



## INSTRUCTION

# GoBio Mini Dsalt

GoBio™ Mini Dsalt columns 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. GoBio Mini 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



## Intended use

WorkBeads resins are developed and supported for both research and production-scale chromatography. WorkBeads resins are produced according to ISO 9001:2015, and Regulatory Support Files (RSF) are available to assist the process validation and submissions to regulatory authorities.

The GoBio prepacked column family has been developed for convenient, reproducible, and rapid results and can be used for small scale purification and all the way up to process development and full-scale manufacturing.

## Safety

Please read the Safety Data Sheet (SDS) for WorkBeads Dsalt, and the safety instructions for any equipment to be used.

## Unpacking and inspection

Unpack the shipment as soon as it arrives and inspect it for damage. Promptly report any damage or discrepancies to [complaints@bio-works.com](mailto:complaints@bio-works.com)

## Short protocol

This general short protocol is for usage of GoBio Mini 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 GoBio Mini Dsalt 1 mL and from 1.25 mL for GoBio Mini Dsalt 5 mL.

Low molecular weight components start to elute from 0.7 mL for GoBio Mini Dsalt 1 mL and from 3.2 mL for GoBio Mini 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. GoBio Mini 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 GoBio Mini 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. GoBio Mini 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.

GoBio Mini 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  $\mu\text{m}$  filter (e.g., a syringe filter) to avoid inadvertently applying any remaining particles onto the column. Alternatively, clarify the sample by centrifugation at 10000 – 20000  $\times g$  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 (GoBio Mini 1 mL column) or 5 mL/min (GoBio Mini 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 GoBio Mini 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 (GoBio Mini 1 mL) or 6 mL/min (GoBio Mini 5 mL) before the storage solution has been removed to avoid overpressure due to high viscosity of the 20% ethanol solution.

#### 4. Equilibrate the column

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  $\mu$ L sample on a GoBio Mini Dsalt 1 mL or 0.1 – 1.5 mL sample on a GoBio Mini Dsalt 5 mL column. Apply the sample at 0.5 – 1 mL/min (GoBio Mini 1 mL) or 2 – 5 mL/min (GoBio Mini 5 mL).

The recommended sample volume range for GoBio Mini Dsalt 1 mL is 20  $\mu$ L – 300  $\mu$ L and for GoBio Mini 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 GoBio Mini Dsalt 1 mL and GoBio Mini Dsalt 5 mL, respectively. A schematically drawing of how to use GoBio Mini 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 GoBio Mini Dsalt 1 mL and GoBio Mini Dsalt 5 mL, respectively. Low molecular weight components start to elute from 0.7 mL and 3.2 mL for GoBio Mini Dsalt 1 mL and GoBio Mini 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 start after applying a total volume of the void volume of the column, even if sample volume is larger than the void volume.

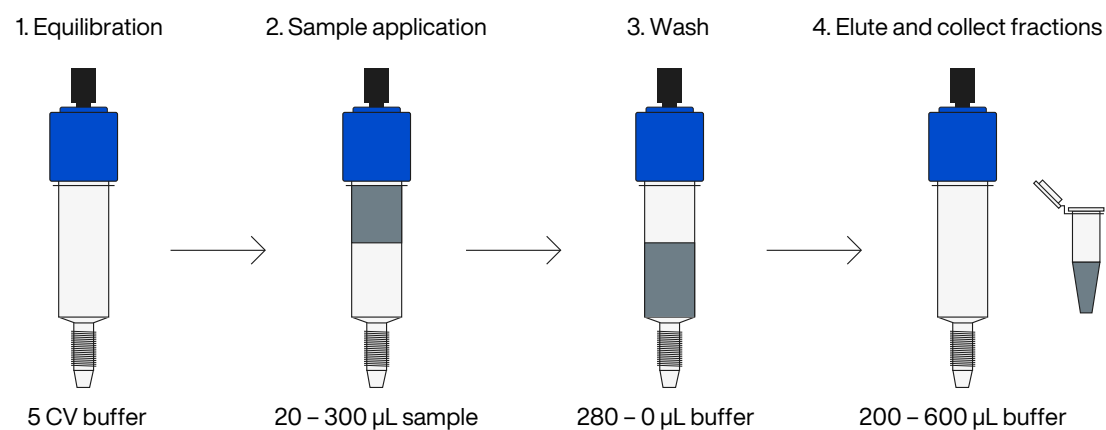
#### 7. Re-equilibrate

Re-equilibrate the column with 5 CV buffer.

