

WorkBeads Dsalt

BabyBio Dsalt 1 ml

BabyBio Dsalt 5 ml

WorkBeads™ Dsalt resin and BabyBio™ Dsalt prepacked columns allow quick and easy separation of high- and low molecular weight substances for efficient desalting and/or buffer exchange of proteins, large peptides or nucleic acids. This technique is easy and allows fast scale-up.

- Designed for rapid and efficient desalting and/or buffer exchange
- Group separation of high molecular weight substances from low molecular weight substances
- Conveniently prepacked 1 ml and 5 ml BabyBio columns, as well as pre-swollen bulk resin for packing larger columns



Resin description

WorkBeads Dsalt is a pre-swollen cross-linked dextran-based resin, optimized for desalting, and/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.

Substances that are larger than M_r 5 000 do not enter the porous beads and are therefore eluted in the void volume (early elution). Substances smaller than M_r 5 000 (e.g., salts, buffer substances and other low-molecular weight additives or impurities) enter the bead pores. These substances are delayed (late elution).

WorkBeads Dsalt allow desalting or buffer exchange by group separation of protein samples before or after various purification steps. The chromatographic desalting technique can be run at high flow rates and can be scaled up for bioproduction.

The main characteristics of WorkBeads Dsalt resin are shown in Table 1. For additional information, see instructions, IN 40 360 010.

BabyBio 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 semi-crude feed with minimal clogging.

The ready-to-use BabyBio prepacked 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 providing a convenient way to perform smaller scale-up experiments.

The main characteristics of BabyBio Dsalt columns are shown in Table 2. For additional information, see instruction IN 45 360 010.

Table 1. Main characteristics of WorkBeads Dsalt.

| | WorkBeads Dsalt |
|--|---|
| Target substance | Proteins, large peptides ($M_r > 5\,000$), nucleic acids and other biomolecules of similar size |
| Matrix | Highly cross-linked dextran |
| Average particle size ¹ (D_{V50}) | 150 μm |
| Typical sample volume | 20 to 30% of the column volume (0.3 CV) |
| Typical flow rate | 150 to 300 cm/h |
| Chemical stability | Compatible with all standard aqueous buffers used for protein purification, 0.2 M NaOH, 0.2 M HCl, 1 M acetic acid, 8 M urea, 6 M guanidine HCl |
| pH stability | 2 to 12 |
| Storage | 2 to 25 °C in 20% ethanol or other suitable storage solution |
| Shipping solution | 0.15% ProClin™ 150 in deionized water |

1. The median particle size of the cumulative volume distribution.

ProClin is a trademark of the Dow Chemical Company (Dow) or an affiliated company of Dow.

Table 2. Main characteristics of BabyBio Dsalt 1 ml and BabyBio Dsalt 5 ml columns.

| | BabyBio Dsalt |
|----------------------------|--|
| Target substance | Proteins, large peptides ($M_r > 5\,000$), 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) 13 x 38 mm (5 ml) |
| Typical sample volume | |
| BabyBio Dsalt 1 ml | 20 - 300 μl |
| BabyBio Dsalt 5 ml | 100 - 1500 μl |
| 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, 0.2 M NaOH, 0.2 M HCl, 1 M acetic acid, 8 M urea, 6 M guanidine HCl |
| pH stability | 2 - 12 |
| Storage | 2 to 25 °C in 20% ethanol |
| Shipping solution | 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 or presence of additives, e.g., use half of the maximum flow rate for 20% ethanol

Applications

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, function or for further purification or use. 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 1, 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)

