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 chromatins 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
- Guard column before Protein A-based column to reduce fouling during mAb purification

Short protocol

This is a general short protocol for the use of BabyBio columns. Detailed instructions and recommendations for optimization are given later in this instruction.

1. Connect the column to the chromatography system, syringe or pump.
2. Equilibrate with binding buffer.
3. Apply the sample.
4. After sample application, remove unbound material by washing the column with e.g., 20 - 30 columns volumes (CV) washing buffer.

   Note: If the resin is used in “negative mode” the target molecule with elute in the wash step.
5. Elute with elution buffer containing a competing agent or by changing the pH.
6. Wash the column with 5 CV deionized water to remove the elution buffer.
7. Equilibrate the column with 5 CV 20% ethanol for storage. Close the column using the included cap and plug.

Principle

BabyBio TREN columns are prepacked WorkBeads 40 TREN resin. WorkBeads 40 TREN resins contain ligands based on Tris(2-aminoethyl)amine (TAEA). The structure of the ligand used in WorkBeads 40 TREN is shown in Figure 1.

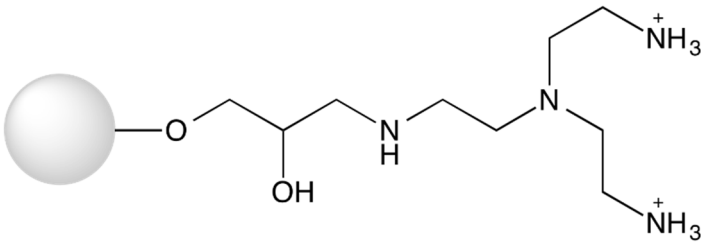

Figure 1. Structure of the ligand used in WorkBeads 40 TREN.

Multimodal chromatography

Multimodal chromatography separates protein, peptides and other biomolecules via a ligand acting with more than one interaction sites. The interaction utilizes two, or more, different properties for example charge and hydrophobicity. Depending on the chromatographic conditions the interactions differs, and work either together or separate in the purification procedure.

Using multimodal chromatography can be an excellent alternative, when the common techniques (e.g., ion exchange chromatography, size exclusion chromatography or affinity chromatography) are insufficient. However, to reach the optimal purification using multimodal chromatography, the purification process need to be optimized according to the target molecule properties.

WorkBeads 40 TREN in mAb purification

Purification of monoclonal antibodies usually involves purification on Protein A chromatography resins followed by polishing steps based on anion- or cation exchange chromatography. The presence of chromatin fragments (fragments of chromosomes, based on histone proteins and DNA) is a major cause for fouling of Protein A columns, and is also a key impurity in to be removed during mAb purification. Chromatin particles are heavily charged structures with massive negative net charges. Due to this, these can easily be adsorbed on WorkBeads 40 TREN, which has proved to be useful for removal of chromatin and other impurities such as nucleic acids, endotoxins and host cell proteins (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 WorkBeads 40 TREN after the Protein A purification step. Notice that the majority of mAbs are basic, thus are mainly positively charged at neutral pH or low pH, and therefore do not bind to the resin.

The characteristics of WorkBeads 40 TREN can be exploited in several ways in mAb purification process:

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. Only 0.5 - 5 g resin/ml feed is generally needed.

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.

Multimodal IEX

WorkBeads 40 TREN resin is positively charged below approx. pH 9. Choose a suitable pH and buffer for the binding of the target protein or the impurities (if the purification is done in “negative mode”). One pH unit above pI, for WorkBeads 40 TREN is a good starting point. The binding conditions should be optimized to achieve binding of the target protein or the impurities. When scouting for the best conditions it is important to start with sufficiently low ionic strength. Guideline for a starting point for designing the experiment is given in Table 1.

Table 1. Typical buffer composition for purification using BabyBio TREN.

Product	Buffer	Buffer composition
BabyBio TREN	Binding buffer	50 mM Tris-HCl, pH 7.4
	Elution buffer	50 mM Tris-HCl, 1 M NaCl, pH 7.4

Purification

Applications can be carried out at room temperature or at temperatures down to 4°C. Operation at a low temperature may require a reduced flow rate due to the increased viscosity of the buffer. All steps can be carried out with a syringe, a peristaltic pump or a chromatography system. If the chromatography system has a pressure limit functionality, set the maximum pressure over the column to 3 bar (remember to take the system fluidics contribution to the pressure into account).