#### 8. Re-equilibrate

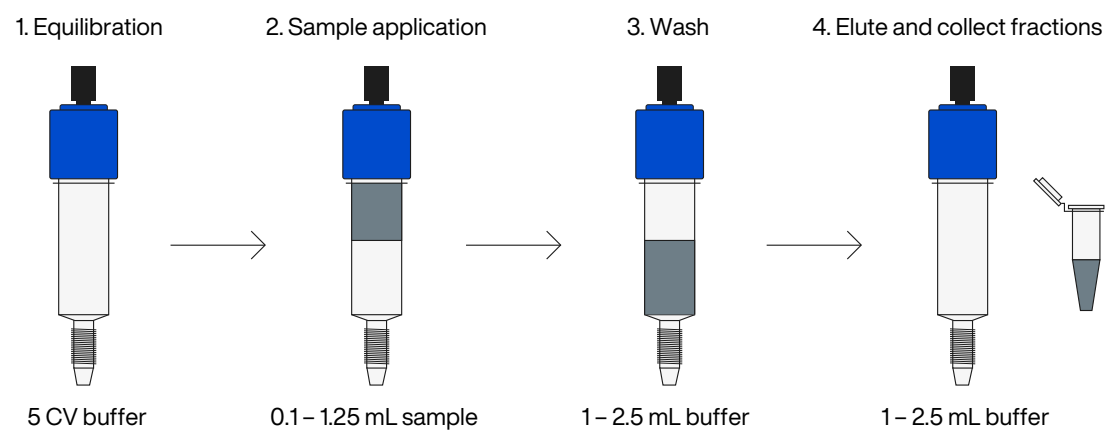
Wash the column with 5 CV deionized water to remove the buffer. Equilibrate the column with 10 CV 20% ethanol for storage. Close the column using the included cap and plug.

## GoBio Mini Dsalt 1 mL

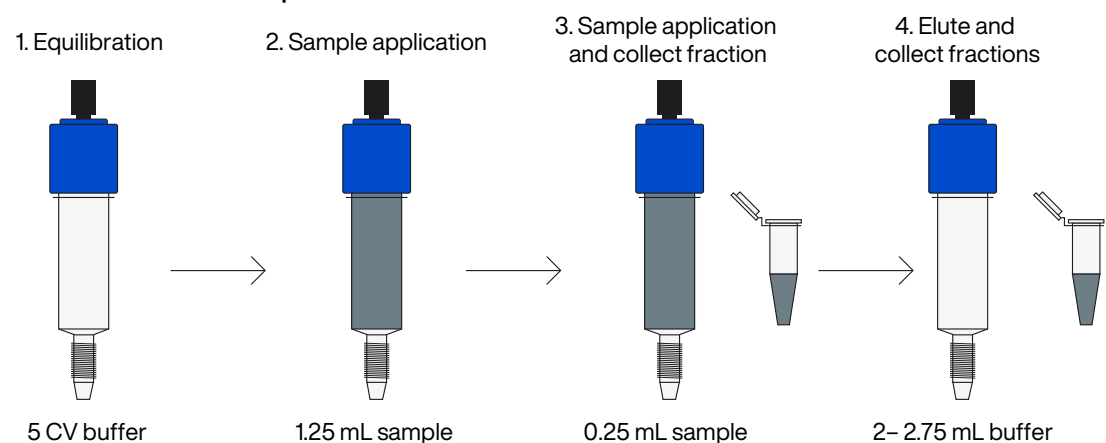


## GoBio Mini Dsalt 5 mL

### Alternative 1: 0.1 – 0.25 mL sample



### Alternative 2: 1.5 mL sample



**Figure 2.** Schematical drawing of how to use GoBio Mini Dsalt 1 mL and GoBio Mini Dsalt 5 mL columns. The procedure depends on the sample volume during sample application.