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

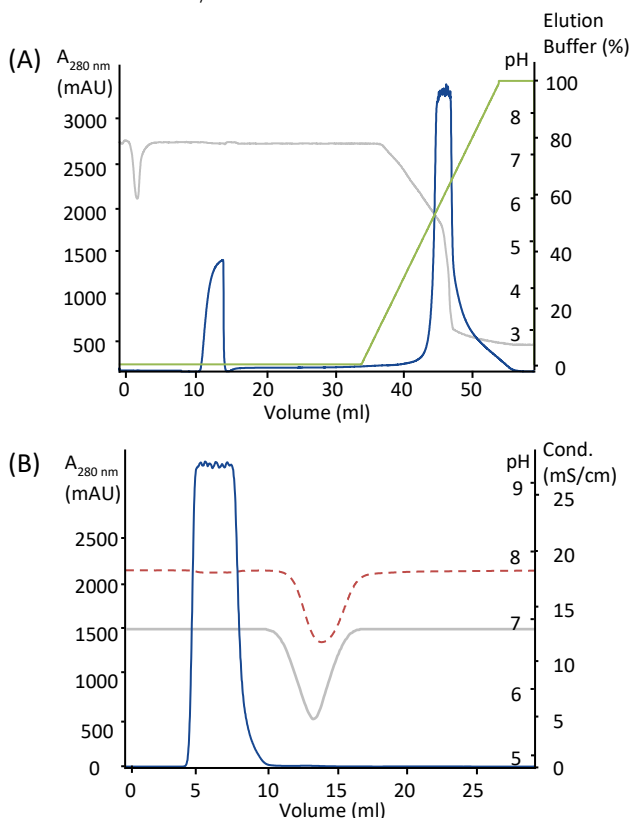


Figure 1. (A) Human polyclonal IgG adsorbed on a BabyBio A 1 ml column and eluted by low pH. (B) Buffer exchange of 3 ml of the eluted IgG pool applied on 3 x BabyBio Dsalt 5 ml Blue line: absorbance at 280 nm, grey line: pH, green line: concentration of elution buffer and dashed red line: conductivity.

A BabyBio Ni-NTA 1 ml column (packed with WorkBeads 40 Ni-NTA) was used for the purification of His-tagged Green Fluorescent Protein (His₆-GFP) expressed in *E. coli* (Figure 2). After elution with high concentration of imidazole, the His₆-GFP may aggregate due to the presence of high imidazole. The imidazole was therefore rapidly removed by buffer exchange using WorkBeads Dsalt packed in a 7.9 ml column.

Column A: BabyBio Ni-NTA 1 ml
 Binding buffer: 50 mM sodium phosphate, 300 mM NaCl, 10 mM imidazole, pH 8.0
 Elution buffer: 50 mM sodium phosphate, 300 mM NaCl, 300 mM imidazole, pH 8.0
 Sample: 10 ml clarified extract His₆-GFP expressed in *E. coli*
 Flow rate: 1 ml/min (150 cm/h)
 Linear gradient: 0 - 100% elution buffer, 20 CV

Column B: WorkBeads Dsalt, 7.9 ml, 10 x 100 mm
 Running buffer: PBS, pH 7.4
 Sample: 2 ml elution pool from BabyBio Ni-NTA 1 ml
 Flow rate: 1 ml/min (75 cm/h)

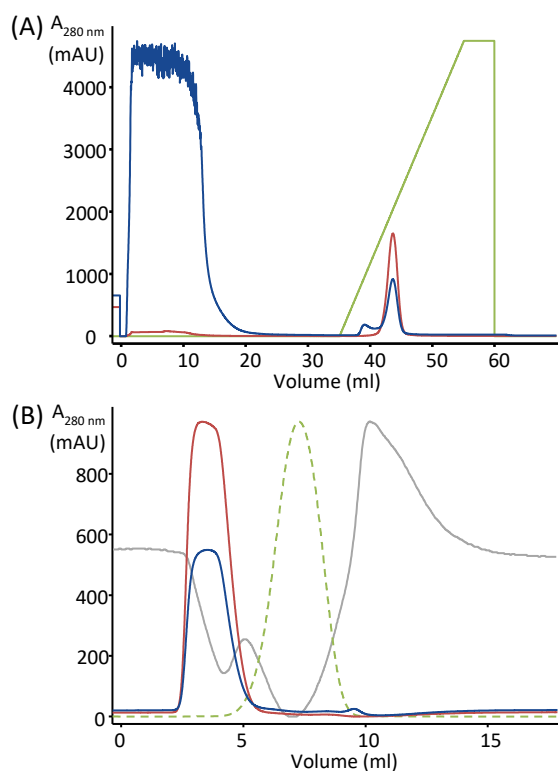


Figure 2. (A) His₆-GFP purified on BabyBio Ni-NTA 1 ml. (B) Desalting using WorkBeads Dsalt. Blue line: absorbance at 280 nm, red line: absorbance at 465 nm, grey line: pH, green line: concentration of elution buffer and dashed green line: conductivity.