1. Prepare the sample

After cell disruption or extraction, clarify the sample by centrifugation at 10 000 - 20 000 × *g* for 15 - 30 minutes. It is generally recommended to pass the sample through a 0.22 - 0.45 µm filter (e.g. a syringe filter) to avoid inadvertently applying any remaining particles onto the column. If the sample contains only small amounts of particles, centrifugation may be omitted and it is enough only to carry out filtration. Application of a sample that has not been properly clarified may reduce the performance and lifetime of the column. The sample should be applied under conditions similar to those of the binding buffer. The sample should be applied at 0.5 - 1 ml/min for the BabyBio 1 ml or 2 - 5 ml/min for the BabyBio 5 ml columns. A too high flow rate may reduce the yield.

2. Connect the column

Cut off or twist off the end at the outlet of the column, see Figure 2.

Note: It is of high importance to cut off the tip at the very end of the cone, preferable using a scalpel. Incorrect removal of the end piece will affect the performance of the column.

Connect the column to your equipment using the recommended connectors shown in Table 2. Fill the equipment with deionized water or buffer and make drop-to-drop connection with the column to avoid getting air into the column. Carry out all steps, except for sample application, at 1 ml/min (BabyBio 1 ml column) or 5 ml/min (BabyBio 5 ml column).

Figure 2. Removal of the cut-off end at the column outlet should be done by cutting or by twisting (A) not bending (B).

Table 2. Recommended connectors for coupling BabyBio columns to the equipment of choice.

Equipment	Accessories for connection
Syringe	Female luer/male coned 10 - 32 threads
Chromatography system	Fingertight connectors (coned 10 - 32 threads) for 1/16" o.d. tubing

3. Remove the storage solution

The column contains 20% ethanol on delivery. This storage solution should be washed out before use. Wash the column with 5 CV deionized water or buffer. Avoid flow rates higher than 2 ml/min for BabyBio 1 ml columns or 10 ml/min for BabyBio 5 ml columns before the storage solution has been removed to avoid overpressure due to the relatively high viscosity of the 20% ethanol solution.

4. Equilibrate the column

Equilibrate the column with 5 - 10 CV suitable running buffer.
Note: To avoid bacterial growth and poor column performance, use only freshly prepared and filtered buffers.

5. Apply the sample

Apply the sample at 0.5 - 1 ml/min for the BabyBio 1 ml or 2 - 5 ml/min for the BabyBio 5 ml columns. A too high flow rate may reduce the yield.

6. Wash

After sample application, remove unbound material by washing the column with 20 - 30 CV washing buffer or until desired $A_{280\,nm}$ absorbance of the wash fractions (e.g., 0.01 - 0.02) is obtained.
Note: If the resin is used in "negative mode" the target molecule with elute in the wash step.

7. Elute

Alternative 1: Desorb the target protein with 5 CV elution buffer.
Alternative 2: For high purity, gradient elution is recommended. For example, use a linear gradient from 0 to 100% with 20 CV.
Alternative 3: Target protein is eluted in the wash step and impurities are eluted using elution buffer

8. Re-equilibrate

Clean the column with 0.5 - 1 M NaOH for 15 - 30 minutes and re-equilibrate the column with 10 CV binding buffer to restore the pH.

9. Column storage

Wash the column with 5 CV deionized water to remove the remaining buffer. Equilibrate the column with 10 CV 20% ethanol for storage. Close the column using the cap and plug (included).

Scale-up

Scale-up from a BabyBio 1 ml column can easily be done by using a BabyBio 5 ml column and applying a sample volume five times larger. BabyBio columns can be connected in series with a maximum of five columns. This will increase the capacity accordingly. By connecting BabyBio columns in series, column volumes from 1 ml to 25 ml can be obtained.

BabyBio columns are easily connected together without accessories. The pressure drop across each column bed will be the same as for a single column, but the upstream columns will be exposed to a higher internal pressure since it is affected by the added pressure drops across the downstream columns. It may therefore be necessary to decrease the flow rate accordingly to avoid reaching the maximum pressure limit in the first column. If possible, the maximum pressure of the chromatography system should be set according to Table 3. Remember to take the system fluidics contribution to the pressure into account.

Table 3. Recommended maximum pressure settings for BabyBio columns connected in series. Notice that the maximum pressure over each column is always 3 bar.

