

## BabyBio TREN

The ready-to-use BabyBio™ TREN columns are prepacked with WorkBeads™ 40 TREN resin and are available in two column sizes, 1 ml and 5 ml. WorkBeads 40 TREN resin for multimodal Ion Exchange Chromatography (IEX) has a ligand which is positively charged below approx. pH 9. The resin can be used for several different applications, e.g., for multimodal IEX, for sample cleanup in monoclonal antibody (MAb) purification processes in order to guard the Protein A column from chromatin and other host cell impurities, or as a polishing step in the MAb purification process.

- Prepacked and ready-to-use columns for fast and reliable results
- Improved selectivities through multimodal IEX separation
- Reduced fouling of Protein A resins by chromatin and host cell impurity removal

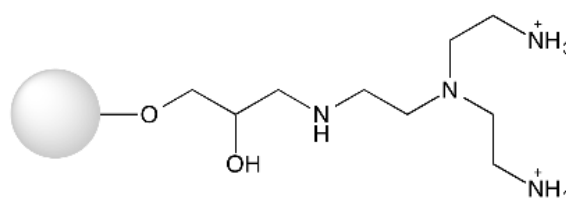


Figure 1. Structure of the ligand used in WorkBeads 40 TREN.

### Resin description

BabyBio TREN 1 ml and 5 ml columns are prepacked with WorkBeads 40 TREN resin. WorkBeads are agarose-based chromatographic resins manufactured using a proprietary method that results in porous beads with a tight size distribution and very 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 a ligand based on Tris(2-aminoethyl) amine (TAEA). The structure of the ligand used in WorkBeads 40 TREN is shown in Figure 1.

WorkBeads 40 TREN resin can be used for the separation of biomolecules exploiting surface charge to purify proteins, peptides and oligonucleotides. BabyBio TREN columns can be used to adsorb impurities (negative chromatography mode), allowing clean-up of samples before Protein A-based purification of MAbs to reduce fouling of the Protein A resin.

The main characteristics of BabyBio TREN columns are shown in Table 1. For more detailed instructions of how to use BabyBio TREN, see instruction IN 45 655 030.

Table 1. Main characteristics of BabyBio TREN 1 ml and BabyBio TREN 5 ml columns.

BabyBio TREN	
Target substance	Proteins, peptides and oligonucleotides. Chromatin fragments.
Resin	WorkBeads 40 TREN
Matrix	Rigid, highly cross-linked agarose
Average particle size <sup>1</sup> (D <sub>v50</sub> )	45 µm
Ligand	Tris(2-aminoethyl)amine (TAEA)
Column volume	1 ml 5 ml
Column dimension	7 x 28 mm (1 ml) 13 x 38 mm (5 ml)
Recommended flow rate	
BabyBio 1 ml	1 ml/min (150 cm/h)
BabyBio 5 ml	5 ml/min (225 cm/h)
Maximum flow rate <sup>2</sup>	
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. 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).

## Column description

The column is made from biocompatible polypropylene which does not significantly interact with biomolecules. The top and bottom filters are made from polyethylene. The ready-to-use BabyBio 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).

## Applications

### Multimodal ion exchange chromatography

BabyBio TREN columns can be used for multimodal ion exchange chromatography. Due to the ligand structure of the WorkBeads 40 TREN resin the selectivity is slightly different compared to WorkBeads 40 Q and WorkBeads 40 DEAE anion exchange resins. In Figure 2, an example of separation of the acidic proteins apo-transferrin, α-lactalbumin and soybean trypsin inhibitor using BabyBio TREN 1 ml is presented. Figure 3 shows a separation comparison between BabyBio TREN 1 ml and BabyBio DEAE 1 ml (a weak anion exchange chromatography column).

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

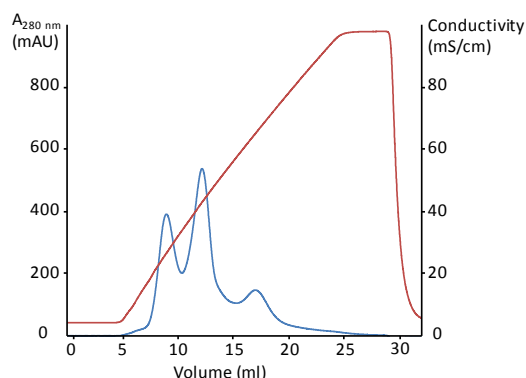


Figure 2. Separation BabyBio TREN 1 ml column of, peaks from left to right, apo-transferrin, α-lactalbumin and soybean trypsin inhibitor. The blue line corresponds to the absorbance at 280 nm and the red line to the conductivity.

