# BabyBio Dsalt

BabyBio™ Dsalt are designed to enable quick and easy separations of high- and low molecular weight substances. These ready-to-use columns enables efficient desalting or buffer exchange of proteins, large peptides and nucleic acids. BabyBio Dsalt columns are available in two column sizes, 1 ml and 5 ml.

- Ready-to-use prepacked columns
- Designed for rapid and efficient desalting or buffer exchange
- Convenient scale-up by connecting columns in series


## Short protocol

This general short protocol is for usage of BabyBio Dsalt columns. Detailed instructions and recommendations for optimization are given later in this instruction. For sample volume recommendations and detailed instructions on how to collect samples see instructions below.

1. Connect the column to the chromatography system, syringe or pump.
2. Equilibrate the column using 5 column volumes (CV) buffer with the desired final-composition for the target protein.
3. Apply a sample corresponding to 0.02 - 0.3 CV.
4. Elute the target protein by applying 5 CV of the same buffer as in step 2 and collect fractions.

High molecular weight components start to elute from 0.3 ml for BabyBio Dsalt 1 ml and from 1.25 ml for BabyBio Dsalt 5 ml.
Low molecular weight components start to elute from 0.7 ml for BabyBio Dsalt 1 ml and from 3.2 ml for BabyBio Dsalt 5 ml.

## Principle

Proteins and many other biomolecules differ greatly in size from salts and other small molecules. Size exclusion chromatography is an efficient technique for the separation of components according to size. BabyBio Dsalt columns have an exclusion limit of approximate molecular weights ($M_r$) 5000 for globular proteins and large peptides, and 10 base pairs (bp) for nucleic acids. Substances that are larger than $M_r$ 5000 do not enter the porous beads, and are therefore eluted in the void of the column (early elution). Substances smaller than $M_r$ 5000 (e.g., salts, buffer substances and other low molecular weight additives or impurities) enter the bead pores. Consequently, these substances are delayed (late elution). This mechanism allows group separation of large substances from the small substances. A protein can therefore be transferred from salt or buffer substances in the sample, into a solution containing another buffer or salt composition. Buffer exchange and desalting are common techniques in laboratories working with purification and analysis.
The prepacked BabyBio Dsalt columns are excellent tools for sample preparation before or after a subsequent purification step, or for formulation of the sample before use in research.

Buffer exchange or desalting can be used to prepare a sample for mass spectrometry analysis or lyophilisation and before/after e.g., ion exchange chromatography. BabyBio Dsalt columns are a useful alternative to dialysis when larger sample volumes are processed or when samples need to be processed rapidly to avoid degradation.

BabyBio Dsalt columns are designed for optimal desalting by group separation of a protein sample. The prepacked column can also be used for rapid buffer exchange after certain applications. To minimize the dilution and still retain good separation, sample volumes up to approximately 30% of the total bed volume are recommended. Desalting can be performed at high flow rates as the flow rate has minor impact on the resolution. The chromatographic desalting technique is perfectly scalable to allow sample modifications in larger scale purification processes.

## Instructions

Desalting or buffer exchange 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

If large particles are present 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. Alternatively, clarify the sample by centrifugation at 10 000 - 20 000 × *g* for 15 - 30 minutes. If the sample contains only small amounts of particles it may be 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 buffer.

### 2. Connect the column

Cut off or twist off the end at the outlet of the column, see Figure 1.
**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).

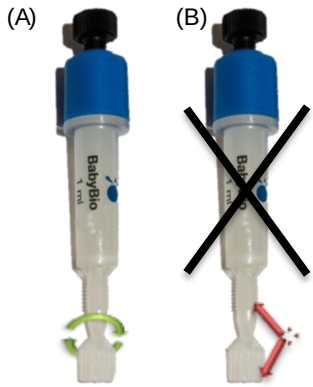


Figure 1. Removal of the cut-off end at the column outlet should be done by cutting or by twisting (A), not bending (B).

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 10 CV deionized water or buffer. Avoid flow rates higher than 2 ml/min (BabyBio 1 ml) or 6 ml/min (BabyBio 5 ml) before the storage solution has been removed to avoid overpressure due to high viscosity of the 20% ethanol solution.

