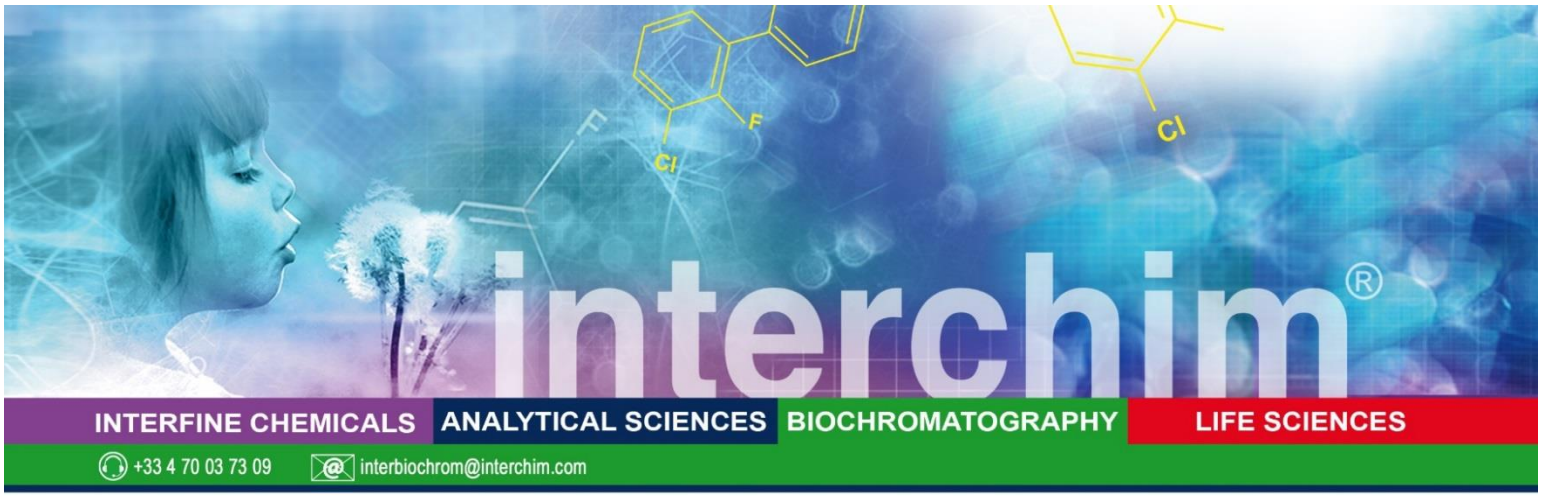

Bio-Works

BabyBio Dsalt™
Mini columns for desalting

BabyBio Dsalt are ready to use mini column available in 1 ml and 5 ml that allows quick, easy and convenient group separation of high and low molecular weight substances.

- **Designed for swifter and efficient desalting and buffer exchange applications**
- **Stronger, reproducible and easy to use column**
- **Easy scaling-up, columns can be coupled in series**

Media description
Cross-linked beads based on dextran which gives high flow properties and low protein adsorbtion.

Desalting
Proteins and other biomolecules differ greatly in size from salts and other small molecules. Size exclusion is efficient technique to separates the components in high molecular weight substances and low molecular weight substances. The M_r cut off is 5 000. Buffer exchange and desalting are common uses in laboratories working with purification and analysis, BabyBio Dsalt is excellent for this.
Buffer exchange or desalting of a sample can be used to prepair for mass spectroscopy analysis, lyophilization and after certain procedures such as ion exchange chromatography.
BabyBio Dsalt is a useful alternative to dialysis when larger sample volumes are used or when samples need to be processed rapidly to avoid degradation.

Column description
The BabyBio column body is made from biocompatible polypropylene, which does not significantly interact with biomolecules. The top and bottom filters are made from polyethylene.
These ready to use columns are delivered with plugs in the inlet and a snap-off end at the outlet. A cap for the outlet is included for closing the column during storage. The columns can be connected a syringe, pump or chromatography system using fingertight fittings (coned 10- 32) for 1/16” o.d. tubing.

Using the column

1. Installation of the column.
2. Removal of storage solution.
3. Equilibrate the column using 5 column volumes (CV) of buffer with desired end-composition for the protein.
4. Apply 20-300 µl sample.
5. Elute the sample by applying 5 CV of buffer and collect fractions.

Applications

Columns are useful in desalting or buffer exchange of protein samples or high or low molecular weight substances. In desalting applications is the sample-to-gel volume ratio affects resolution. To minimize 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 flow rate has a minor impact on resolution.

Scale-up

Scale-up can conveniently be performed from a 1 ml column to a 5 ml column. Larger sample volumes can be applied by coupling columns in series. Note that back pressure will then increase.

Equipment

BabyBio Dsalt can generally be used together with most equipment available for chromatography.

