

BabyBio Dsalt

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

- Ready-to-use columns
- Designed for rapid and efficient desalting or buffer exchange
- Convenient scale-up by connecting columns in series
- Filters optimized to allow crude feed application with minimal clogging



Resin Description

BabyBio Dsalt columns are prepacked with a cross-linked dextran based resin.

The resin is optimized for desalting, or buffer exchange. This is achieved by group separation of high molecular weight substances, such as proteins, and low molecular weight substances, such as salts. The exclusion limit of the resin is M_r 5000 for proteins and 10 bp for nucleic acids.

Column description

The column is made from biocompatible polypropylene which does not significantly interact with biomolecules. The top and bottom filters are made from low protein binding polyethylene. The filters in the top and the bottom of the column have a pore size optimized to allow loading of crude feed with minimal clogging.

The ready-to-use BabyBio columns are delivered with a plug in the inlet, a cut-off outlet and a cap for storage. The columns can be connected to a syringe, pump or chromatography system using finger tight fittings (coned 10 - 32) for 1/16" o.d. tubing (standard HPLC PEEK tubing), with a female and a male connection at the top and bottom respectively.

BabyBio columns can be connected in series allowing a convenient way to perform scale-up experiments.

The main characteristics of BabyBio Dsalt columns are shown in Table 1. For additional information, see instruction IN 45 360 010.

Table 1. Main characteristics of BabyBio Dsalt 1 ml and BabyBio Dsalt 5 ml columns.

	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 5 ml
Column dimension	7 x 28 mm (1 ml) 13x38 mm (5 ml)
Typical sample volume	
BabyBio Dsalt 1 ml	20 - 300 μ l
BabyBio Dsalt 5 ml	100 - 1500 μ l
Recommended flow rate	
BabyBio Dsalt 1 ml	1 ml/min (150 cm/h)
BabyBio Dsalt 5 ml	5 ml/min (225 cm/h)
Max flow rate ¹	
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
pH stability	2 - 12
Storage	2 to 25 °C in 20% ethanol

1. 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).

Applications

BabyBio Dsalt columns are designed for desalting or rapid buffer exchange which is often necessary before protein analysis, before or after a chromatographic purification. Desalting is often carried out before an ion exchange chromatography step to condition the sample or after an ion exchange chromatography step to remove the salt used for elution.

High molecular weight components start to elute at 0.30 ml and 1.25 ml for BabyBio Dsalt 1 ml and BabyBio Dsalt 5 ml, respectively. Low molecular weight components start to elute at 0.7 ml and 3.2 ml for BabyBio Dsalt 1 ml and BabyBio Dsalt 5 ml, respectively.

Principle

Proteins and many other biomolecules differ greatly in size from salts and other small molecules. Size exclusion chromatography is an efficient technique to separate components according to size. Using BabyBio Dsalt columns, substances that are larger than M_r 5000 do not enter the porous beads, and are therefore eluted in the void volume (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. These substances are delayed (late elution). This

mechanism allows group separation of the 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 technics in laboratories working with purification and analysis.

Buffer exchange or desalting are also useful for preparing a sample for mass spectrometry analysis or lyophilisation and after certain procedures such as ion exchange chromatography. BabyBio Dsalt columns are a fast and easy alternative to dialysis when larger sample volumes are used 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, as well as for rapid buffer exchange before/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 large scale purification processes.

Sample volume range

In Figure 1, the effect of increasing sample volumes is exemplified on a BabyBio Dsalt 5 ml column. For efficient desalting in this case, sample volume should not exceed 1.5 ml.