Sample volume range

WorkBeads Dsalt is used for efficient separation of high- and low-molecular weight substances. The amount of low-molecular weight substances in the sample will depend on sample volume and pooling of the eluted fraction. Figure 3 shows a comparison of different sample loads on WorkBeads Dsalt.

Column: WorkBeads Dsalt, 25 × 250 mm (123 ml)
 Buffer: PBS, pH 7.4
 Sample: 1 mg/ml BSA, 1 M NaCl in PBS, pH 7.4
 Sample volume: 30 ml (25 % of CV)
 37 ml (30 % of CV)
 43 ml (35 % of CV)
 Flow rate: 25 ml/min (300 cm/h)

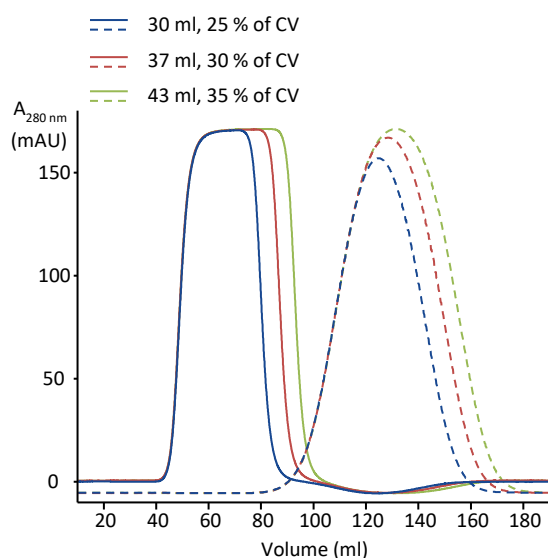


Figure 3. Desalting of 1 mg BSA/ml, 1 M NaCl in PBS, pH 7.4 on WorkBeads Dsalt, 25 × 250 mm column. Sample volume range of 25 to 35 % of column volume. The solid lines correspond to absorbance at 280 nm (protein) and the dashed lines to the conductivity (salt).

BabyBio Dsalt columns

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 for the BabyBio Dsalt 1 ml and the low-molecular weight components at 0.7 ml. For the BabyBio Dsalt 5 ml column corresponding values are 1.25 ml and 3.2 ml. Increasing the sample volumes increases the tail of the peaks of both the high- and low- M_r components, exemplified here on a BabyBio Dsalt 5 ml column (Figure 4). For efficient desalting in this column the sample volume should not exceed 1.5 ml.

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

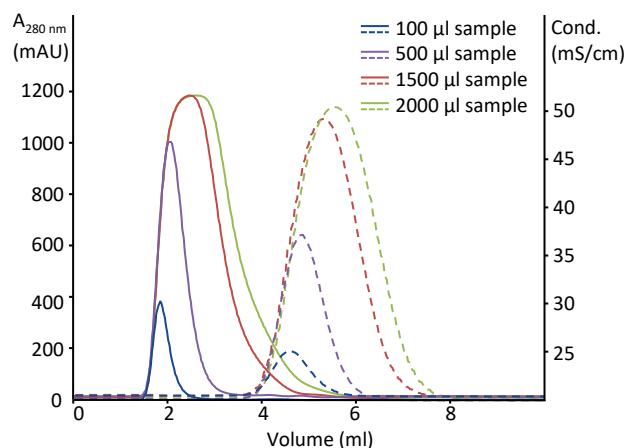


Figure 4. Desalting of 2 mg BSA/ml in 25 mM sodium phosphate, 0.5 M NaCl, pH 7.0 on BabyBio Dsalt 5 ml. Sample volume range of 100 - 2000 µl. The solid lines correspond to absorbance at 280 nm (protein) and the dashed lines to the conductivity (salt).

