Bio-Works

DATA SHEET

WorkBeads 40 TREN GoBio prepacked columns

WorkBeads™ 40 TREN resin for multimodal ion exchange chromatography (IEX) has a ligand that is positively charged below approx. pH 9. This resin 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 viruses and different host cell impurities, or as a polishing step in the mAb purification process.

This unique resin is also available in several different prepacked column sizes for fast screening to optimize purification conditions and purifications from laboratory to production scale. GoBio™ Mini 1 mL and 5 mL, GoBio Screen 7x100, GoBio Prep 16x100 and GoBio Prep 26x100, as well as GoBio Prod columns starting from 1 L.

- Differential selectivity due to higher salt tolerance and multimodal properties
- Reduced fouling of e.g., protein A resins by viruses and host cell impurity removal
- High binding capacity and purity
- 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 high 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 TREN resin contains ligands based on Tris(2-aminoethyl)amine (TAEA). The structure of the ligand used in WorkBeads 40 TREN is shown in Figure 1.

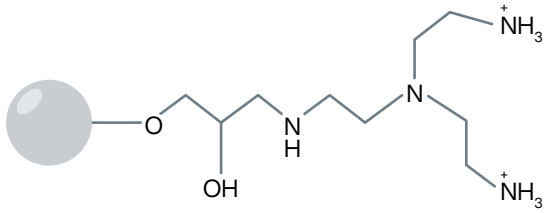

Figure 1. Structure of the ligand used in WorkBeads 40 TREN.

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 main characteristics of WorkBeads 40 TREN resin are shown in Table 1. For more detailed instructions of how to use WorkBeads 40 TREN, see instruction IN 40 600 020.

Table 1. 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)amine (TAEA)
Ionic capacity	130 – 200 µmol Cl^-/mL resin
Dynamic binding capacity	50 mg BSA/mLresin[2]
Max flow rate (20 cm bed height and 5 bar)[3]	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 range[4]	2 – 13
CIP and screening pH range[4]	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 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
Maximal 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 and GoBio Prod columns.

	GoBio Prod 80x200, GoBio Prod 130x200, GoBio Prod 200x200, GoBio Prod 280x200, GoBio Prod 360x250
Column hardware	Acrylic
Top and bottom filters	Polyamide
Top and bottom plugs	Polypropylene
Connections	TC-connections
Column volumes	1 L, 2.5 L, 5 L, 10 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) and 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.

Applications

Multimodal ion exchange chromatography

WorkBeads 40 TREN resin can be used for similar applications to those when using ion exchange chromatography resins. In Figure 2, an example of separation of the acidic proteins apo-transferrin, α-lactalbumin and soybean trypsin inhibitor are separated on GoBio Mini TREN 1 mL, prepacked with WorkBeads 40 TREN. Figure 3 shows a separation comparison between GoBio Mini TREN 1 mL and GoBio Mini DEAE 1 mL (a weak anion exchange chromatography column). As can be seen in the chromatograms, the selectivity is different. WorkBeads 40 TREN is a high-salt-tolerant multimodal AIEX resin.

Column: 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: 2.5 mL of 0.3 mg/mL apo-transferrin, 0.2 mg/mL α-lactalbumin, 0.6 mg/mL soybean trypsin inhibitor in binding buffer
Flow rate: 1 mL/min (150 cm/h)
Gradient: 0 – 100% elution buffer in 20 column volumes (CV)

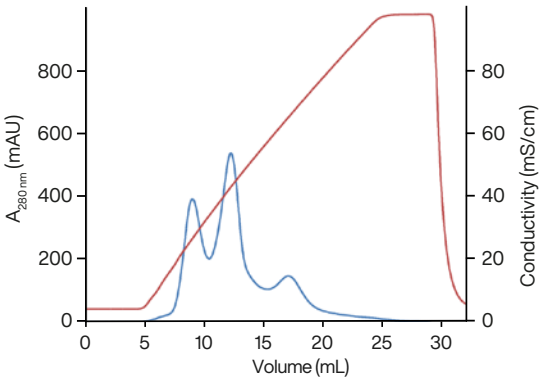

Figure 2. Separation of a protein mix on a GoBio Mini TREN 1 mL column. The peaks from left to right corresponds to apo-transferrin, α-lactalbumin and soybean trypsin inhibitor. The blue line corresponds to the absorbance at 280 nm and the red line to the conductivity.

