Bio-Works

INSTRUCTION

WorkBeads Dsalt

WorkBeads™ Dsalt resin is designed to enable quick and easy separations of high and low molecular weight substances. This resin is pres-wollen and enables efficient desalting and/or buffer exchange of proteins, large peptides and nucleic acids.

- Designed for rapid and efficient desalting and/or buffer exchange
- Group separation of high molecular weight substances from low molecular weight substances
- Pre-swollen resin for packing larger columns
- 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 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

Short protocol

This short protocol is for column packing and running of WorkBeads Dsalt resin. Detailed instructions and recommendations for optimization are given later in this instruction. Recommended flow rates are listed in Table 1 and examples of running buffers are listed in Table 2.

1. Make a slurry of the desired resin concentration.
2. Pour the slurry into the column.
3. Pack the resin with an appropriate flow rate.
4. Apply an axial compression of less than 2%.
5. Wash out the storage solution with 3 column volumes (CV) of deionized water or running buffer.
6. If deionized water was used in step 5, equilibrate the column with 3 column volumes of running buffer.
7. Apply sample.
8. Elute the target protein with running buffer.
9. Wash the column with deionized water.
10. Equilibrate the column with 20% ethanol or other desired storage solution.

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. 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). 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 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.

WorkBeads Dsalt resin can be used for buffer exchange or desalting to prepare a sample, e.g., before mass spectrometry analysis, lyophilization and before/after ion-exchange chromatography. The separation is convenient and very fast and is an excellent alternative to dialysis when samples need to be processed rapidly to avoid degradation. The chromatography format 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 has only a minor impact on the resolution.

Note: WorkBeads Dsalt resin is supplied pre-swollen for convenient preparation for column packing.

Column packing

When packing a column follow both this general advice and the column manufacturer's instructions. Preferably, use a column with an adjustable adaptor to allow a final minimal axial compression. In some instances, a packing reservoir or column extension may be needed.

Note: Always make sure that the maximum pressure of the column hardware is not exceeded. Backpressure caused by the chromatography system components connected downstream of the column may reduce the maximum flow that can be used. Wear eye protection.

1. Wash the resin

The resin is provided in 0.15% ProClin™ 150 in deionized water. To remove the storage solution, wash the desired amount of resin with at least three column volumes of deionized water or running buffer before packing.

2. Make a slurry

Add deionized water to the washed resin to obtain a 50% to 75% slurry concentration. Make approximately 15% extra slurry to compensate for the compression during packing. The amount of slurry to be prepared can be calculated according to:

$$\text{Slurry volume} = \frac{\text{bed volume} \times 100}{\%\ \text{slurry}} \times 1.15$$

3. Pour the slurry into the column

Pour the slurry slowly down the side of the column to avoid formation of air bubbles. Preferably, use a packing reservoir or an extra column tube (and connector between the columns) to extend the column volume to accommodate the entire slurry volume during packing.

4. Pack the bed

Pack the resin with a downward flow higher than the intended operational flow. We recommend 375 cm/h. Make sure the packing flow rate does not exceed the maximum pressure of the column hardware or the resin. The intended operational flow should not be more than 75% of the packing flow rate. For high-flow applications in shorter columns (200 – 300 mm bed height) a packing flow rate of 600 cm/h may be used.

5. Close the column

When the bed height is constant, mark the bed height on the column. Stop the flow and remove the packing reservoir or extra tube. Note that the bed height will increase temporarily when the flow is stopped. If needed, adjust the bed height by removing excess resin. Be careful not to remove too much resin. Gently fill the column with packing solution to its rim without disrupting the packed bed. Open the adaptor inlet if needed to let packing solution out during insertion of the adapter on top of the packed bed. Adjust the adaptor to the mark. Apply a small axial compression of less than 2% of the final bed height by lowering the adapter further below the mark.

6. Apply a flow

Apply the operating flow of 300 cm/h, considering step 4. Check for any gap formation above the surface of the resin bed. If a gap is observed, stop the flow and adjust the adaptor to eliminate the gap.

Evaluation of the packed column

Test the column efficiency to check the quality of packing. Testing should be done after packing, at regular intervals during the usage of the column or when separation performance is seen to deteriorate.