Scale-up

Purification using BabyBio columns can be scaled up conveniently from a 1 ml column to a 5 ml column. Further scale-up can be done by connecting up to five columns (1 or 5 ml columns) in series to increase the volume capacity (Figure 5). Sample volumes of up to 7.5 ml can be treated.

Further scale-up of the desalting separation can be done by packing the WorkBeads Dsalt resin in larger columns up to bioprocess scale. The ratio between sample volume and column volume should be kept constant. The column volume is scaled up by increasing the column diameter while keeping the bed height the same. The linear flow rate should remain the same while the volumetric flow rate increases.

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. The example below shows efficient salt removal using five BabyBio Dsalt 5 ml connected in series with a sample volume of 5 ml (Figure 5).

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

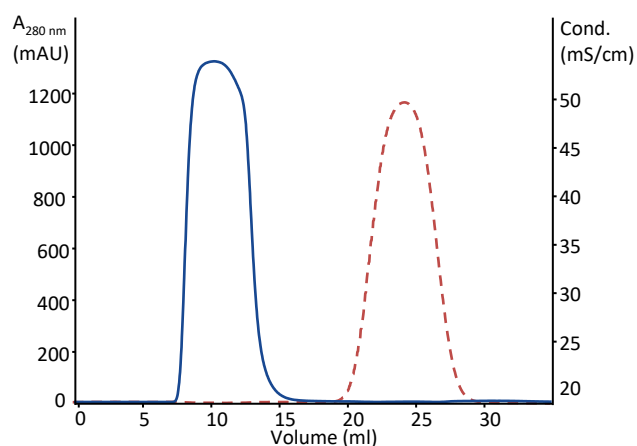


Figure 5. Chromatogram of efficient desalting of 5 ml sample using five BabyBio Dsalt 5 ml connected in series, giving a total column volume of 25 ml. The blue line corresponds to the absorbance at 280 nm (protein) and the red dashed line to conductivity (salt).

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 degree to which this happens 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.

Regular cleaning of the column is recommended, for example treatment with two column volumes of 0.2 M NaOH. Preferably this should be done using reversed flow through the column.

Storage

Store WorkBeads Dsalt and BabyBio Dsalt columns at 2 to 25°C in 20% ethanol or another suitable storage solution containing a bacteriostatic. Make sure that the BabyBio column is securely closed upon storage.

Related products

| Product name | Pack size ¹ | Article number |
|---|------------------------|----------------|
| Prepacked columns | | |
| BabyBio S 5 ml | 5 ml × 5 | 45 200 107 |
| BabyBio Q 5 ml | 5 ml × 5 | 45 100 107 |
| BabyBio DEAE 5 ml | 5 ml × 5 | 45 150 107 |
| BabyBio affimAb 1 ml | 5 ml × 5 | 45 800 107 |
| BabyBio NTA His-tag screening kit 1 ml ² | 1 ml × 4 | 45 700 101 |
| BabyBio NTA His-tag Screening kit 5 ml ² | 5 ml × 4 | 45 700 102 |
| BabyBio IDA His-tag Screening kit 1 ml ² | 1 ml × 4 | 45 700 001 |
| BabyBio IDA His-tag Screening kit 5 ml ² | 5 ml × 4 | 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 |
|--------------------|------------|----------------|
| WorkBeads Dsalt | 300 ml | 40 360 003 |
| | 1 L | 40 360 010 |
| | 5 L | 40 360 050 |
| | 10 L | 40 360 060 |
| BabyBio Dsalt 1 ml | 1 ml × 1 | 45 360 101 |
| | 1 ml × 2 | 45 360 102 |
| | 1 ml × 5 | 45 360 103 |
| | 1 ml × 10 | 45 360 104 |
| | 1 ml × 100 | 45 360 110 |
| BabyBio Dsalt 5 ml | 5 ml × 1 | 45 360 105 |
| | 5 ml × 2 | 45 360 106 |
| | 5 ml × 5 | 45 360 107 |
| | 5 ml × 10 | 45 360 108 |
| | 5 ml × 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



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