## 4. Equilibrate the columns

Equilibrate the column with 5 CV buffer. The buffer should be selected according to the target proteins final condition and stability requirements, and according to the requirements in subsequent use of the protein preparation.

**Note:** To avoid bacterial growth and poor column performance, use only freshly prepared and filtered buffers.

## 5. Apply the sample

Apply 20 - 300 µl sample on a BabyBio Dsalt 1 ml or 0.1 - 1.5 ml sample on a BabyBio Dsalt 5 ml column. Apply the sample at 0.5 - 1 ml/min (BabyBio 1 ml) or 2 - 5 ml/min (BabyBio 5 ml).

The recommended sample volume range for BabyBio Dsalt 1 ml is 20 µl - 300 µl and for BabyBio Dsalt 5 ml is 0.1 ml - 1.5 ml. Depending on the sample volume and the collected fraction volume, the dilution, protein yield and remaining low molecular weight substances (e.g., salt) content in the collected fraction will vary. Typical example of sample volumes, and the effect on the mentioned factors are shown in Table 2 and Table 3 for BabyBio Dsalt 1 ml and BabyBio Dsalt 5 ml, respectively. A schematically drawing of how to use BabyBio Dsalt columns are shown in Figure 2.

The desalting effect when changing the sample volume is exemplified in Figure 3. It is recommended to apply less than 0.3 CV of sample to get a baseline separation between protein and salt. This enables collection of a larger volume to capture the entire protein fraction without contamination of the salt. This approach will usually result in a larger dilution. This can be avoided by connecting two or more columns in series to improve the separation between the protein and the salt. This is also a good way to scale-up the separation.

## 6. Elute and collect fractions

Elute the protein with 5 CV buffer and collect fractions. High molecular weight components start to elute at the void volume of the columns which is from 0.3 ml and 1.25 ml after sample application, for BabyBio Dsalt 1 ml and BabyBio Dsalt 5 ml, respectively. Low molecular weight components start to elute from 0.7 ml and 3.2 ml for BabyBio Dsalt 1 ml and BabyBio Dsalt 5 ml, respectively. When applying a smaller sample than the void volume of the column, addition of buffer up to the total void volume of the column, is required. However, the fraction collection must always starts after applying a total volume of the void volume of the column, even if sample volume is larger than the void volume.

## 7. Re-equilibrate

Re-equilibrate the column with 5 CV buffer.

