WorkBeads 40 TREN
BabyBio TREN 1 ml
BabyBio TREN 5 ml

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 chromatins, viruses, endotoxins and other host cell impurities, or as a polishing step in the mAb purification process. This unique resin is also available in prepacked BabyBio™ 1 ml and 5 ml columns for fast and easy small-scale purifications as well as fast screening for optimizing purification conditions.

- Differential selectivity due to higher salt tolerance and multimodal properties
- Reduced fouling of *e.g.* protein A resins by chromatin, viruses, endotoxins and host cell impurity removal
- High binding capacity and purity
- Available in prepacked BabyBio 1 ml and 5 ml columns

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.

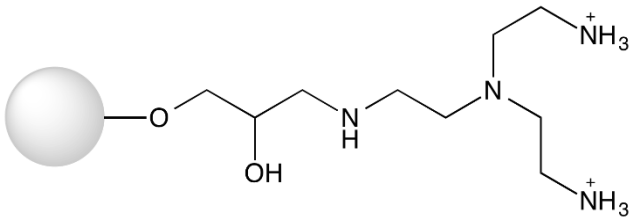

Figure 1. Structure of the ligand used in WorkBeads 40 TREN.

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, endotoxins and chromatin fragments
Matrix	Rigid, highly cross-linked agarose
Average particle size[1] (D_{v50})	45 µm
Ligand	Tris(2-ethylaminoethyl)amine (TAEA)
Dynamic binding capacity	50 mg BSA/ml resin[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.
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 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.

BabyBio description

The column is made from biocompatible polypropylene which does not significantly interact with biomolecules. The top and bottom filters are made from low-protein-binding polyethylene. The filters in the top and the bottom of the column have a pore size optimized to allow loading of semi-crude feed with minimal clogging.

The ready-to-use BabyBio prepacked columns are delivered with a plug in the inlet, a cut-off outlet and a cap for storage. The columns can be connected to a syringe, pump or chromatography system using finger tight fittings (coned 10 - 32) for 1/16" o.d. tubing (standard HPLC PEEK tubing), with a female and a male connection at the top and bottom respectively. BabyBio columns can be connected in series providing a convenient way to perform smaller scale-up experiments.

The main characteristics of BabyBio TREN columns are shown in Table 2. For additional information, see instruction IN 45 655 030.

Table 2. Main characteristics of BabyBio TREN columns.

	BabyBio TREN
Target substance	Proteins, peptides, oligonucleotides, viruses, endotoxins, and chromatin fragments.
Resin	WorkBeads 40 TREN
Matrix	Rigid, highly cross-linked agarose
Average particle size[1] (D_{v50})	45 µm
Ligand	Tris(2-aminoethyl)amine (TAEA)
Dynamic binding capacity	50 mg BSA/ml resin[2]
Column volume	1 ml 5 ml
Column dimension	7 x 28 mm (1 ml) 13 x 38 mm (5 ml)
Recommended flow rate[3]	
BabyBio 1 ml	0.25 - 1 ml/min (37 - 150 cm/h)
BabyBio 5 ml	1.25 - 5 ml/min (56 - 225 cm/h)
Maximum flow rate[4]	
BabyBio 1 ml	5 ml/min (780 cm/h)
BabyBio 5 ml	20 ml/min (900 cm/h)
Maximum back pressure	0.3 MPa, 3 bar, 43 psi
Chemical stability	Compatible with all standard aqueous buffers used for protein purification. Do not keep the column at low pH for prolonged time.
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 determined at 4 minutes residence time (0.25 ml/min in 1 ml column) in 50 mM Tris-HCl, 50 mM NaCl, pH 8.0.
3. Optimal flow rate during binding is depending on the sample. During column wash and elution, a flow rate of 1 ml/min and 5 ml/min can be used for 1 ml and 5 ml columns, respectively. Note: The maximum pressure the packed bed can withstand depends on the sample/liquid viscosity and chromatography resin characteristics. The pressure also depends on the tubing used to connect the column and the system restrictions after the column outlet.
4. 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).

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 BabyBio TREN 1 ml, prepacked with WorkBeads 40 TREN. Figure 3 shows a separation comparison between BabyBio TREN 1 ml and BabyBio 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.

