 1	55 700 031
Bulk resins		
WorkBeads 100S	25 mL 200 mL 1 L 5 L	10 200 001 10 200 002 10 200 010 10 200 050
WorkBeads 100Q	25 mL 200 mL 1 L 5 L	10 100 001 10 100 002 10 100 010 10 100 050
WorkBeads Dsalt	300 mL 1 L 5 L	40 360 003 40 360 010 40 360 050
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] Packed on request.

Ordering information

Product name	Pack size	Article number
Prepacked column		
GoBio Mini IEX Screening Kit[1]	1 mL × 4	45 900 001
GoBio Mini Peptide Purification Kit[2]	1 mL × 2	45 300 102
GoBio Mini S 1 mL	1 mL × 1 1 mL × 5 1 m × 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

Product name	Pack size	Article number
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 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[3]	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[3]	20 mL × 1	55 415 021
GoBio Prep 16x100 40 TREN	20 mL × 1	55 463 021
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[3]	53 mL × 1	55 415 031
GoBio Prep 26x100 40 TREN[3]	53 mL × 1	55 463 031
GoBio Prod 80x200 40S[3]	1 L	55 420 042
GoBio Prod 80x200 40Q[3]	1 L	55 410 042
GoBio Prod 80x200 40 DEAE[3]	1 L	55 415 042
GoBio Prod 80x200 40 TREN[3]	1 L	55 463 042
GoBio Prod 130x200 40S[3]	2.7 L	55 420 062
GoBio Prod 130x200 40Q[3]	2.7 L	55 410 062
GoBio Prod 130x200 40 DEAE[3]	2.7 L	55 415 062
GoBio Prod 130x200 40 TREN[3]	2.7 L	55 463 062
GoBio Prod 200x200 40S[3]	6 L	55 420 072
GoBio Prod 200x200 40Q[3]	6 L	55 410 072
GoBio Prod 200x200 40 DEAE[3]	6 L	55 4150 72
GoBio Prod 200x200 40 TREN[3]	6 L	55 463 072
GoBio Prod 240x200 40S[3]	9 L	55 420 082
GoBio Prod 240x200 40Q[3]	9 L	55 410 082
GoBio Prod 240x200 40 DEAE[3]	9 L	55 415 082
GoBio Prod 240x200 40 TREN[3]	9 L	55 463 082
GoBio Prod 330x250 40S4[3]	21.4 L	55 420 093
GoBio Prod 330x250 40Q[3]	21.4 L	55 410 093
GoBio Prod 330x250 40 DEAE[3]	21.4 L	55 415 093
GoBio Prod 330x250 40 TREN[3]	21.4 L	55 463 093
Bulk resins		
WorkBeads 40S	25 mL 200 mL 1 L 5 L 10 L	40 200 001 40 200 002 40 200 010 40 200 050 40 200 060
WorkBeads 40Q	25 mL 200 mL 1 L 5 L 10 L	40 100 001 40 100 002 40 100 010 40 100 050 40 100 060
WorkBeads 40 DEAE	25 mL 200 mL 1 L 5 L 10 L	40 150 001 40 150 002 40 150 010 40 150 050 40 150 060
WorkBeads 40 TREN	25 mL 150 mL 1 L	40 603 001 40 603 003 40 603 010

[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.
[2] GoBio Mini Peptide Purification kit is a bundle of: GoBio Mini S 1 mL × 1 and GoBio Mini Q 1 mL × 1.
[3] 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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