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

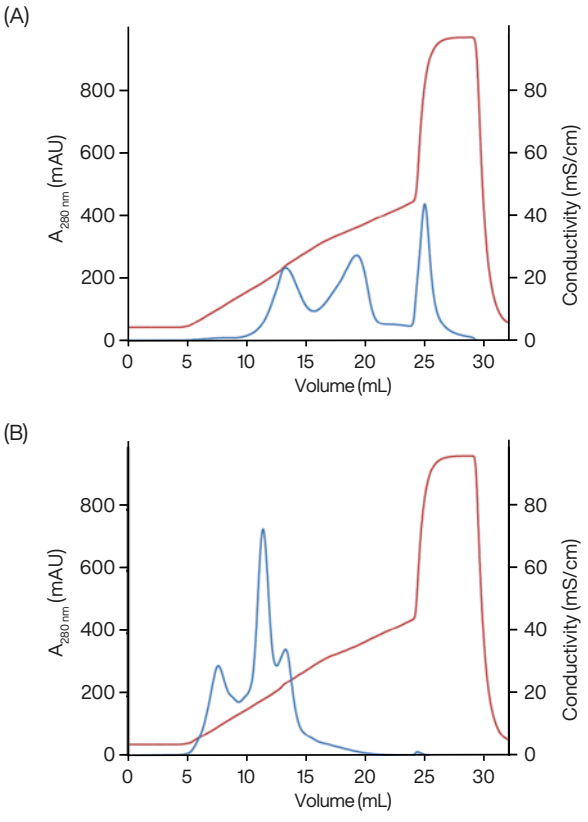

Figure 3. Comparison of separation of a protein mix on a GoBio Mini TREN 1 mL (A) and GoBio Mini DEAE 1 mL (B). A more shallow salt gradient was used compared to the separation in Fig. 2. The peaks from left to right corresponds to apo-transferrin, α-lactalbumin and soybean trypsin inhibitor. The blue line corresponds to the absorbance at 280 nm and the red line to the conductivity.

Viruses bind to WorkBeads 40 TREN

For biopharmaceutical processes that result in products for use in humans, virus clearance studies need to be conducted. Viruses in the final product can prove to be fatal, and therefore need to be removed in the purification process. This virus removal can be performed in a pre-treatment step using WorkBeads 40 TREN as shown below.

A feed (clarified supernatant containing mAbs expressed from CHO cells) was spiked with two viruses, MVM and

X-MuLV (which may be present in CHO cells), one at a time and in duplicates.

MVM, Minute Virus of Mice, is a small (18 – 26 nm) non-enveloped parvovirus and X-MuLV, Xenotropic Murine Leukemia Virus is a moderately large (80 – 120 nm) enveloped retrovirus. The virus titers pre- and post-purification on WorkBeads 40 TREN were measured and compared to control samples that showed the amount of virus applied to the columns. A logarithmic reduction value (LRV) was calculated.

The capacity of WorkBeads 40 TREN resin for reduction of virus in the flow through was high for both viruses tested. The average logarithmic reduction factor (LRV) of the parvovirus MVM in two runs was 4.89 Log_{10} $TCID_{50}$ units which is deemed effective removal. The average LRV of X-MuLV in two runs was > 3.67 Log_{10} $TCID_{50}$ units. This is moderately effective to effective removal, see Table 4 below.

Two different viruses were here shown to efficiently bind to WorkBeads 40 TREN, showing it can be used for virus clearance from feeds or for enrichment of viruses to be used for gene therapy and characterization studies etc.

Loading capacities for WorkBeads 40 TREN

In Table 4 loading capacities (determined at 4 minutes residence times) for two different viruses are presented. For proteins, peptides, viruses and ssDNA, all molecules that bind to this resin, see Table 5 below.

Table 4. Virus removal using WorkBeads 40 TREN.

Virus type	Reduction of MVM (Log_{10} $TCID_{50}$)	Reduction of X-MuLV (Log_{10} $TCID_{50}$)
Run 1	4.87	3.44
Run 2	4.91	3.82
Average	4.9	3.7

Table 5. Loading capacities of different molecules on WorkBeads 40 TREN.

Molecule	Dynamic binding capacity (mg/mL resin)	Resin/Column tested
MVM	4.9[1]	WorkBeads 40 TREN (10 × 100 mm)
X-MuLV	3.7[1]	WorkBeads 40 TREN (10 × 100 mm)
BSA	50	GoBio Mini TREN 1 mL
Peptide[3]	31	GoBio Mini TREN 1 mL
ssDNA[4]	21	WorkBeads 40 TREN (6.6 × 100 mm)

[1] Log_{10} $TCID_{50}$ in complex mixtures/feeds.
[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] Synthesized acidic 39 aa-long peptide.
[4] Synthesized 20 nt-long ssDNA.