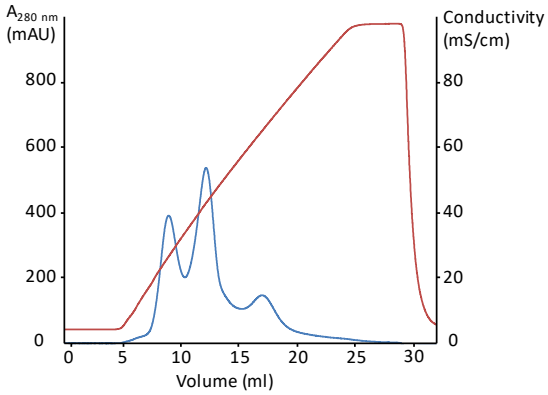

Figure 1. Separation of a protein mix on a BabyBio TREN 1 ml column. The peaks from left to right corresponds to apo-transferrin, α-lactalbumin and soybean trypsin inhibitor. The blue line corresponds to the absorbance at 280 nm and the red line to the conductivity.

Column:	BabyBio 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)

Column:	(A) BabyBio TREN 1 ml (B) BabyBio 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

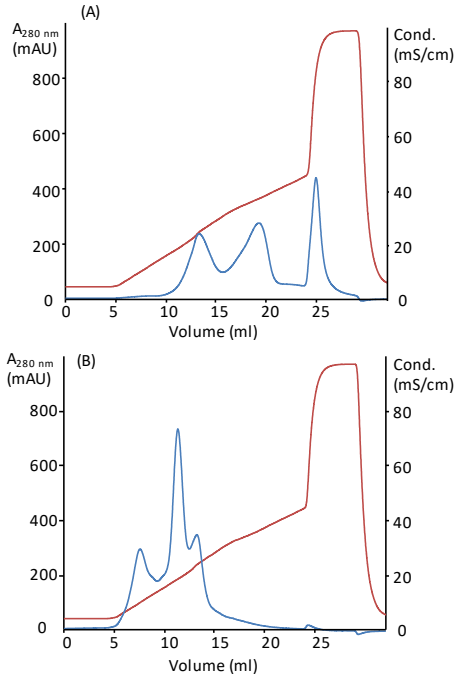

Figure 2. Comparison of separation of a protein mix on a BabyBio TREN 1 ml (A) and BabyBio DEAE 1 ml (B). A more shallow salt gradient was used compared to the separation in Fig. 2. The peaks from left to right corresponds to apo-transferrin, α-lactalbumin and soybean trypsin inhibitor. The blue line 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 Log10 TCID50 units which is deemed effective removal. The average LRV of X-MuLV in two runs was > 3.67 Log10 TCID50 units. This is moderately effective to effective removal, see Table 3 below.

Table 3. 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

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

WorkBeads 40 TREN has been shown to bind chromatin and other host-cell derived impurities in a mammalian cell culture (Nian et al., J. Chromatogr. A, 1431 (2016) 1-7; Chen et al., J. Biotechnol., 236 (2016) 128-140). In Table 4 loading capacities (determined at 4 minutes residence times) for proteins, peptides, viruses and ssDNA, all molecules that bind to this resin, are shown.

Table 4. Loading capacities of different molecules on WorkBeads 40 TREN

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

1. Log10 TCID50 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 presence of chromatin fragments (fragments of the chromosomes, based on histone proteins and DNA) is a major cause for fouling of protein A columns, and is also a key impurity after the protein A step. Chromatin particles are heavily charged structures with massive negative net charges. Due to this, they can easily be adsorbed on WorkBeads 40 TREN at neutral or low pH, which has proved to be useful for removal of chromatin and other impurities.

The use of WorkBeads 40 TREN in binding or flow through mode will also facilitate removal of nucleic acids, endotoxins, 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 precipitation agent added to the feed to induce chromatin precipitation for easy removal by continuous centrifugation followed by depth filtration before the protein A step. 0.5 - 5% g resin/ml cell supernatant is often enough for chromatin removal from cell supernatant feed.

2. As a guard column for removal of chromatin and other impurities before the protein A column.

3. In a polishing step after the Protein A purification step.

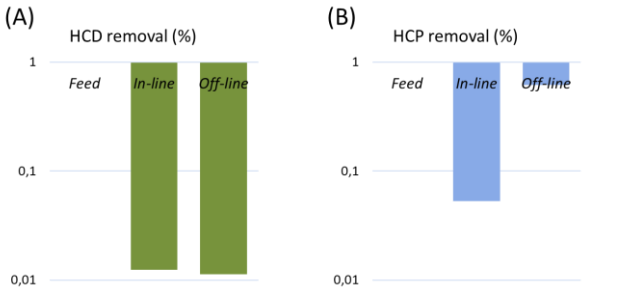

Figure 4. Removal of host cell impurities. (A) HCD removal pre- and post-WorkBeads 40 TREN for inline *vs* offline purifications. Feed value is set to 1. (B) HCP removal pre- and post-WorkBeads 40 TREN for inline *vs* offline purifications. Feed value is set to 1.