The best method of expressing the efficiency of a packed column is in terms of the height equivalent to a theoretical plate (HETP) and the asymmetry factor (A_s). These values are easily determined by applying a sample such as 1% acetone solution to the column.

For optimal results, the sample volume should be 2.5% of the column volume (CV) and the flow rate 30 cm/h.

If an acceptance limit is defined in relation to column performance, the column plate number can be used as one of the acceptance criteria for column use.

Note: The calculated number of plates will vary according to the test conditions and should only be used as a reference value. Keep test conditions and equipment constant so that results are comparable. Changes of for example solute, solvent, eluent, sample volume, flow velocity, liquid pathway, temperature, etc. will influence the results.

Measuring HETP and A_s

Calculate HETP and A_S from the UV curve (or conductivity curve).

$$HETP = \frac{L}{N}$$

$$N = 5.54 \times \left(\frac{V_R}{W_h}\right)^2$$

L = bed height (cm)

N = number of theoretical plates

V_R = volume eluted from the start of sample application to the peak maximum

W_h = peak width measured as the width of the recorded peak at half of the peak height

V_R and W_h are in the same units

The concept of reduced plate height is often used for comparing column performance. The reduced plate height, h, is calculated:

$$h = \frac{HETP}{d_{50v}}$$

d_{50v} = Median particle size of the cumulative volume distribution (cm)

As a guideline, a value of < 3 is very good.

The peak must be symmetrical, and the asymmetry factor as close to 1 as possible. (A typical acceptable range is $0.7 < A_S < 1.3$). A change in the shape of the peak is usually the first indication of bed deterioration.

Peak asymmetry factor calculation:

$$A_s = \frac{a}{b}$$

a = ascending part of the peak width at 10% of peak height

b = descending part of the peak width at 10% of peak height

Figure 1 below shows a UV trace for acetone in a typical test chromatogram from which the HETP and A_s values are calculated.

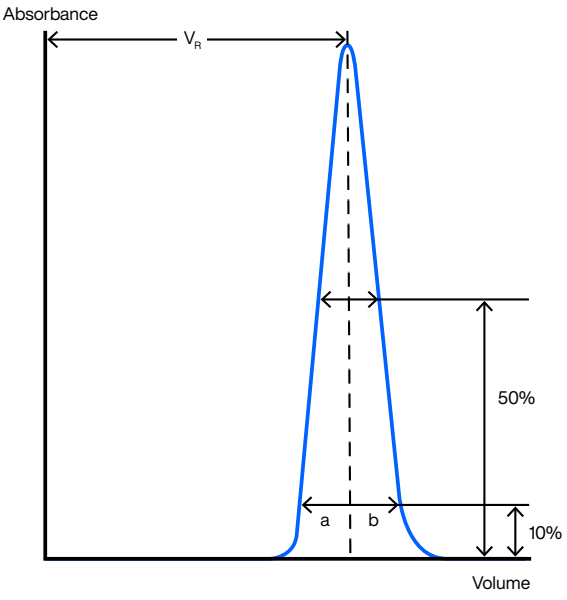

Figure 1. A typical test chromatogram showing the parameters used for HETP and A_s calculations.

Desalting and/or buffer exchange

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.

Table 1. Recommended operating flow rates.

Resin	Recommended flow rate (cm/h)	Typical range of flow rate (cm/h)
WorkBeads Dsalt	150	100 to 300

Table 2. Recommended operating flow rates.

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

Equilibration

Before loading the sample, the column must be equilibrated with a suitable buffer. First the packing solution is removed by applying 0.2 column volumes (CV) of water, then with 1 – 2 CV of running buffer. It is recommended that absorbance is monitored at 280 nm (A_{280}), conductivity and pH of the column effluent. Stable signals are a strong indication of completed equilibration. Equilibration can be done at elevated flow, although it may then require a slightly bigger volume to establish equilibrium.

Sample preparation

If the sample does not come from an earlier chromatography step it is generally recommended that it be passed through a 0.22 – 0.45 µm filter (e.g., a syringe filter) to avoid inadvertently applying any remaining particles onto the column. Alternatively, clarify the sample by centrifugation at 10 000 – 20 000 × g for 15 – 30 minutes. If the sample contains only small amounts of particles it may be enough to only carry out filtration. Application of a sample that has not been properly clarified may reduce the performance and lifetime of the column.