Use of WorkBeads 40 TREN in mAb purification

Purification of monoclonal antibodies usually involves purification on chromatography resins with protein A ligands followed by polishing steps based on anion- or cation exchange chromatography.

The use of WorkBeads 40 TREN in binding or flow through mode will also facilitate removal of nucleic acids, viruses, host cell proteins and other cell-derived impurities. As protein A ligands may be cleaved by proteases, leached protein A ligands can be removed by a polishing step using a WorkBeads 40 TREN column after the protein A purification step. Notice that the majority of mAbs are basic, thus are mainly positively charged at neutral pH, and therefore do not bind to the resin.

Figure 4 and 5 shows that the of majority of HCD, HMWS and HCP from a CHO cell feed containing mAbs were adsorbed to the WorkBeads 40 TREN and thus removed from the sample before it was loaded onto the WorkBeads affimAb column. Viruses are also efficiently removed as has been shown above. No significant yield loss of mAbs was detected.

The characteristics of WorkBeads 40 TREN can be exploited in several ways in a mAb purification process, see Figure 6 and below.

1. As a guard column for removal of impurities before the protein A column.
2. In a polishing step after the Protein A purification step.

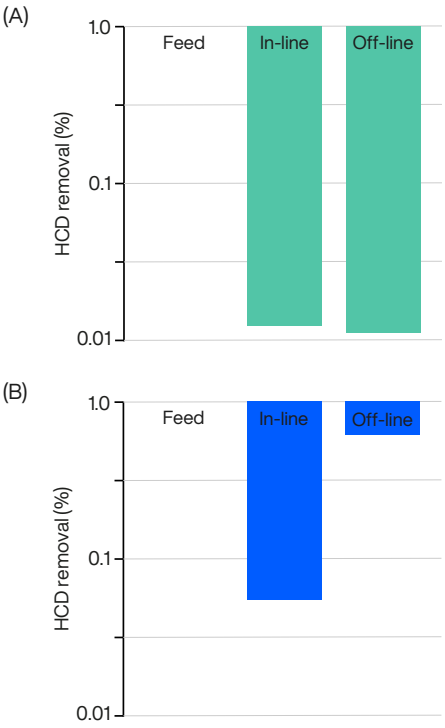

Figure 4. Removal of host cell impurities. (A) HCD removal pre- and post-WorkBeads 40 TREN for inline vs offline purifications. Feed value is set to 1. (B) HCP removal pre- and post-WorkBeads 40 TREN for inline vs offline purifications. Feed value is set to 1.

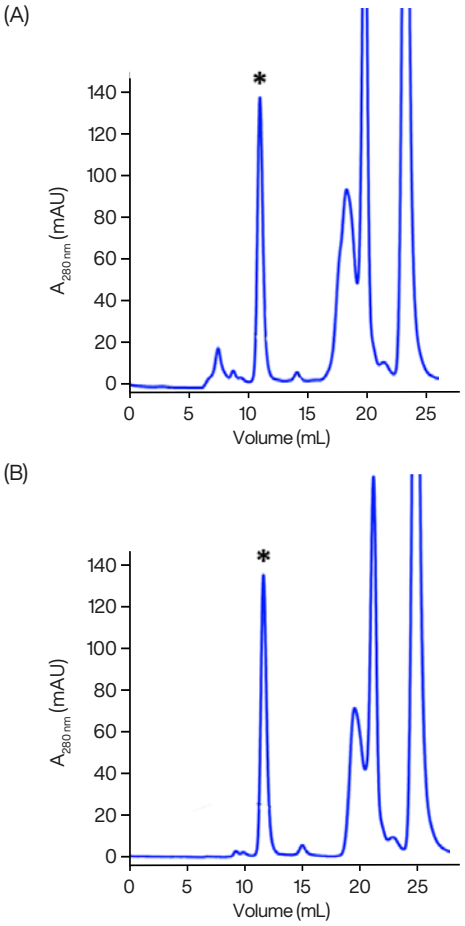

Figure 5. Analytical SEC profiles of CHO cell supernatants. (A) Before pre-treatment with WorkBeads 40 TREN and after (B). The asterisk (*) highlights the target mAb, and the high-molecular weight substances (HMWS) are marked with a circle.

