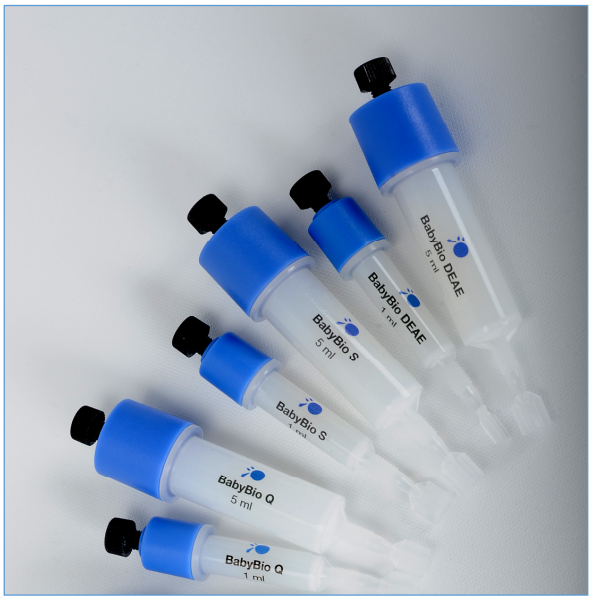
BabyBio S
BabyBio Q
BabyBio DEAE

BabyBio™ S, BabyBio Q and BabyBio DEAE are ready-to-use ion exchange chromatography columns for easy and convenient purification of proteins, peptides and oligonucleotides, by utilizing the difference in these molecules surface charge. BabyBio S works as a strong cation exchanger, BabyBio Q as a strong anion exchanger and BabyBio DEAE as a weak anion exchanger. The columns are prepacked with WorkBeads™ 40S, WorkBeads 40Q and WorkBeads 40 DEAE resins, and are available in two column sizes, 1 ml and 5 ml.



- Prepacked ready-to-use columns for fast and reliable results
- High binding capacity and purity
- Easy-to-use for screening of conditions

Short protocol

This general short protocol is for the use of BabyBio S, BabyBio Q and BabyBio DEAE columns. Detailed instructions and recommendations for optimization are given later in this instruction. Recommended and useful buffers are listed in Table 3. BabyBio S columns are suitable for basic proteins, i.e. proteins with a high isoelectric point (pI), while BabyBio Q and BabyBio DEAE columns are suitable for purification of acidic proteins, i.e. proteins with low pI.

1. Choose a suitable pH and buffer for the binding of the target protein. One pH unit below pI (BabyBio S columns) or above pI (BabyBio Q and BabyBio DEAE columns) is a good starting point.
2. Connect the column to the chromatography system, syringe or pump.
3. Equilibrate the column with 10 column volumes (CV) 20 - 50 mM binding buffer at the chosen pH.
4. Apply a clarified sample to the column at low ionic strength and the chosen pH (similar to the binding buffer) to allow binding of the target protein.
5. Wash the column using 10 - 20 CV binding buffer.
6. Elute the target protein.
   Alternative 1: Desorb the target protein with 5 CV elution buffer.
   Alternative 2: For increased purity, gradient elution is recommended. For example, use a gradient from 0 to 100% with 20 CV binding buffer including 1 M NaCl.
7. Clean the column using 0.5 - 1.0 M NaOH for 15 - 30 minutes (optional).
8. Wash the column with 5 CV deionized water to remove the cleaning solution.
9. Equilibrate with 10 CV 20% ethanol for storage. Close the column using the included cap and plug.

Principle

Ion exchange chromatography (IEX) can be used for purification of biomolecules, such as proteins, peptides and oligonucleotides, by utilizing the difference in their surface charge. The biomolecules interact with the immobilized ion exchange groups with opposite charge on the chromatography resin. WorkBeads resins are available with sulfonate groups (WorkBeads 40S), quaternary amines (WorkBeads 40Q) or tertiary amines (WorkBeads 40 DEAE) as the ion exchange groups. WorkBeads 40S is a strong cation exchanger and will bind positively charged molecules. WorkBeads 40Q and WorkBeads 40 DEAE are strong and weak anion exchangers respectively and will bind negatively charged molecules. The structure of the ligands used in WorkBeads 40S, WorkBeads 40Q and WorkBeads 40 DEAE are shown in Figure 1.