Column characteristics - BabyBio Dsalt

Target substance	Proteins and other biomolecules of similar size
Medium	Highly cross-linked dextran
Column volumes	1 ml, 5 ml
Column dimensions	7 × 28 mm (1 ml), 13 × 38 mm (5 ml)
Recommended flow rate	1 ml/min (BabyBio Dsalt 1 ml) 5 ml/min (BabyBio Dsalt 5 ml)
Max flow rate[1]	5 ml/min (BabyBio Dsalt 1 ml) 20 ml/min (BabyBio Dsalt 5 ml)
Maximum back pressure	0.3 MPa, 3 bar, 43 psi
Chemical stability	Compatible with all standard aqueous buffers used for protein purification.
Recommended working range	2-12
pH Stability	
Storage	+2°C to +25°C in 20% ethanol

1 Aqueous buffers at 20°C. Decrease the max flow if the liquid has a higher viscosity. Higher viscosities can be caused by low temperature (use max flow/2 at 4°C), or by additives (e.g, use max flow/2 for 20% ethanol).

Instruction of use

Short protocol

1. Equilibrate the column using 5 column volumes (CV) of buffer with desired end composition for the protein.
2. Apply 20-300 µl sample.
3. Elute the sample by applying 5 CV of buffer and collect fractions.

   The composition of the sample and selection of buffer may affect the results. See later in this instruction for more details.

Instructions

Desalting or buffer exchange can usually be performed at room temperature, but can also be done at 4°C at reduced flow rate to keep a sample stable. No sample preparation are required, but the sample should be clear from particles.

1. **Connection of the column**
   Connect the column to a chromatography system using finger tight connectors (coned 10-32) for 1/16" o.d. tubing. Fill the system with water and make a drop-to-drop connection with the column to avoid air getting into the column. Perform all steps at 1 ml/min (BabyBio Dsalt 1 ml) or 5 ml/min (BabyBio Dsalt 5 ml). Higher flow rates can be used if desired (see table below).
2. **Removal of storage solution**
   When the column is delivered it contains a storage solution of 20% ethanol. This solution should be washed out before use. Wash the column with 3 column volumes (CV) of water. Avoid higher flow rate before the storage solution has been removed to avoid over pressure due to the high viscosity of the 20% ethanol solution.
3. **Equilibration**
   Equilibrate the column with 10 CV selected buffer. The buffer should be selected according to protein stability requirements, and according to the requirements in subsequent use of the protein preparation.
4. **Sample application**
   Usually the protein sample to be desalted or buffer exchanged are relatively pure, and has no particles. If particles are present it is recommended to clarify the sample by passing the sample via a 0.45 µm syringe filter when it is applied on the column, or alternatively clarify the sample by centrigugation at 10 000-20 000 × g for 15-30 minutes.
   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.
5. **Elution**
   Elute the protein with 5 CV of buffer, collect fractions. Removal of the elution buffer Wash with 5 CV deionised water to remove the salts of the elution buffer.
6. **Equilibrate** with 10 CV 20% ethanol for storage. Close the column using the included caps.

Optimization

Selection of column

BabyBio Dsalt 1 ml and BabyBio Dsalt 5 ml can be used to desalt up to 300 µl and 1.5 ml samples, respectively. Scale-up can thus be done by changing from BabyBio 1 ml to a BabyBio Dsalt 5 ml, or by combining up to 5 columns in series. This will increase the capacity accordingly. The BabyBio columns can easily be connected together without accessories.
By connecting columns in series any sample volume from 20 µl to 7.5 ml can be treated. With several columns connected in series the upper columns will be expose to higher internal pressure. It may be necessary to decrease the flow rate to avoid passing the maximum hardware pressure over the top column. The pressure across each column bed will be the same for all columns.

Optimization of desalting or buffer exchange

Desalting or buffer exchange can be done under most any conditions suitable for the protein. The selection of buffer should be aimed at high protein stability, and suitability for next step of the work. Desalting can be done to reduce ionic strength or to change the pH of the protein sample. Buffer exchange are often needed between purification steps in order to stabilize the sample, or preparing it for the next separation step. For example, a high ionic strenght of the sample may hinder adsorption in ion-exchange chromatography, or a specific pH is needed for binding in an affinity chromatographic separation. BabyBio Dsalt columns can also be used to remove remaining low-molecular weight reagents used for labelling or other treatments of a protein.
When using high-viscosity solution the flow rate must be reduced in relation to the increase in viscosity compared to dilute aqueous solutions. Similarly, the viscosity of an aqueous solution will increase when the temprature is decreased, e.g., when working at 4°C, reduce the flow rate to ca half of the flow used at room temperature.

Ordering information

Product Name	Pack Size	Article Number
BabyBio Dsalt 1 ml	1 × 1 ml	45 360 101
	2 × 1 ml	45 360 102
	5 × 1 ml	45 360 103
	10 × 1 ml	45 360 104
	100 × 1 ml	45 360 110
BabyBio Dsalt 5 ml	1 × 5 ml	45 360 105
	2 × 5 ml	45 360 106
	5 × 5 ml	45 360 107
	10 × 5 ml	45 360 108
	100 × 5 ml	45 360 109

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