WorkBeads Dsalt

WorkBeads™ Dsalt resin is designed to enable quick and easy separations of high and low molecular weight substances. This resin is preswollen 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

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 6, 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 M_r 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 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.

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.

Notice: 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:

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

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.

Purification 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 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

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. 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	Linear flow rate (cm/h)	Volumetric flow rate (ml/min)
10 × 300	150	1.93
25 × 300	150	12.3
50 × 300	150	49

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 when possible 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

Unpacking and inspection

Unpack the shipment as soon as it arrives and inspect it for damage. Please, report any damage or discrepancies to your local supplier.

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.

Storage

Store at 2 to 25 °C in 20% ethanol or other desired storage solution.

Additional information

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[1] (D_{v50})	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.

Intended use

WorkBeads resins are developed and supported for both research and production-scale chromatography. WorkBeads resins are produced with validated methods and Regulatory Support Files (RSF) are available to assist the process validation and submissions to regulatory authorities.

Safety

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

Related products

Related product	Pack size[1]	Article number
Prepacked columns		
BabyBio™ Dsalt 1 ml	1 ml × 5	45 360 103
BabyBio Dsalt 5 ml	5 ml × 5	45 360 107
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 TREN	5 ml x 5	45 655 217
BabyBio affimAb 5 ml	5 ml × 5	45 800 107
BabyBio Ni-NTA 5 ml	5 ml × 5	45 655 107
BabyBio NiMAC	5 ml x 5	45 655 317
Bulk resins		
WorkBeads 40S	25 ml	40 200 001
	200 ml	40 200 002
WorkBeads 40Q	25 ml	40 100 001
	200 ml	40 100 002
WorkBeads 40 DEAE	25 ml	40 150 001
	200 ml	40 150 002
WorkBeads affimAb	25 ml	40 800 001
	200 ml	40 800 002
WorkBeads 40 Ni-NTA	25 ml	40 651 001
	150 ml	40 651 003
WorkBeads NiMAC	25 ml	40 653 001
	150 ml	40 653 003

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

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

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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