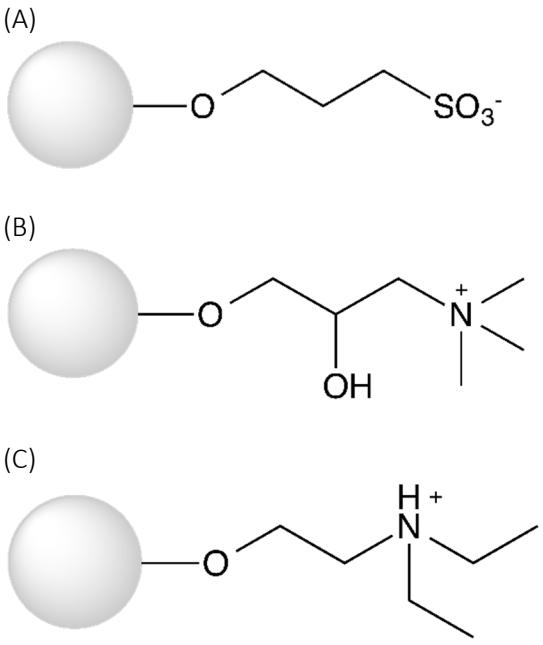


Figure 1. Structure of the ligand used in WorkBeads 40S (A), WorkBeads 40Q (B) and WorkBeads 40 DEAE.

The surface charge of proteins depends on the pH of their environment. When the pH is equal to the isoelectric point (pI) of the protein the net charge is zero. At pH values below the pI the net charge will be positive, and at a pH above the pI the net charge will be negative. It should be noted that the interaction of the protein depends on the presence and distribution of both positive and negative charged groups on the surface (net charge). A protein may therefore interact with an ion exchange resin also at the pI. The likelihood of binding to either the cation or the anion exchange resin will increase when moving away from the pI.

Ion exchange chromatography begins with equilibration of the column to establish the desired pH and charging the resin with suitable counter ions to the charged ligands on the resin (e.g., the negative sulfonate groups can interact with Na^+ ions and the positive trimethyl amine groups can interact with Cl^- ions). It is common to use an equilibration buffer composed of a buffer substance to control the pH, and NaCl to include suitable counter ions. When the sample is applied, proteins with suitable charge will bind to the charged groups of the resin while displacing the counter ions. Desorption of the proteins (elution) is carried out by increasing the concentration of counter ion (salt gradient elution). The counter ions will displace the proteins as the salt concentration increases. Various additives (e.g., enzyme inhibitors, non-ionic detergents, urea and low concentrations of organic solvent) can be used in samples and buffers for IEX as long as they do not strongly interact strongly with the charged groups on the resin or on the protein which could interfere with the protein binding to the resin.

Ion exchange chromatography is one of the most frequently used chromatography techniques because of its versatility and ability to separate proteins even with small differences in charge. It is also one of the more cost-effective chromatography techniques and is therefore excellent for scale-up.

Instructions

Purification 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 (resin bed) 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.

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

Equipment	Accessories for connection
Syringe	Female luer or 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 10 CV of binding buffer. The buffer should be selected to provide a good buffering capacity, with pK_a within 0.5 units from the intended pH for capturing the target protein on the selected ion exchange chromatography column. Examples of buffers to be used are listed in Table 3.

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.

Applied samples should have a pH that gives the target protein a charge that is opposite the charge of the column resin. The pH together with the ionic strength in the sample solution might need adjustment for optimal binding.

6. Wash

After sample application, remove unbound impurities 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.

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 binding buffer including 1 M NaCl.
A stringent wash step with, for instance, 2 M NaCl can be included after elution, to ensure desorption of all interacting proteins.

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. If additional separation will be carried out, restart at step 5.