**Table 2.** Typical sample volume, buffer volume for wash, fraction collection volume, dilution factor, remaining protein yield and salt content in fraction for GoBio Mini 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

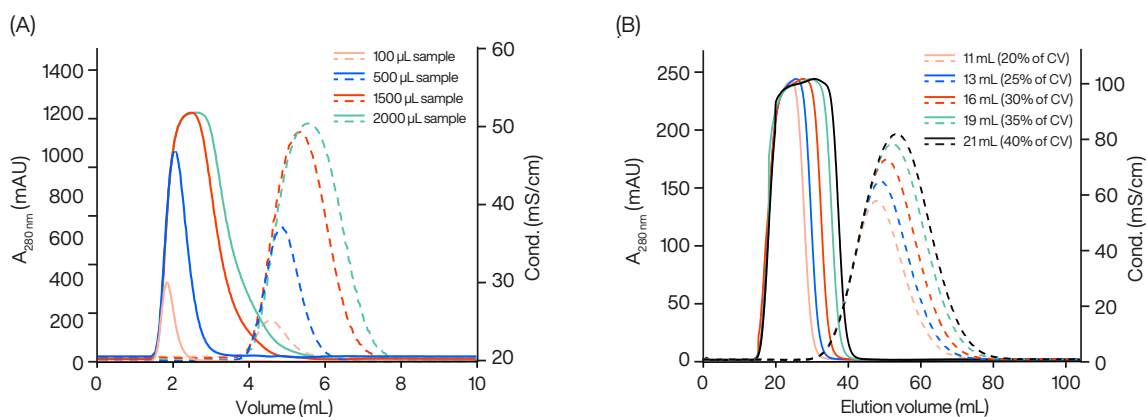
**Table 3.** Typical sample volume, buffer volume for wash, fraction collection volume, dilution factor, remaining protein yield and salt content in fraction for GoBio Mini Dsalt 5 mL.

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 <sup>1</sup>	2 <sup>2</sup>	1.5	90 – 95	0.2
1.5	0.25 <sup>1</sup>	2.75 <sup>3</sup>	2	>95	3

<sup>1</sup> 1.25 mL is the void volume of the column. After 1.25 mL the collection of the 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.

<sup>2</sup> The total fraction size will be 2.25 mL, for this example.

<sup>3</sup> The total fraction size will be 3 mL, for this example.



**Figure 3.** Effects of varying the sample volume. (A) GoBio Mini Dsalt 1 mL, sample volume ranges from 50 µL – 300 µL sample. (B) GoBio Mini 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 GoBio Mini Dsalt 1 mL and GoBio Mini Dsalt 5 mL, respectively. The solid lines correspond to the absorbance at 280 nm. The dashed lines correspond to the conductivity.

## Scale-up

GoBio Mini Dsalt 1 mL and GoBio Mini Dsalt 5 mL columns can be used for up to 300  $\mu$ L 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  $\mu$ L to 7.5 mL can be desalted or buffer exchanged.

Connection of the columns is 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 larger sample volumes there are larger GoBio Prep columns available such as GoBio Prep 16x100 Dsalt for samples volumes  $\leq$ 6 mL and GoBio Prep 26x100 Dsalt for samples volumes  $\leq$ 16 mL.

WorkBeads Dsalt is also available in prepacked GoBio Prod columns starting from 1 L, see "Related products".

**Table 4.** Recommended maximum pressure settings for GoBio Mini columns connected in series. Notice that the maximum pressure over each column is always 3 bar.

No. of columns in series	Max pressure GoBio Mini 1 mL (bar)	Max pressure GoBio Mini 5 mL (bar)
1	3.0	3.0
2	6.0	6.0
3	9.0	9.0
4	12	10 <sup>1</sup>
5	15	10 <sup>1</sup>

<sup>1</sup> 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 to stabilize the sample or prepare 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. GoBio Mini 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 diluted 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 products visit [www.bio-works.com](http://www.bio-works.com)

## Maintenance of the column

### 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.

## Product information

GoBio Mini 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 × 28 mm (1 mL) 13 × 38 mm (5 mL)
Typical sample volume	
GoBio Mini Dsalt 1 mL	20 µL – 300 µL
GoBio Mini Dsalt 5 mL	100 µL – 1500 µL
Recommended flow rate <sup>1</sup>	
GoBio Mini Dsalt 1 mL	0.25 – 1 mL/min (37 – 150 cm/h)
GoBio Mini Dsalt 5 mL	1.25 – 5 mL/min (56 – 225 cm/h)
Max flow rate <sup>2</sup>	
GoBio Mini Dsalt 1 mL	5 mL/min (780 cm/h)
GoBio Mini 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

<sup>1</sup> 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.

