Bio-Works

INSTRUCTION

GoBio Prep 16x100 Dsalt
GoBio Prep 26x100 Dsalt

GoBio™ Prep 16x100 Dsalt and GoBio Prep 26x100 Dsalt are prepacked columns with WorkBeads™ Dsalt resin. These ready-to-use columns are designed for fast and easy group separation of high (M_r > 5000) from low (M_r < 1000) molecular weight substances which enables efficient desalting and/or buffer exchange of proteins, large peptides, and nucleic acids.

- Prepacked, ready-to-use columns for fast and easy buffer exchange and desalting
- Reliable and reproducible results
- Easy scale-up

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.

GoBio prepacked column family is developed for convenient, reproducible, and fast results and can be used from small scale purification through process development to full-scale manufacturing.

Safety

Please read the associated Safety Data Sheet (SDS) for WorkBeads Dsalt resin, 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. Please, report any damage or discrepancies to complaints@bio-works.com

Principle

Proteins and many other biomolecules differ greatly in size from salts and other small molecules. Size exclusion chromatography is an efficient technique for separation of components according to size. 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 retained (late elution). This mechanism allows group separation of large substances from small substances. A common guideline is that there should be at least a tenfold size difference between the substances/ molecules to achieve an efficient group separation. A protein can therefore be separated from salt and/or buffer substances in the sample and in the process be transferred into a solution of choice.

The GoBio Prep Dsalt prepacked columns are designed to be used for buffer exchange or desalting to prepare a sample, before lyophilization and before or after ion-exchange chromatography. The separation is convenient and fast and is an excellent alternative to dialysis when samples need to be processed rapidly to avoid degradation.

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's native structure and activity and is suitable for the next process step or final use of the protein.

Product description

GoBio Prep column characteristics

Make sure when using GoBio Prep columns that the connectors are tightened to prevent leakage. The pressure over the packed bed varies depending on parameters such as the resin characteristics, sample/buffer viscosities and the tubing used. Make sure that the flow through the column is in the direction of the arrow on the column.

These columns should not be opened and refilled.

Note: GoBio Prep column hardware is compatible with most aqueous chemicals, but NOT with concentrated alcohol. Maximal alcohol concentration is 20%.

Table 1. GoBio Prep 16x100 and GoBio Prep 26x100 columns characteristics.

Column characteristics	
Column hardware	Acrylic
Top and bottom plugs	Polypropylene
Top and bottom filters	Polyamide
Connections	1/16" female thread in both ends
Column volumes	20 mL (GoBio Prep 16x100) 53 mL (GoBio Prep 26x100)
Column dimensions	16 × 100 mm (GoBio Prep 16x100) 26 × 100 mm (GoBio Prep 26x100)
Maximal column hardware pressure	5 bar, 0.5 MPa, 70 psi

Resins characteristics

WorkBeads Dsalt resin has an approximate exclusion limit of M_r 5 000 for globular proteins and large peptides, and 10 base pairs (bp) for nucleic acids. Substances that are larger than Mr 5 000 do not enter the porous beads and are therefore eluted in the void of the column (early elution).

The chromatography resin is also completely scalable up to production scale. To minimize the dilution and still retain good separation, sample volumes up to approximately 30% of the total column bed volume are recommended. Desalting can be performed at high flow rates as the flow rate only has a minor impact on the resolution.

Note that for scaling up, WorkBeads Dsalt is supplied pre-swollen for convenient preparation for column packing.

The characteristics of GoBio Prep 16x100 Dsalt and GoBio Prep 26x100 Dsalt are listed in section “Product description”.

Purification planning

Unpacking and connecting GoBio Prep 16x100 and GoBio Prep 26x100 columns to a chromatography system

*Each packed column is sealed with a pressure syringe on the **bottom** of the column. It is then placed in a sealed plastic bag.*

1. Cut the plastic bag and remove the column with care.
2 Follow the flow direction (indicated by an arrow on the column label) to clamp the column onto the chromatography system or to a vertical stand.
3. Prepare the chromatography system for connecting the column. The GoBio Prep 16x100 and GoBio Prep 26x100 columns are compatible with 1/16” male connectors with narrow heads. The length of the connector thread must be at least 7 mm to avoid leakage.