Sample application

Sample volumes up to 20 – 30% of the column volume can be loaded for an efficient desalting, e.g., up to 30 mL on a 100 mL column.

Elution

The elution is done with the same buffer as used for the equilibration. Normally, all sample components are eluted within 1 CV, where molecules larger than the exclusion limit are expected in the void. It is therefore recommended that 1.3 CV is used for elution. If an additional desalting will be done using the same sample and separation conditions, the column can be re-equilibrated with a small volume, e.g., 0.5 CV, before application of the next sample.

Preparation for storage

Wash the column with 0.5 CV water, then with 1.5 CV 20% ethanol or other desired storage solution.

Cleaning-in-place, CIP

After elution, apply 1 CV 0.2 M NaOH over 1 – 2 hours. Preferably restore the pH with equilibration buffer before applying 0.5 CV water followed by 1.5 CV 20% ethanol or 0.15% ProClin 150 for storage.

Optimization

Optimization of desalting and buffer exchange

Buffer exchange is often needed between purification steps to stabilize the sample, or to prepare it for the next separation step. For example, a high ionic strength sample may prevent binding of the target protein using ion exchange chromatography, or a low pH is needed for elution during affinity chromatography. WorkBeads Dsalt resin can also be used to remove remaining low-molecular weight reagents used for labelling or for 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's native structure and activity and is suitable for the next process step or final use of the protein.

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 dilute 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 used at room temperature.

Scale-up

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 the same (e.g., 200 – 300 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 doing scale-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 smaller column has been used, the flow rate for the larger column can be calculated from the flow that was established on the small column, using the equation above by keeping the residence time of the small 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 3 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 3. 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

Combining techniques for purification

We recommend a purification strategy based on three phases: Capture, Enhance and Polish. In the capture phase, usually one purification step, it is common to use affinity chromatography or ion exchange chromatography, to remove bulk impurities and to concentrate and stabilize the target substance. The Enhance phase (one or several purifications steps) aims at further removing impurities. The polish phase aims at removing any final impurities, and adjusting the conditions of the product for subsequent use. If the capture is performed using a sufficiently selective method, the enhance phase may be omitted and it may suffice to carry out a polishing step. Note that changing order of the traditional phases Capture, Enhance, Polish (CEP) to Enhance, Capture, Polish (ECP) instead can in many chromatography processes optimize both the purity of the target molecule and the resin lifetime. One example of this is during purification of monoclonal antibodies (mAbs) where adding the IEX/multimodal IEX step before the affinity chromatography step will enhance both the purity of the mAb and the lifetime usage of the protein A resin.

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

Maintenance of the resin

Cleaning

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 clean, reduces the rate of further build-up of impurities on the resin, and prolongs the capacity, resolution and flow properties of the column. A regular cleaning of a packed column is recommended, for example using 2 CV of 0.2 M NaOH.

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 or other desired storage solution.

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

Product description

	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 (D_{v50})[1]	150 µm
Typical sample volume	20 to 30% of 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 13
Storage	2 to 25 °C in 20% ethanol
Shipping solution	0.15% ProClin 150 in deionized water

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

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 × 1 1 mL × 5 1 mL × 10 1 mL × 100	45 360 101 45 360 103 45 360 104 45 360 110
GoBio Mini Dsalt 5 mL	5 mL × 1 5 mL × 5 5 mL × 10 5 mL × 100	45 360 105 45 360 107 45 360 108 45 360 109
GoBio Prep 16x100 Dsalt[1]	20 mL × 1	55 700 021
GoBio Prep 26x100 Dsalt	53 mL × 1	55 700 031
GoBio Prod 80x200 Dsalt[1]	1 L	55 700 042
GoBio Prod 130x200 Dsalt[1]	2.7 L	55 700 062
GoBio Prod 200x200 Dsalt[1]	6 L	55 700 072
GoBio Prod 240x200 Dsalt[1]	9 L	55 700 082
GoBio Prod 330x250 Dsalt[1]	21.4 L	55 700 093

[1] Packed on request.

Ordering information

Product name	Pack size	Article number
WorkBeads Dsalt	300 ml 1 L 5 L 10 L	40 360 003 40 360 010 40 360 050 40 360 060

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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