Number of columns in series	Max pressure BabyBio 1 ml (bar)	Max pressure BabyBio 5 ml (bar)
1	3.0	3.0
2	6.0	6.0
3	9.0	9.0
4	12	10[1]
5	15	10[1]

1 The maximum pressure is limited by the column hardware maximum pressure.

The column size should be selected based on the estimated amount of protein to be purified or impurities to be bound. A test run with a defined small volume of sample on a BabyBio 1 ml column can be used to estimate the concentration of the target protein or impurities in the sample. A general recommendation is to use 70 - 80% of the column binding capacity. For large sample volumes with low concentrations of the target protein, it may be suitable to use a larger column than the calculated one to allow higher sample flow rates, and consequently shorter application time. For example, a 5 ml column allows a flow rate five times higher than a 1 ml column due to the larger cross-section of the column. Have in mind that too high flow rate may reduce the binding capacity.

For columns larger than 20 ml, it is recommended to pack a single column using bulk resin. To find out more about bulk chromatography resins, please visit www.bio-works.com

Optimization

The following paragraphs will give indications on some parameters that can be tuned to get the optimal conditions for purification of using BabyBio TREN.

Selection of buffer

Selecting a buffer with optimal conditions for the target protein will improve the result of the purification. The buffer should be chosen with a pK_a-value within 0.5 units from the intended pH to obtain a high enough buffering capacity. Table 1 shows one example of buffers which can be used for ion exchange chromatography, however the buffer choice will be depending on the target molecule and aim of the purification procedure. For other useful buffers and their pK_a-values at 25 °C see reference: Methods in Enzymology, Volume 463, pp 46-47, Burgess, R.R and Deutcher M.P.

The buffer substance should be selected to have the same charge as the resin. A buffer with opposite charge will interact with the charged groups in the resin and may cause local pH disturbances that destroys the separation. Usually, low conductivity in the binding buffer is preferred but optimization with regard to pH and conductivity can improve binding capacity. An increase in ionic strength may decrease the ability of contaminants to bind while the target protein remains bound. However, chromatographic conditions should be chosen so that the protein is stable during purification.

Optimization of binding

The key conditions to be optimized is usually pH and conductivity (by addition of NaCl or other salts, or dilution). The conditions must also be selected to keep the protein in its native state.

The flow rate during sample loading affects the binding capacity and resolution during the elution. A low flow rate during sample application promotes binding capacity since more time is allowed for mass transport of the target substance into the pores of the resin. A small substance, e.g., a peptide, that has a high diffusion rate will have fast mass transport into the resin and can thus be adsorbed efficiently at high flow rates. A large target substance (e.g., a large protein) has a lower diffusion rate and is more hindered by the walls in the pores giving slow mass transport, causing reduced dynamic binding capacity. A high binding capacity of this substance may thus require a lowered flow rate. If only a part of the binding capacity of the column is used the sample application can be at a higher flow rate without loss of the target substance.

For scale-up planning it is useful to use the expression r*esidence time* instead of flow rate. The residence time can be defined as the time between entering and exiting the column of a specific part of the sample or buffer. It can be calculated as column volume divided by the volumetric flow (e.g., the residence time for 1 ml column at 0.5 ml/min is 1 ml / 0.5 ml/min = 2 minutes). The residence time is typically 1 to 5 minutes in IEX. When a suitable residence time has been selected using BabyBio TREN column, this value can be used for calculation of a suitable flow rate on a larger column with higher bed. The linear flow rate can be increased if the bed height is increased while keeping the residence time constant.

Optimization of washing

A continuously decreasing UV signal during washing is an indication of unbound material being washed out. Washing should be continued until the UV absorbance signal has reached 0.01-0.02 (“10-20 mAU”). A BabyBio column should be washed with at least 10 CV buffer.

Optimization of elution conditions when target molecule is bound

Elution can be carried out using a high salt concentration or by altering the pH to change the charge of the adsorbed protein. A stronger binding may require higher salt concentration for elution.

Step elution

The optimal salt concentration is dependent on purity and recovery requirements as well as properties of the target protein and the sample. Using a gradient elution gives increased purity than step elution, but step elution may be necessary to obtain the highest possible concentration of the target protein. In order to optimize the salt concentration for step elution an initial gradient test run can be carried out to identify a suitable step elution conditions for purification of the target protein, see Figure 3.

Note: Remember to take the system dead volume into account when comparing the print out of the gradient and the trace.