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

BabyBio S 1 ml, BabyBio Q 1 ml and BabyBio DEAE 1 ml can be used for purification of up to 50 - 100 mg of protein. Scale-up from a BabyBio 1 ml column can be carried out 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 (column stacking). This will increase the total capacity of the run accordingly. By connecting BabyBio columns in series, any column volumes from 1 ml to 25 ml can be obtained. This means a binding capacity of 1000 - 2000 mg of protein can be achieved. Column size selection should be based on estimated amount of target protein in each run.

Another easy-to-use alternative for scaling up is by using the prepacked OptioBio™ 40S 10x100 or OptioBio 40Q 10x100 columns. OptioBio are prepacked glass columns with WorkBeads 40S or WorkBeads 40Q and with a column volume of 7.9 ml, see *Related products*.

BabyBio columns are easily connected together without accessories. Up to five columns may be connected in series (column stacking). The pressure drop across each column bed will be the same as for a single column, but the upstream columns will be subjected to a higher internal pressure from the added pressure drops from downstream columns. It may therefore be necessary to decrease the flow rate accordingly in order to avoid exceed the maximum pressure limit onto the first column. If possible, the maximum pressure of the chromatography system should be set according to Table 2. Remember always to take the system fluidics contribution to the pressure into account.

Table 2. Recommended maximum pressure settings for BabyBio columns connected in series. Notice that the maximum pressure over each column is always 3 bar.

No. 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 defined by the column hardware maximum pressure.

Column size selection should be based on the estimated amount of protein to be purified. A test run with a defined small volume of sample on a BabyBio 1 ml column should be used to estimate the concentration of the target protein 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 one calculated to allow higher sample flow rates, and consequently shorter application time. For example, using a 5 ml column instead of a 1 ml column allows a flow rate five times higher due to the larger cross-section of the column. Have in mind that too high flow rate may reduce binding capacity.

For columns larger than 20 ml, it is recommended to pack a single column using bulk resin as the limitations of column stacking will then impact chromatographic performance. To find out more about Bio-Works bulk chromatography resins, please visit www.bio-works.com

Optimization

The following paragraphs will give indications on some parameters that can be tuned to find the optimal conditions for the purification.

Buffer selection

Choosing a buffer with optimal binding and elution conditions for the target protein will improve the result of the purification. The buffer should be selected to provide an optimal capacity and with a pK_a-value within 0.5 units from the intended pH. Table 3 shows examples of buffers that 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.

Table 3. Example of buffers for model protein purification using BabyBio S, BabyBio Q and BabyBio DEAE. Other buffers can possible be used.

Buffer	Product	Buffer composition
Binding buffer	BabyBio S	50 mM Na-phosphate, pH 7.0
Binding buffer	BabyBio Q	50 mM Tris-HCl, pH 7.4
Binding buffer	BabyBio DEAE	50 mM Tris-HCl, pH 7.4
Elution buffer	BabyBio S	50 mM Na-phosphate, 1 M NaCl, pH 7.0
Elution buffer	BabyBio Q	50 mM Tris-HCl, 1 M NaCl, pH 7.4
Elution buffer	BabyBio DEAE	50 mM Tris-HCl, 1 M NaCl, pH 7.4

Preferably, select buffer substances with opposite charge to the resin. A buffer substance that interacts with the charged groups in the resin may cause local pH disturbances that disturbs 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 bind while the target protein remains bound. However, chromatographic conditions selection should be such that the target protein is stable during purification.

Optimization of binding

The key conditions to be optimized is usually pH and conductivity (by addition of NaCl, other salts, or dilution). Conditions selection should be to maximize purity and/or yield of the target protein, while keeping it in a native/active state.

pH and salt optimization

Strong ion exchangers (S and Q) are used over a broad pH range. The useful pH range is limited by the target protein pH stability and solubility window. Weak ion exchangers have a narrower pH range for usage. The weak DEAE anion exchanger must be used at a pH below its pK_a value of 9.0 - 9.5 to retain its positive charge. The difference between the Q and DEAE ligands may give desired differences in selectivity. Purification is often done by combining an anion exchange column and a cation exchange column with or without changing the pH.