Column: (A) 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  $\alpha$ -lactalbumin, 0.6 mg/ml soybean trypsin inhibitor in binding buffer  
Flow rate: 150 cm/h (1 ml/min)  
Gradient: 0 - 40% elution buffer in 20 CV

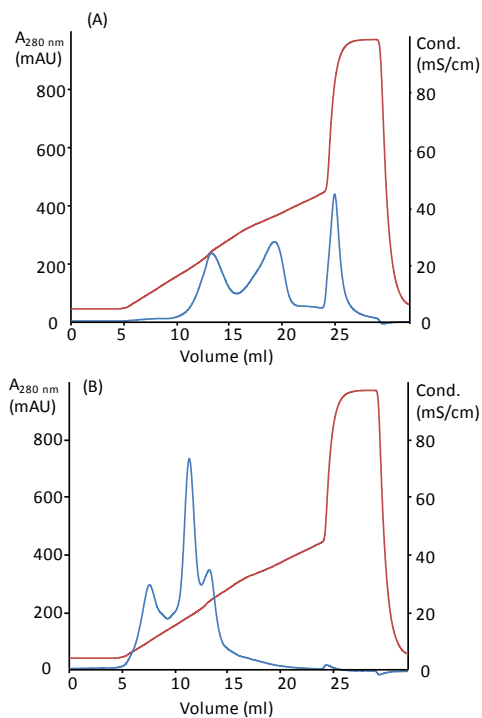


Figure 3. Separation comparison between BabyBio TREN 1 ml (A) and BabyBio DEAE 1 ml (B). The blue line corresponds to the absorbance at 280 nm and the red line to the conductivity.

### Use of BabyBio TREN in MAb purification

Purification of monoclonal antibodies usually involves purification on chromatography resins derivatized 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 it is also a key impurity remaining after the Protein A step. Chromatin particles are large structures with massive negative net charges. Due to this, they can easily be adsorbed on WorkBeads 40 TREN.

The use of BabyBio 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 BabyBio 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.

The characteristics of BabyBio TREN can be exploited in several ways in MAb purification process, see Figure 4.

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

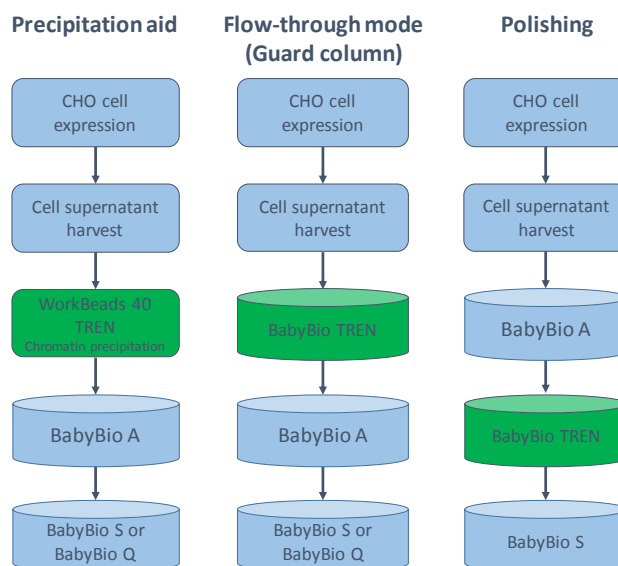


Figure 4. Use of BabyBio TREN and WorkBeads 40 TREN in MAb purification processes.

For scale-up WorkBeads 40 TREN (see Data sheet, DS 140 600 020) can be packed in larger columns. Alternatively, WorkBeads 40 TREN can be used for clean-up of MAb feeds, e.g., CHO cell supernatants, by incubation of a suspension of resin (0.5 - 5%) in the feed before application to the Protein A column. This allows significant reduction of the rate of fouling of the Protein A column. (Nian et al., *J. Chromatogr. A*, 1431 (2016) 1-7; Chen et al., *J. Biotechnol.*, 236 (2016) 128-140.)

### Scale-up

Scale-up can conveniently be carried out from a 1 ml column to a 5 ml column. Column binding capacity may also be increased by coupling up to five columns in series. Note that the back pressure generated by the stacked columns will increase. Further scale-up can be achieved by packing bulk WorkBeads 40 TREN resin in larger columns (see *Related products*). For more detailed description, please see instructions IN 45 655 030.

## 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. The severity of this process depends on the type of sample applied to the column, and the pretreatment of the sample. The impurities may reduce the performance of the column over time. Regular cleaning (Cleaning-in-place, CIP) keeps the resin clean, reduces the rate of further contamination, 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.

Sanitization (reduction of microorganisms) can be achieved 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 needs to be evaluated for each case.

## Equipment

Prepacked BabyBio TREN ready-to-use columns can be used with most standard liquid chromatography equipment. Purification can also be carried out using a syringe connected to the column by a luer/std HPLC connector.

## Storage

Equilibrate the column in 20% ethanol and close it securely using the included plug and cap. Store the column at 2 to 25°C.

## Related products

Product name	Pack size <sup>1</sup>	Article Number
<b>Prepacked columns</b>		
BabyBio Dsalt 5 ml	5 ml x 5	45 360 107
BabyBio S 1 ml	1 ml x 5	45 200 103
BabyBio Q 1 ml	1 ml x 5	45 100 103
BabyBio DEAE 1 ml	1 ml x 5	45 150 103
<b>Bulk resins</b>		
WorkBeads 40 TREN	25 ml	40 603 001
WorkBeads 40 TREN	150 ml	40 603 003
WorkBeads 40 SEC	25 ml	40 300 001
<b>Accessories</b>		
Column plug male 1/16"	10	70 100 010
Column cap female 1/16"	10	70 100 020

1. Other pack sizes can be found in the complete product list on [www.bio-works.com](http://www.bio-works.com)

## Ordering information

Product name	Pack size	Article number
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](mailto:sales@bio-works.com) or contact your local distributor.

For more information about local distributor and products please visit [www.bio-works.com](http://www.bio-works.com) or contact us at [info@bio-works.com](mailto:info@bio-works.com)



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