<sup>2</sup> 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).<sup>1</sup>



## GoBio prepacked column family

GoBio prepacked column family is developed for convenient, reproducible and fast results and includes columns with different sizes and formats.

GoBio Mini 1 mL and GoBio Mini 5 mL for small scale purification and screening using a shorter packed bed.

GoBio Screen 7x100 (3.8 mL) for reproducible process development including fast and easy optimization of methods and parameters.

GoBio Prep 16x100 (20 mL) and GoBio Prep 26x100 (53 mL) for lab-scale purifications and scaling up.

GoBio Prep 16x600 (120 mL) and GoBio Prep 26x600 (320 mL) for preparative lab-scale size exclusion chromatography.

GoBio Prod 80x200 (1 L), GoBio Prod 130x200 (2.7 L), GoBio Prod 200x200 (6 L), GoBio Prod 240x200 (9 L) and GoBio Prod 330x250 (21.4 L) for production-scale purifications.

## Related products

Product name	Pack size <sup>1</sup>	Article number
<b>Prepacked columns</b>		
GoBio Mini S 1 mL	1 mL × 5	45 200 103
GoBio Mini Q 1 mL	1 mL × 5	45 100 103
GoBio Mini DEAE 1 mL	1 mL × 5	45 150 103
GoBio Mini TREN 1 mL	1 mL × 5	45 655 213
GoBio Mini NiMAC 1 mL	1 mL × 5	45 655 113
GoBio Mini affmAb	1 mL × 5	45 800 103
GoBio Prep 16x100 Dsalt <sup>2</sup>	20 mL	55 700 021
GoBio Prep 26x100 Dsalt	53 mL	55 700 031
GoBio Prod 80x200 Dsalt <sup>2</sup>	1 L	55 700 042
GoBio Prod 130x200 Dsalt <sup>2</sup>	2.7 L	55 700 062
GoBio Prod 200x200 Dsalt <sup>2</sup>	6 L	55 700 072
GoBio Prod 240x200 Dsalt <sup>2</sup>	9 L	55 700 082
GoBio Prod 360x250 Dsalt <sup>2</sup>	21.4 L	55 700 094
<b>Bulk resin</b>		
WorkBeads Dsalt	300 mL	40 360 003
WorkBeads 40S	25 mL 200 mL	40 200 001 40 200 002
WorkBeads 40Q	25 mL 200 mL	40 100 001 40 100 002
WorkBeads 40 DEAE	25 mL 200 mL	40 150 001 40 150 002
WorkBeads 40 TREN	25 mL 150 mL	40 603 001 40 603 003
WorkBeads NiMAC	25 mL 150 mL	40 653 001 40 653 003
WorkBeads affmAb	25 mL 200 mL	40 800 001 40 800 002

<sup>1</sup> Other pack sizes can be found in the complete product list on [www.bio-works.com](http://www.bio-works.com)

<sup>2</sup> Packed on request.

## Ordering information

Product name	Pack size	Article number
GoBio Mini Dsalt 1 mL	1 mL × 1	45 360 101
	1 mL × 5	45 360 103
	1 mL × 10	45 360 104
	1 mL × 100	45 360 110
GoBio Mini Dsalt 5 mL	5 mL × 1	45 360 105
	5 mL × 5	45 800 107
	5 mL × 10	45 360 108
	5 mL × 100	45 360 109

Orders: [sales@bio-works.com](mailto:sales@bio-works.com) or contact your local distributor.

For more information about local distributor and products visit [www.bio-works.com](http://www.bio-works.com) or contact us at [info@bio-works.com](mailto:info@bio-works.com)

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