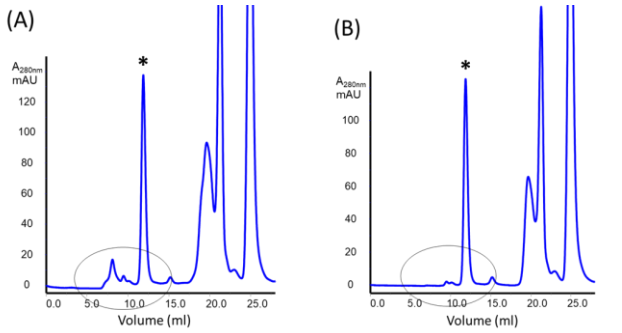

Figure 5. Analytical SEC profiles of CHO cell supernatants. (A) Before pre-treatment with WorkBeads 40 TREN and after (B). The asterisk (*) highlights the target mAb, and the high-molecular weight substances (HMWS) are marked with a circle.

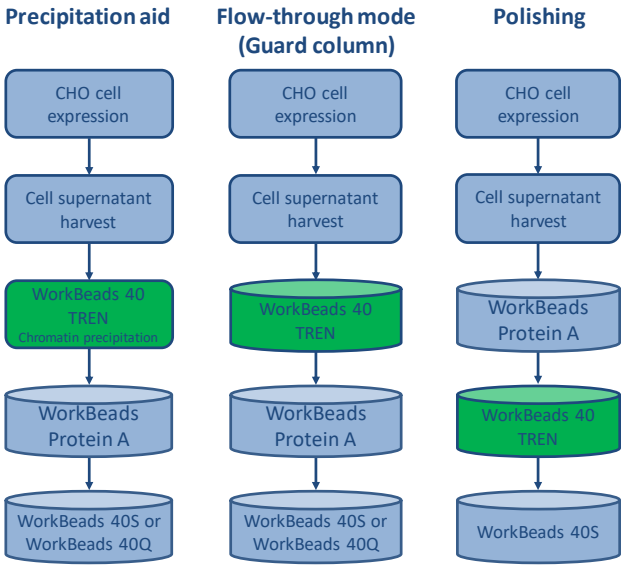

Figure 6. Use of WorkBeads 40 TREN in mAb purification processes.

Cleaning-in-place

After chromatin removal from cell supernatant/feeds WorkBeads 40 TREN can usually not be cleaned but must be discarded. During other purification schemes, 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.

Storage

Store WorkBeads 40 TREN resin and BabyBio TREN columns at 2 to 25°C in 20% ethanol.

Related products

Product name	Pack size[1]	Article number
Prepacked columns		
BabyBio IEX Screening Kit[2]	1 ml x 4	45 900 001
BabyBio S 1 ml	1 ml x 5	45 200 103
BabyBio S 5 ml	5 ml x 5	45 200 107
BabyBio Q 1 ml	1 ml x 5	45 100 103
BabyBio Q 5 ml	5 ml x 5	45 100 107
BabyBio affimAb 1 ml	1 ml x 5	45 800 103
BabyBio affimAb 5 ml	5 ml x 5	45 800 107
BabyBio Dsalt 1 ml	1 ml x 5	45 360 103
BabyBio Dsalt 5 ml	5 ml x 5	45 360 107
Bulk resins		
WorkBeads 40S	25 ml	40 200 001
	200 ml	40 200 002
WorkBeads 40Q	25 ml	40 100 001
	200 ml	40 100 002
WorkBeads affimAb	25 ml	40 800 001
	200 ml	40 800 002
WorkBeads Dsalt	300 ml	40 360 003

1. All different pack sizes are available on www.bio-works.com
2. BabyBio IEX Screening Kit includes one of each: BabyBio S 1 ml, BabyBio Q 1 ml, BabyBio DEAE 1 ml and BabyBio TREN 1 ml.

Ordering information

Product name	Pack size	Article number
WorkBeads 40 TREN	25 ml	40 603 001
	150 ml	40 603 003
	1 L	40 603 010
BabyBio TREN 1 ml	1 ml x 1	45 655 211
	1 ml x 2	45 655 212
	1 ml x 5	45 655 213
	1 ml x 10	45 655 214
BabyBio TREN 5 ml	5 ml x 1	45 655 215
	5 ml x 2	45 655 216
	5 ml x 5	45 655 217
	5 ml x 10	45 655 218

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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