Column: BabyBio Dsalt 5 ml
 Buffer: 25 mM Na-phosphate, 150 mM NaCl, pH 7.0
 Sample: 2 mg/ml BSA in 20 mM Na-phosphate, 0.5 M NaCl, pH 7.0
 Flow rate: 5 ml/min

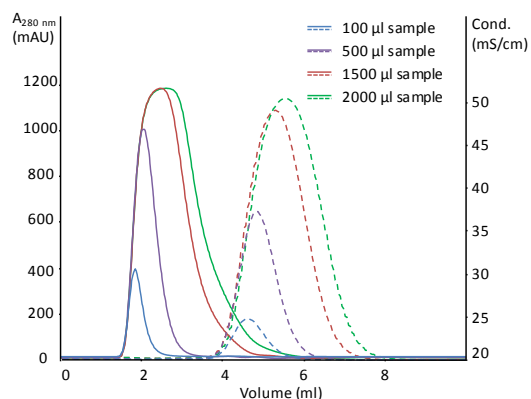


Figure 1. Desalting of 2 mg BSA/ml in 25 mM Na-phosphate, 0.5 M NaCl, pH 7.0 on BabyBio Dsalt 5 ml. Sample volume range of 100 - 2000 µl. The solid lines correspond to absorbance at 280 nm and the dashed lines to the conductivity.

Scale-up using columns in series

For treatment of larger samples, the volume capacity of the column can be increased by connecting up to five BabyBio Dsalt columns in series. The connection can be carried out without any accessories. An example of efficient salt removal using five BabyBio Dsalt 5 ml connected in series with a sample volume of 5 ml is shown in Figure 2.

Column: BabyBio Dsalt 5 ml (5 columns connected in series)
 Buffer: 25 mM Na-phosphate, 150 mM NaCl, pH 7.0
 Sample: 5 ml 2 mg/ml BSA in 20 mM Na-phosphate, 0.5 M NaCl, pH 7.0
 Flow rate: 5 ml/min

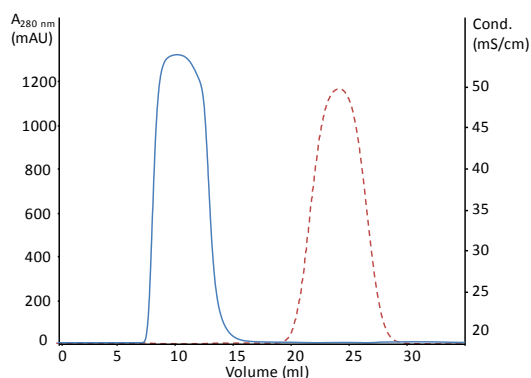


Figure 2. Chromatogram of efficient desalting of 5 ml sample using five BabyBio Dsalt 5 ml connected in series, giving a total column volume of 25 ml. The blue line corresponds to the absorbance at 280 nm and the red dashed line to conductivity.

Buffer exchange of protein solutions

In some applications it may be necessary to expose the target protein to conditions that are not optimal for its structure and function. This is exemplified by purification of IgG by Protein A affinity chromatography where the elution is performed by decreasing pH. The low pH tends to cause aggregation of the purified antibodies unless the pH is quickly restored to neutral.

In Figure 3, a BabyBio A 1 ml column (packed with WorkBeads™ Protein A) was used for the purification of human polyclonal IgG. After low pH elution, the pH of the IgG fraction was rapidly restored to neutral by buffer exchange using three BabyBio Dsalt 5 ml connected in series.

Column A: BabyBio A 1 ml
 Binding buffer: PBS, pH 7.4
 Elution buffer: 100 mM glycine-HCl, pH 2.7
 Sample: 20 ml 1 mg/ml human, polyclonal IgG in PBS pH 7.4
 Flow rate: 1 ml/min
 Linear gradient: 0 - 100% elution buffer, 20 column volumes (CV)

Column B: BabyBio Dsalt 5 ml (3 columns connected in series)
 Running buffer: 25 mM Na-phosphate, 150 mM NaCl, pH 7.0
 Sample: 3 ml of elution pool from BabyBio A 1 ml
 Flow rate: 5 ml/min