It should be noted that the binding capacity and purity depends on the combination of pH and counter ion concentration (i.e., salt concentration or ionic strength). Therefore, it is recommended to investigate the combination effects of pH and salt concentration during optimization. A low salt concentration is considered to give strong binding with high capacity, but it should be noted that it is often observed that an intermediate concentration of salt gives a better binding capacity. For example, a concentration of 50 mM NaCl in the buffer may give a better capacity than 20 mM NaCl. This may be attributed to improved mass transport of target substance into the pores of the resin, obtained by reduced pore exclusion. Pore exclusion can be explained as a hindrance of diffusion caused by strong interaction of substance on the walls of the outer pores, causing, in effect, a “traffic jam” and thus reduced diffusion rate into the pores. A slightly elevated salt concentration reduces, but does not eliminate the interactions with the resin by creating a dynamic adsorption-desorption equilibrium that allows further diffusion into the resin, thus increasing the binding capacity.

Tuning the flow rate

Flow rate is another factor that can be optimized to improve the binding capacity during sample application or the resolution during 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) has a high diffusion rate and is not hindered by the walls in the pores and will have fast mass transport into the resin and thus be adsorbed at a high flow rate. A large target substance (e.g., a large protein) has a lower diffusion rate and is held back by the walls of the pores slowing its mass transport. A high binding capacity of this substance may require a lower flow rate. If only a part of the binding capacity of the resin is used the sample application can be done 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 Q, BabyBio S or BabyBio DEAE columns, 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 and elution

Prolonged wash

A continuously decreasing UV signal during washing is an indication of unbound material being washed out (it may be target substance if it is weakly bound). 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.

Optimizing elution conditions

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.

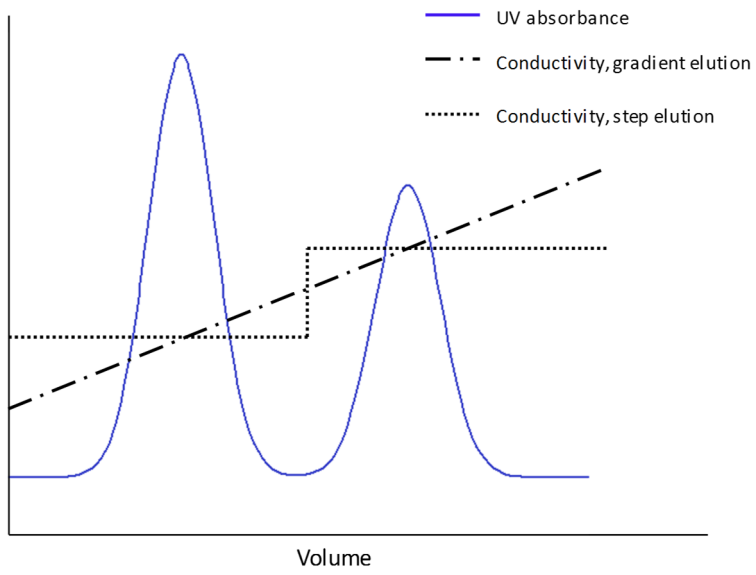


Figure 3. Optimization of step gradient elution with salt. A test run with linear gradient elution gives information about suitable salt concentrations to be used during the step elution.

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*).

Desalting and buffer exchange

Buffer exchange or desalting of a sample are useful before analysis and/or after chromatography steps such as ion exchange chromatography. This can be carried out quickly and easily using BabyBio Dsalt 1 ml 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.

Additional purification

Ion exchange chromatography is a powerful single protein purification step or combined with other chromatography techniques. The overall process needs to be optimized for each purification step.

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

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 pre-treatment 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 resin 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 done 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 S, BabyBio Q and BabyBio DEAE 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 back pressure of BabyBio S, BabyBio Q and BabyBio DEAE columns is 0.3 MPa (3 bar, 43 psi).