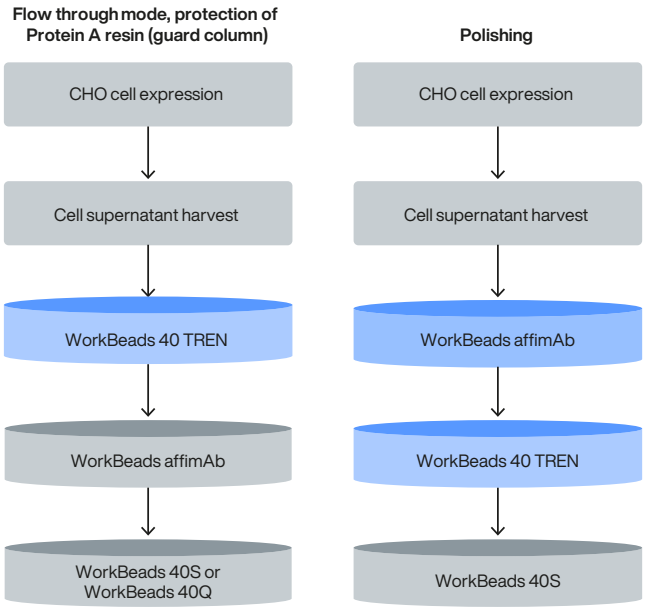

Figure 6. Use of WorkBeads 40 TREN in mAb purification processes.

Cleaning-in-place (CIP)

During purification impurities such as cell debris, lipids, nucleic acids and protein precipitates from the samples may gradually build-up in the resin, (cause fouling). 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. Cleaning of a column using 1 M NaOH applied by a low reversed flow for 2 hours or overnight is often sufficient.

According to internal studies WorkBeads 40 TREN has very good tolerance towards commonly used CIP solutions (168 h at 40°C).

The alkali tolerance has been investigated for WorkBeads 40 TREN by determination of the dynamic binding capacity (DBC) between cleaning-in-place cycles, see Figure 7.

Resin:	WorkBeads 40 TREN
Column:	6.6 × 100 mm
Sample:	*E coli* lysate, 50 runs loaded and eluted
CIP:	1 M NaOH, 15 min contact time, 50 cycles (CIP performed after each sample run)
Frontal analysis to calculate DBC as $Q_{B,10\%}$:	1 mg/mL BSA in 50 mM Tris, 50 mM NaCl, pH 7.4

Result: Reduction to 93% of DBC after 50 CIP cycles with 1 M NaOH and 50 cycles of *E coli* lysate loading & elution. Used column turned slightly yellowish, but capacity & function were not affected by the color change.

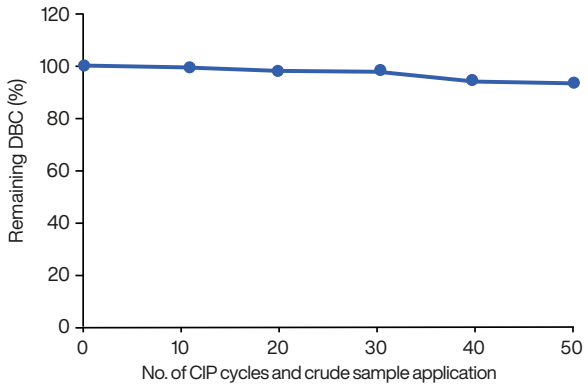

Figure 7. Alkali tolerance of WorkBeads 40 TREN.

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 S 1 mL	1 mL × 5	45 200 103
GoBio Mini Q 1 mL	1 mL × 5	45 100 103
GoBio Mini affimAb 1 mL	1 mL × 5	45 800 103
GoBio Mini Dsalt 1 mL	1 mL × 5	45 360 103
GoBio Mini S 5 mL	5 mL × 5	45 200 107
GoBio Mini Q 5 mL	5 mL × 5	45 100 107
GoBio Mini affimAb 5 mL	5 mL × 5	45 800 107
GoBio Mini Dsalt 5 mL	5 mL × 5	45 360 107
GoBio Prep 16x100 Dsalt[3]	20 mL × 1	55 700 021
GoBio Prep 26x100 Dsalt	53 mL × 1	55 700 031

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 mLand GoBio Mini TREN 1 mL.
3 Packed on request.

Ordering information

Product name	Pack size	Article number
Prepacked columns		
GoBio 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 40 TREN	3.8 mL × 1	55 463 001
GoBio Prep 16x100 40 TREN	20 mL × 1	55 463 021
GoBio Prep 26x100 40 TREN[1]	53 mL × 1	55 463 031
GoBio Prod 80x200 40 TREN[1]	1 L	55 463 042
GoBio Prod 130x200 40 TREN[1]	2.7 L	55 463 062
GoBio Prod 200x200 40 TREN[1]	6 L	55 463 072
GoBio Prod 280x200 40 TREN[1]	9 L	55 463 082
GoBio Prod 330x200 40 TREN[1]	21.4 L	55 463 093
Bulk resin		
WorkBeads 40 TREN	25 mL 150 mL 1 L	40 603 001 40 603 003 40 603 010

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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