   Note: It is recommended to use the two red connectors attached to the transport syringe when connecting the column to a chromatography system. One red connector should be used in each end of the column.
4. Gently unhook the springs from the shaft top of the transport syringe using even force.
5. Remove the syringe and keep it for further use during storage.
6. Unscrew the top plug, some liquid may come out. Connect the column to the chromatography system using one of the red connectors “drop-to-drop” avoiding introducing air into the packed column.
7. Connect the bottom of the column to the chromatography system using the second red connector.

Buffer preparation

The buffer composition and buffer concentration are important for robust and reproducible methods. Choose a pH and buffer that is gentle for the target molecule and keeps it in its native form or do a buffer exchange to the buffer which will be used in the next purification step. See Table 2 for examples of common buffers.

Table 2. Examples of common buffer compositions.

Buffer
20 mM sodium phosphate, 150 mM NaCl, pH 7.4 (PBS)
50 mM sodium phosphate buffer, pH 7.0
20 mM Tris-HCl, 100 mM NaCl, pH 8.0

Sample preparation

If necessary, clarify the sample by passing it through a 0.22 – 0.45 µm filter, e.g., a syringe filter, to avoid transferring any remaining contaminants onto the column or do a centrifugation at 10 000 - 20 000× g for 10 - 15 minutes. Application of a sample that has not been properly clarified may reduce the performance and lifetime of the column.

The sample concentration should not exceed 70 mg/mL for proteins and 5 mg/mL for dextran.

Maximal sample volume is 30% of the column volume (CV). Larger sample volumes may be used but it will have a negative impact on the desalting result.

Table 3. Recommended sample volumes and flow rates.

	GoBio Prep 16x100	GoBio Prep 26x100
Recommended sample volume	≤ 6 mL (Max. 30% of CV)	≤ 16 mL (Max. 30% of CV)
Recommended flow rate[1]	5-10 mL/min (150 - 300 cm/h)	13-26 mL/min (150 - 300 cm/h)

[1] At room temperature in H_2O.

Desalting/Buffer exchange runs

Desalting and/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.

Note: Do not exceed the maximum recommended flow rate and back pressure for the column.

1. Wash out the storage solution 20% ethanol and equilibrate with 5 - 8 column volumes (CV) using the buffer of choice and a flow rate of 5 mL/min for GoBio 16x100 Dsalt and 13 mL 7 min for GoBio 26x100 Dsalt.
2. Apply the sample. Do not exceed the recommended sample volume as this may have a negative impact on the desalting result – see table 3.
3. Elution is performed with the same buffer as used for the wash/equilibration. Elute the sample with 1.3 - 2 CV.
4. If a new desalting is performed equilibrate the column with at least 5 CV buffer.
5. For storage, equilibrate the column with at least 3 - 5 CV 20% ethanol.

   Use a reduced flow rate, 50% of the maximum flow rate when equilibrating with the storage solution.
6. Make sure that the stop plugs are tight to prevent leakage.

   For prolonged storage, connect the included syringe filled with storage solution to the bottom end of the column.

Optimization

High viscosity

Although most aqueous buffers have a viscosity close to that of water, some samples or elution buffers may have additives that raise the viscosity. When using high viscosity solutions, the flow rate must be reduced in proportion to the increase in viscosity over that of diluted aqueous solutions. Similarly, the viscosity of an aqueous solution will increase when the temperature is decreased (e.g., when working at 4°C), in that case reduce the flow rate to half of the flow rate used at room temperature.

Improve resolution

The easiest and most effective way to improve the resolution is to decrease the sample volume. The drawback is that the target molecule will be more diluted. It can also be useful to decrease the flow rate or decrease the sample concentration if it is close to 70 mg/mL.

Scale-up

Note: WorkBeads Dsalt resin is delivered pre-swollen for fast and easy packing of larger columns.

Several prepacked Dsalt columns are available in the GoBio prepacked column family for convenient, fast and easy scaling up. These columns are also very useful alternatives when samples need to be processed rapidly to avoid degradation. For larger sample volumes prepacked GoBio Prod columns starting from 1 L are available or diafiltration can be used, see “Related products”.