Product information

	BabyBio S	**BabyBio Q**	**BabyBio DEAE**
Target substance	Proteins, peptides	Protein, peptides, oligonucleotides	Protein, peptides, oligonucleotides
Resin	WorkBeads 40S	WorkBeads 40Q	WorkBeads 40 DEAE
Matrix	Rigid, highly cross-linked agarose	Rigid, highly cross-linked agarose	Rigid, highly cross-linked agarose
Average particle size[1] (D_{V50})	45 µm	45 µm	45 µm
Ligand	Sulfonate ($-SO_3^-$)	Quarternary amine ($-N^+(CH_3)_3$)	Diethylaminoethyl ($-CH_2CH_2N^+H(CH_2CH_3)_2$)
Ion capacity	0.18 - 0.25 mmol Na^+/ml resin	0.18 - 0.25 mmol Cl^-/ml resin	0.11 - 0.16 mmol Cl^-/ml resin
Dynamic binding capacity	130 mg BSA/ml resin[2]	50 mg BSA/ml resin[3]	40 mg BSA/ml resin[3]
Column volume	1 ml 5 ml	1 ml 5 ml	1 ml 5 ml
Column dimension	7 x 28 mm (1 ml) 13 x 38 mm (5 ml)	7 x 28 mm (1 ml) 13 x 38 mm (5 ml)	7 x 28 mm (1 ml) 13 x 38 mm (5 ml)
Recommended flow rate			
BabyBio 1 ml	1 ml/min (150 cm/h)	1 ml/min (150 cm/h)	1 ml/min (150 cm/h)
BabyBio 5 ml	5 ml/min (225 cm/h)	5 ml/min (225 cm/h)	5 ml/min (225 cm/h)
Maximum flow rate			
BabyBio 1 ml	5 ml/min (780 cm/h)	5 ml/min (780 cm/h)	5 ml/min (780 cm/h)
BabyBio 5 ml	20 ml/min (900 cm/h)	20 ml/min (900 cm/h)	20 ml/min (900 cm/h)
Maximum back pressure	0.3 MPa, 3 bar, 43 psi	0.3 MPa, 3 bar, 43 psi	0.3 MPa, 3 bar, 43 psi
Chemical stability	Compatible with all standard aqueous buffers used for protein purification and 70% ethanol. Should not be stored at low pH for prolonged time		
pH stability	2 - 13	2 - 13	3 - 9 recommended pH 3 - 13
Storage	2 to 25 °C in 20% ethanol	2 to 25 °C in 20% ethanol	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 20 mM Na-citrate, 60 mM NaCl, pH 4.0.
3. 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.
4. Maximum flow rate for 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 for 20% ethanol).

Related Products

Product name	Pack size[1]	Article number
Prepacked columns		
BabyBio Dsalt 1 ml	1 ml x 5	45 360 103
BabyBio Dsalt 5 ml	5 ml x 3	45 360 107
OptioBio 40S 10x100	7.9 ml x 1	55 420 011
OptioBio 40Q 10x100	7.9 ml x 1	55 410 011
Bulk resins		
WorkBeads 40S	25 ml	40 200 001
WorkBeads 40Q	25 ml	40 100 001
WorkBeads 40 DEAE	25 ml	40 150 001
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 S 1 ml	1 ml x 1	45 200 101
	1 ml x 2	45 200 102
	1 ml x 5	45 200 103
	1 ml x 10	45 200 104
BabyBio S 5 ml	5 ml x 1	45 200 105
	5 ml x 2	45 200 106
	5 ml x 5	45 200 107
	5 ml x 10	45 200 108
BabyBio Q 1 ml	1 ml x 1	45 100 101
	1 ml x 2	45 100 102
	1 ml x 5	45 100 103
	1 ml x 10	45 100 104
BabyBio Q 5 ml	5 ml x 1	45 100 105
	5 ml x 2	45 100 106
	5 ml x 5	45 100 107
	5 ml x 10	45 100 108
BabyBio DEAE 1 ml	1 ml x 1	45 150 101
	1 ml x 2	45 150 102
	1 ml x 5	45 150 103
	1 ml x 10	45 150 104
BabyBio DEAE 5 ml	5 ml x 1	45 150 105
	5 ml x 2	45 150 106
	5 ml x 5	45 150 107
	5 ml x 10	45 150 108

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

Bio-Works

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