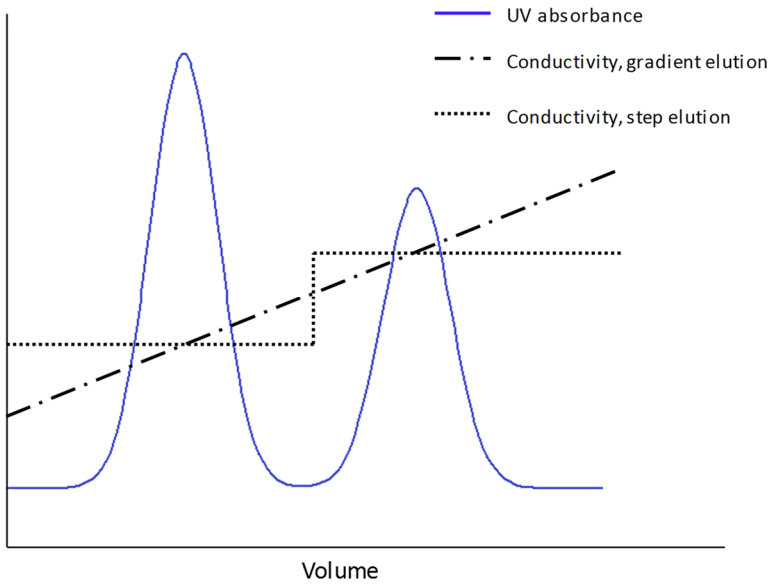

Figure 3. Optimization of step gradient elution with salt. A test run with linear gradient elution gives information about suitable salt concentrations to be used during the step elution.

Extra purification step

Optimization of the overall purification process by tuning the binding, washing and/or elution steps is an option. However, an additional purification step based on another chromatography technique is recommended (see *Additional purification*).

Additional purification steps

Optimization of the purification process by tuning the binding, washing and/or elution conditions of the IEX purification step may not be enough to obtain the required purity. Combining two or more purification step based on additional chromatography techniques is then recommended. For example, cation exchange chromatography and anion exchange chromatography can be combined in a purification process. Other techniques, such as size exclusion chromatography (gel filtration) and hydrophobic interaction chromatography (HIC) are commonly used alternatives. Each purification step should be thoroughly optimized, and preferably in the context of the other steps applied on the overall process.

Desalting and buffer exchange

Buffer exchange or desalting of a sample can be used before analysis and/or after purification by ion exchange chromatography. This can be carried out quickly and easily in lab-scale using BabyBio Dsalt 1 or 5 ml columns (see *Related products*). BabyBio Dsalt columns are also a useful alternative to dialysis for larger sample volumes or when samples need to be processed rapidly to avoid degradation. For large processes diafiltration is recommended.

To find out more about Bio-Works chromatography resins for additional purification, please visit www.bio-works.com

Maintenance of the column

Unpacking and inspection

Unpack the shipment as soon as it arrives and inspect it for damage. Promptly report any damage or discrepancies to your local supplier.

Cleaning and sanitization

During purification impurities such as cell debris, lipids, nucleic acids and protein precipitates from the samples may gradually build-up of contaminants in the resin, or 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. CIP of the column can be carried out as followed:

1. Wash the column with 5 CV deionized water.
2. Apply 3 - 10 CV of 0.5 - 1 M NaOH for 15 - 30 minutes.
   Note: The contact time is the important factor, treatment with NaOH overnight can be necessary if severely fouled.
3. Wash the column with 5 - 10 CV deionized water or binding buffer (until the column is neutral after CIP).
4. Equilibrate the column with 10 CV 20% ethanol (for storage).

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 needs to be evaluated for each case.

Storage

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

Additional information

Intended use

BabyBio TREN columns are intended for research and process development only. The columns shall not be used for the preparation of material for clinical or diagnostic purposes.

Safety

Please, read the associated Safety Data Sheets (SDS) for BabyBio columns, and the safety instructions for any equipment to be used. Note that the maximum backpressure of BabyBio TREN is 0.3 MPa (3 bar, 43 psi).

Product description

	BabyBio TREN
Target substance	Proteins, peptides and oligonucleotides. 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)
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[2]	
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).

Related products

Product name	Pack size[1]	Article Number
Prepacked columns		
BabyBio Dsalt 5 ml	5 ml x 5	45 360 107
BabyBio S 1 ml	1 ml x 5	45 200 003
BabyBio Q 1 ml	1 ml x 5	45 100 003
BabyBio DEAE 1 ml	1 ml x 5	45 150 003
Bulk resins		
WorkBeads 40 TREN	25 ml	40 603 001
WorkBeads 40 TREN	150 ml	40 603 003
WorkBeads 40 SEC	25 ml	40 300 001
Accessories		
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

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 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

Bio-Works
Virdings allé 18
754 50 Uppsala
Sweden