## 8. Column storage

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

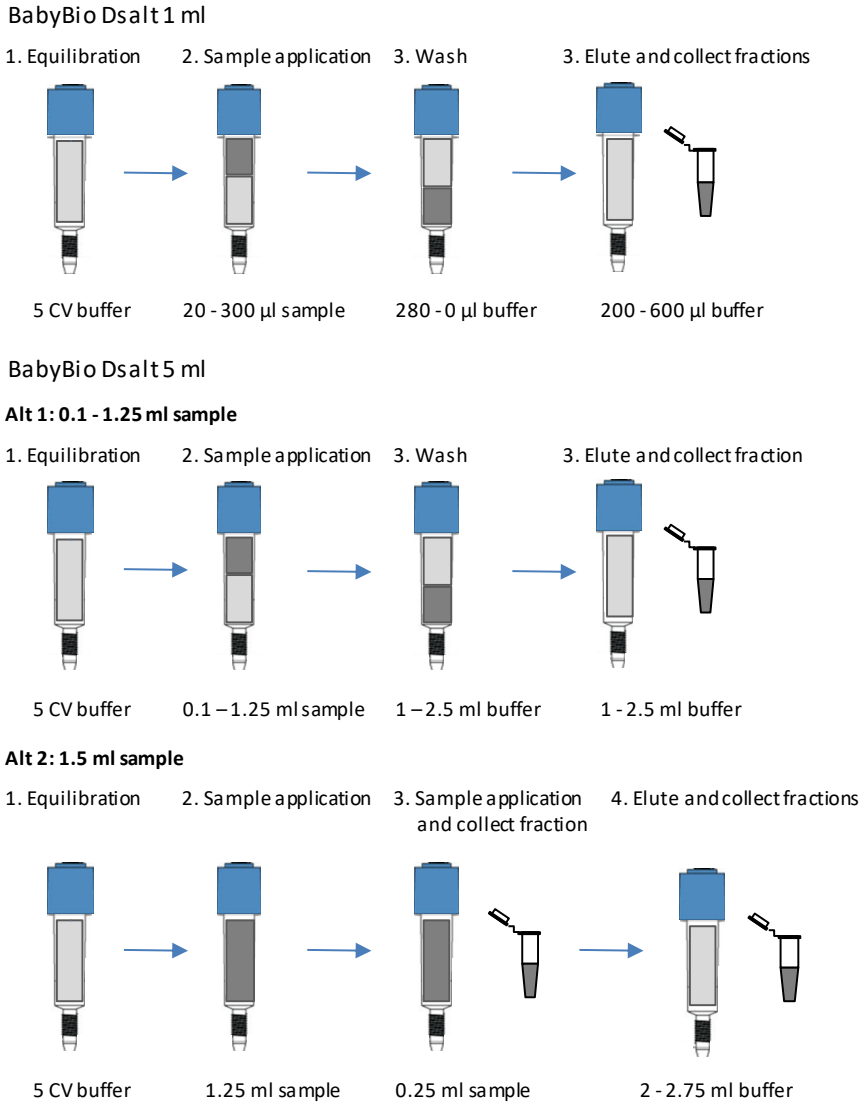


Figure 2. Schematically drawing of how to use BabyBio Dsalt 1 ml and BabyBio Dsalt 5 ml columns. The procedure depends on the sample volume during sample application.

Table 2. Typical example of sample volume, buffer volume for wash, fraction collection volume, dilution factor, remaining protein yield and salt content in fraction for **BabyBio Dsalt 1 ml**.

| Sample volume (µl) | Wash (µl) | Add buffer and collect (µl) | Dilution factor | Protein yield (%) | Salt content in fraction (%) |
|---|---|---|---|---|---|
| 20 | 280 | 250 | 12.5 | 90 - 95 | 0 |
| 20 | 280 | 200 | 10 | 85 - 90 | 0 |
| 50 | 250 | 300 | 6 | 85 - 90 | 0 |
| 100 | 200 | 300 | 3 | 90 - 95 | 0 |
| 200 | 100 | 300 | 1.5 | 85 - 90 | 0 |
| 200 | 100 | 400 | 2 | 90 - 95 | 0.1 |
| 300 | 0 | 500 | 1.7 | 85 - 90 | 0.02 |
| 300 | 0 | 600 | 2 | 90 - 95 | 1 |

Table 3. Typical sample volume, buffer volume for wash, fraction collection volume, dilution factor, remaining protein yield and salt content in fraction for **BabyBio Dsalt 5 ml**.

| Sample volume (ml) | Wash (ml) | Add buffer and collect (ml) | Dilution factor | Protein yield (%) | Salt content in fraction (%) |
|---|---|---|---|---|---|
| 0.1 | 1.15 | 1 | 10 | >95 | 0 |
| 0.2 | 1.05 | 1 | 5 | 90 - 95 | 0 |
| 0.2 | 1.05 | 1.25 | 6.3 | >95 | 0 |
| 0.5 | 0.75 | 1.5 | 3 | >95 | 0 |
| 0.75 | 0.5 | 1.5 | 2 | 90 - 95 | 0 |
| 0.75 | 0.5 | 1.75 | 2.3 | >95 | 0 |
| 1.0 | 0.25 | 2 | 2 | >95 | 0.1 |
| 1.0 | 0.25 | 2.25 | 2.3 | >95 | 0.3 |
| 1.25 | 0 | 2 | 1.6 | 90 - 95 | 0.07 |
| 1.25 | 0 | 2.5 | 2 | >95 | 0.2 |
| 1.5 | 0.25 [1] | 2 [2] | 1.5 | 90 - 95 | 0.2 |
| 1.5 | 0.25 [1] | 2.75 [3] | 2 | >95 | 3 |

1. 1.25 ml is the void volume of the column. After 1.25 ml the collection of sample should start. When applying sample volumes larger than 1.25 ml, the collection should start when 1.25 ml sample has been applied and end after applying the remaining sample and additional buffer according to the table.
2. The total fraction size will be 2.25 ml, for this example.
3. The total fraction size will be 3 ml, for this example.