To find out more about Bio-Works’ chromatography products visit www.bio-works.com

Scale-up principles

During scale-up, 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 constant (e.g. 200 mm). The linear flow rate should remain the same while the volumetric flow rate increases. The volumetric flow rate for each column can be calculated according to:

$$\text{Volumetric flow rate (mL/min)} = \frac{\text{Linear flow rate (cm/h)} \times \text{Column cross sectional area (cm}^2\text{)}}{60}$$

Flow

The concepts of volumetric flow, linear flow rate and residence time are important when scaling-up in chromatography. Volumetric flow is measured in mL/min or L/min, linear flow in cm/h and residence time in minutes. The relationship between these metrics is:

$$\text{Linear flow rate (cm/h)} = \frac{\text{Volumetric flow (mL/min)} \times 60}{\text{Column cross sectional area (cm}^2\text{)}}$$

$$\text{Residence time (minutes)} = \frac{\text{Column bed height (cm)} \times 60}{\text{Linear flow rate (cm/h)}}$$

If a column with smaller bed height has been used, the flow rate for a column with a larger bed height can be calculated from the flow that was established on the smaller column, using the equations above by keeping the residence time of the smaller column the same for the larger column. This will allow an increase of the linear flow in proportion to the increase in bed height between the columns, see Table 4 for examples. If the column bed heights are kept constant during scale-up, the linear flow rate should be kept constant (as well as the residence time).

Table 4. Example of scale-up parameters.

Column dimension	Residence time (minutes)	Linear flow rate (cm/h)	Volumetric flow rate (mL/min)
16x100	4	150	5.0
26x100	4	150	13.3
80x200	8	150	126
130x200	8	150	332
200x200	8	150	785
240x200	8	150	1131
330x250	10	150	2138

Maintenance of the column

Cleaning-in-Place (CIP)

During desalting and/or buffer exchange, 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 fresh and reduces the rate of further build-up of impurities on the resin, and prolongs the capacity, resolution, and flow properties of the column.

After elution, apply 1 CV 0.2 M NaOH 15-30 minutes. Preferably restore the pH with equilibration buffer before applying 0.5 CV water followed by 1.5 CV 20% ethanol for storage.

Storage

Store at 2 to 25 °C in 20% ethanol.

For prolonged storage, connect the included transport syringe filled with storage solution to the bottom end of the column.

Note: Use a reduced flow rate during equilibration with 20% ethanol, maximum 50% of the maximum flow rate.

Product descriptions

	GoBio Prep 16x100 Dsalt **GoBio Prep 26x100 Dsalt**
Target substance	Proteins, large peptides (M_r > 5000), nucleic acids and other biomolecules of similar size
Resin	WorkBeads Dsalt
Matrix	Highly cross-linked dextran
Average particle size (D_{v50})[1]	150 µm
Column volumes	20 mL (16x100) 53 mL (26x100)
Column dimensions	16 × 100 mm 26 × 100 mm
Recommended sample volume (16x100) (26x100)	 ≤ 6mL ≤ 16mL
Recommended flow rates[2] (16x100) (26x100)	 5 - 10 mL/min (150 - 300 cm/h) 13 - 26 mL/min (150 - 300 cm/h)
Maximum flow rate[3] (16x100) (26x100)	 15 mL/min (450 cm/h) 40 mL/min (450 cm/h)
Maximum back pressure	5 bar, 0.5 MPa, 70 psi
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 13
Storage	2 to 25 °C in 20% ethanol

1 The median particle size of the cumulative volume distribution.

2 Optimal flow is depending on the sample. 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.

3 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.

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[1]	Article number
Prepacked columns		
GoBio Mini Dsalt 1 mL	1 mL × 5	45 360 103
GoBio Mini Dsalt 5 mL	5 mL × 5	45 360 107
GoBio Prod 80x200 Dsalt[2]	1 L	55 700 042
GoBio Prod 130x200 Dsalt[2]	2.7 L	55 700 062
GoBio Prod 200x200 Dsalt[2]	6 L	55 700 072
GoBio Prod 240x200 Dsalt[2]	9 L	55 700 082
GoBio Prod 330x250 Dsalt[2]	21.4 L	55 700 093
Bulk resins		
WorkBeads Dsalt	300 mL 1 L 5 L	40 360 003 40 360 010 40 360 050

[1] Other pack sizes can be found in the complete product list on www.bio-works.com
[2] Packed on request.

Ordering information

Product name	Pack size	Article number
GoBio Prep 16x100 Dsalt[1]	20 mL × 1	55 700 021
GoBio Prep 26x100 Dsalt	53 mL × 1	55 700 031

[1] Packed on request.

Orders: sales@bio-works.com or contact your local distributor.

For more information about local distributor and products visit www.bio-works.com or contact us at info@bio-works.com

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