WorkBeads 40 TREN

WorkBeads™ 40 TREN resin for multimodal Ion Exchange Chromatography (IEX) has a ligand that 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 chromatins and other host cell impurities, or as a polishing step in the mAb purification process.

- Improved selectivities through multimodal IEX separation
- Reduced fouling of Protein A resins by chromatin and host cell impurity removal
- High binding capacity and purity

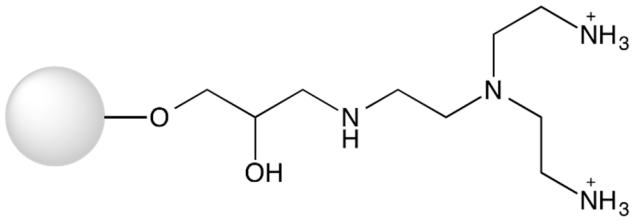

Figure 1. Structure of the ligand used in WorkBeads 40 TREN.

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.

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 and oligonucleotides. Chromatin fragments.
Matrix	Rigid, highly cross-linked agarose
Average particle size[1] (D_{v50})	45 µm
Ligand	Tris(2-ethylaminoethyl)amine (TAEA)
Max flow rate (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.
pH stability	2 - 13
Storage	2 to 25 °C in 20% ethanol

1. The median particle size of the cumulative volume distribution.

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 show 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 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)

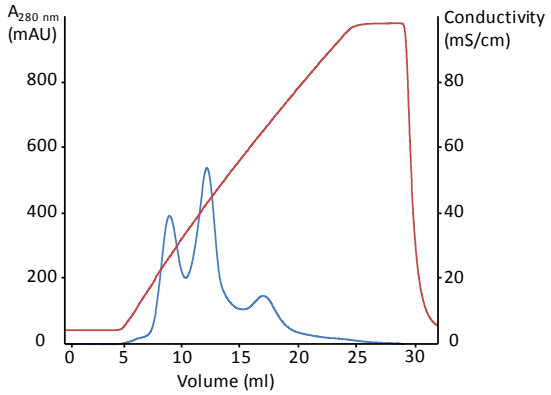

Figure 2. Separation of a protein mix on a BabyBio TREN 1 ml column. The peaks from left to right corresponds to apo-transferrin, α-lactalbumin and soybean trypsin inhibitor. The blue line corresponds to the absorbance at 280 nm and the red line to the conductivity.

Column: (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

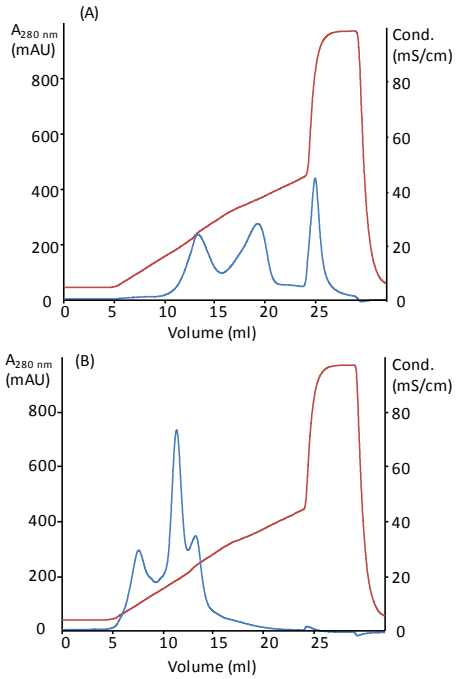

Figure 3. Comparison of separation of a protein mix on a BabyBio TREN 1 ml (A) and BabyBio DEAE 1 ml (B). A more shallow salt gradient was used compared to the separation in Fig. 2. The peaks from left to right corresponds to apo-transferrin, α-lactalbumin and soybean trypsin inhibitor. The blue line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

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 (Nian et al., J. Chromatogr. A, 1431 (2016) 1-7; Chen et al., J. Biotechnol., 236 (2016) 128-140.)

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. The characteristics of WorkBeads 40 TREN can be exploited in several ways in a mAb purification process, see Figure 4 and below.

1. As a precipitation agent added to the feed to induce chromatin precipitation for easy removal by continues 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.

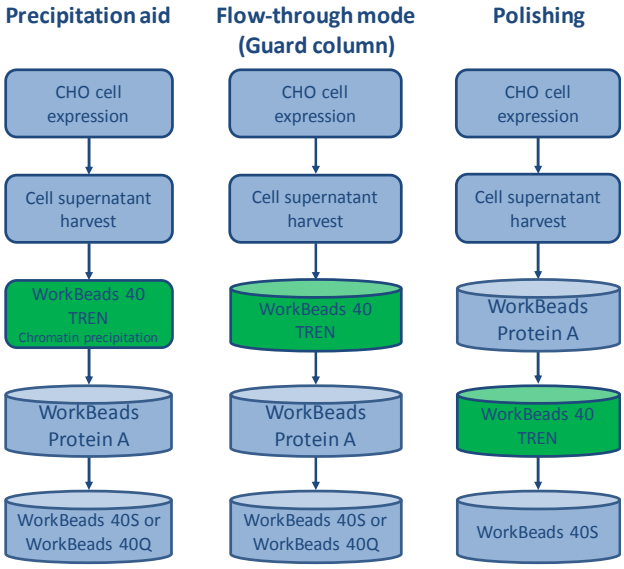

Figure 4. Use of WorkBeads 40 TREN in mAb purification processes.

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 at 2 to 25°C in 20% ethanol.

Related products

Product name	Pack size[1]	Article number
Prepacked columns		
BabyBio TREN 1 ml	1 ml x 5	45 655 213
BabyBio TREN 5 ml	5 ml x 5	45 655 217
BabyBio Dsalt 5 ml	5 ml x 5	45 360 107
Bulk resins		
WorkBeads 40S	25 ml	40 200 001
WorkBeads 40Q	25 ml	40 100 001
WorkBeads 40 DEAE	25 ml	40 150 001

1. Other pack sizes can be found in the complete product list on www.bio-works.com

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

Orders: sales@bio-works.com or contact your local distributor.

For more information about local distributor and products please visit www.bio-works.com or contact us at info@bio-works.com

Bio-Works
Virdings allé 18
754 50 Uppsala
Sweden