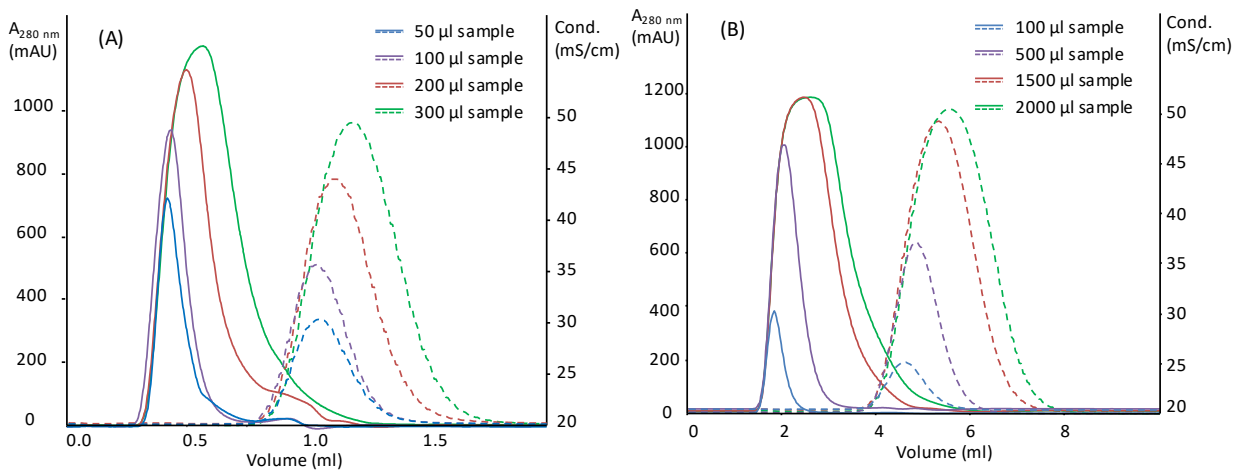


Figure 3. Effects of varying the sample volume. (A) BabyBio Dsalt 1 ml, sample volume ranges from 50 µl - 300 µl sample. (B) BabyBio Dsalt 5 ml, sample volume ranges from 100 µl - 2000 µl. Sample containing 2 mg BSA/ml in 25 mM sodium phosphate, 0.5 M NaCl, pH 7.0. Running buffer 25 mM sodium phosphate, 150 mM NaCl, pH 7.0. Flow rate 1 ml/min and 5 ml/min, for BabyBio Dsalt 1 ml and BabyBio Dsalt 5 ml, respectively. The solid lines correspond to the absorbance at 280 nm. The dashed lines correspond to the conductivity.

## Scale-up

BabyBio Dsalt 1 ml and BabyBio Dsalt 5 ml columns can be used for up to 300 µl and 1.5 ml samples, respectively. Scale-up can thus be carried out by changing from a 1 ml column to a 5 ml column, or by combining up to 5 columns in series. This will increase the capacity accordingly. By connecting columns in series any sample volume from 20 µl to 7.5 ml can be desalted or buffer exchanged.

Connection of the columns are easy 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 over the first column. If possible, the maximum pressure of the chromatography system should be set according to Table 4. Remember to take the pressure contribution of the system tubing downstream the columns into account.
For columns larger than 20 ml, it is recommended to pack a column using WorkBeads Dsalt resin, see *"Related products"*.

Table 4. 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.