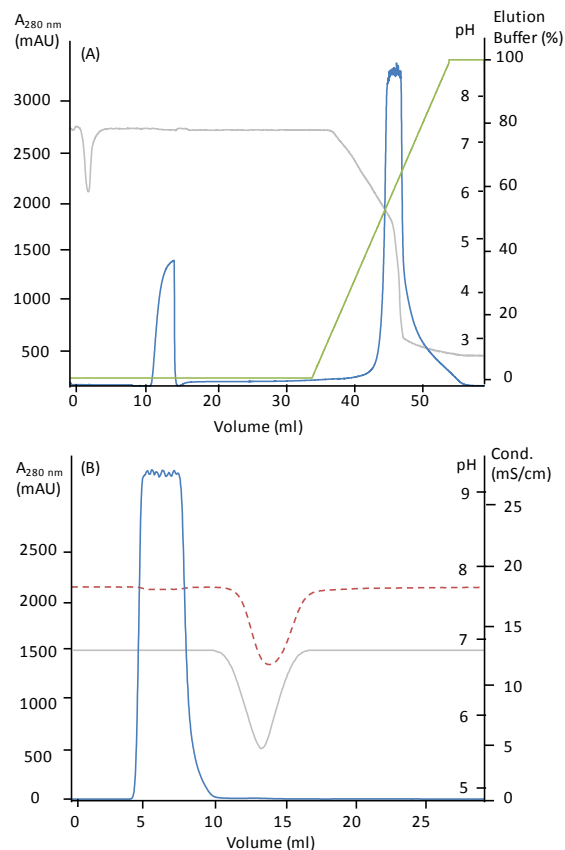


Figure 3. (A) Human polyclonal IgG adsorbed on a BabyBio A 1 ml column and desorbed by low pH. (B) pH restoration by buffer exchange of the collected 3 ml IgG solution applied on three BabyBio Dsalt 5 ml connected in series. Blue line: absorbance at 280 nm, grey line: pH, green line: concentration of elution buffer and dashed red line: conductivity.

Scale-up

Scale-up can conveniently be carried out from a 1 ml column to a 5 ml column. Up to five columns (1 or 5 ml columns) can be connected in series to increase the volume capacity. Sample volumes of up to 7.5 ml can be treated.

Cleaning-in-place

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 with standard buffers is recommended, for example treatment of two column volumes of 0.2 M NaOH.

Equipment

Prepacked BabyBio Dsalt ready-to-use columns can be used with most pumps and standard liquid chromatography equipment's. Purification can also be done using a syringe connected to the column by a luer/std HPLC connector.

Storage

Equilibrate the column in 20% ethanol and store at 2 to 25°C. Make sure that the column is securely closed.

Related products

Product name	Pack size ¹	Article number
Prepacked columns		
BabyBio S 1 ml	1 x 1 ml	45 200 101
BabyBio Q 1 ml	1 x 1 ml	45 100 101
BabyBio DEAE 1 ml	1 x 1 ml	45 150 101
BabyBio A 1 ml	1 x 1 ml	45 605 101
BabyBio NTA His-tag screening kit 1 ml ²	4 x 1 ml	45 700 101
BabyBio NTA His-tag Screening kit 5 ml ²	4 x 5 ml	45 700 102
BabyBio IDA His-tag Screening kit 1 ml ²	4 x 1 ml	45 700 001
BabyBio IDA His-tag Screening kit 5 ml ²	4 x 5 ml	45 700 002

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

2. Includes one column each charged with Ni²⁺, Co²⁺, Cu²⁺ or Zn²⁺

Ordering information

Product name	Pack size	Article number
BabyBio Dsalt 1 ml	1 x 1 ml	45 360 101
	2 x 1 ml	45 360 102
	5 x 1 ml	45 360 103
	10 x 1 ml	45 360 104
	100 x 1 ml	45 360 110
BabyBio Dsalt 5 ml	1 x 5 ml	45 360 105
	2 x 5 ml	45 360 106
	5 x 5 ml	45 360 107
	10 x 5 ml	45 360 108
	100 x 5 ml	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

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