## Optimization

### Optimization of desalting or buffer exchange

Buffer exchange is often needed between purification steps in order to stabilize the sample, or preparing it for the next separation step. For example, a high ionic strength of the sample may prevent binding of the target protein in ion exchange chromatography, or a specific pH is needed for binding during affinity chromatography. BabyBio Dsalt columns can also be used to remove remaining low molecular weight reagents used for labelling or other treatments of a protein. Desalting or buffer exchange can be carried out under almost any conditions suitable for the protein. The aim is usually to select a buffer that maintains the protein native structure and activity, and is a suitable preparation for the next step. Desalting can be carried out to reduce ionic strength or to change pH of the protein sample.
Although most aqueous buffers have a viscosity close to that of water, some samples or elution buffers may have additives resulting in elevated viscosity. When using high viscosity solutions the flow rate must be reduced in proportion to the increase in viscosity compared to dilute aqueous solutions. Similarly, the viscosity of an aqueous solution will increase when the temperature is decreased (e.g., when working at 4°C) then reduce the flow rate to half of the flow used at room temperature.

## Additional purification

To find out more about Bio-Works chromatography resins for additional purification 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. A regular cleaning of the column is recommended, for example using 2 CV of 0.2 M NaOH.

### 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.

## Additional information

### Intended use

BabyBio columns are intended for research use only and shall not be used in any clinical or in vitro procedures for diagnostic purposes.

### Safety

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

### Product information

| | **BabyBio Dsalt** |
|---|---|
| Target substance | Proteins, large peptides ($M_r$ > 5000), nucleic acids and other biomolecules of similar size |
| Matrix | Highly cross-linked dextran |
| Column volume | 1 ml<br>5 ml |
| Column dimension | 7 x 28 mm (1 ml)<br>13 x 38 mm (5 ml) |
| Typical sample volume | |
| BabyBio Dsalt 1 ml | 20 µl - 300 µl |
| BabyBio Dsalt 5 ml | 100 µl - 1500 µl |
| Recommended flow rate[1] | |
| BabyBio Dsalt 1 ml | 0.25 - 1 ml/min (37 - 150 cm/h) |
| BabyBio Dsalt 5 ml | 1.25 - 5 ml/min (56 - 225 cm/h) |
| Max flow rate[2] | |
| BabyBio Dsalt 1 ml | 5 ml/min (780 cm/h) |
| BabyBio Dsalt 5 ml | 12 ml/min (540 cm/h) |
| Maximum back pressure | 0.3 MPa, 3 bar, 43 psi |
| Chemical stability | Compatible with all standard aqueous buffer used for protein purification. |
| Storage | 2 to 25 °C in 20% ethanol |

1. Optimal flow is depending on the sample. During column wash, 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.
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 for 20% ethanol).

## Related products

| Product name | Pack size[1] | Article number |
|---|---|---|
| **Prepacked columns** | | |
| 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 |
| BabyBio TREN 1 ml | 1 ml x 5 | 45 655 213 |
| BabyBio Ni-NTA 1 ml | 1 ml x 5 | 45 655 103 |
| BabyBio affimAb | 1 ml x 5 | 45 800 103 |
| **Bulk resin** | | |
| WorkBeads Dsalt | 300 ml | 40 360 003 |
| WorkBeads 40S | 25 ml | 40 200 001 |
| WorkBeads 40Q | 25 ml | 40 100 001 |
| WorkBeads 40 DEAE | 25 ml | 40 150 001 |
| WorkBeads 40 TREN | 25 ml | 40 603 001 |
| WorkBeads 40 Ni-NTA | 25 ml | 40 651 001 |
| WorkBeads affimAb | 25 ml | 40 800 001 |

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 |
|---|---|---|
| BabyBio Dsalt 1 ml | 1 ml x 1 | 45 360 101 |
| | 1 ml x 2 | 45 360 102 |
| | 1 ml x 5 | 45 360 103 |
| | 1 ml x 10 | 45 360 104 |
| | 1 ml x 100 | 45 360 110 |
| BabyBio Dsalt 5 ml | 5 ml x 1 | 45 360 105 |
| | 5 ml x 2 | 45 360 106 |
| | 5 ml x 5 | 45 360 107 |
| | 5 ml x 10 | 45 360 108 |
| | 5 ml x 100 | 45 360 109 |

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