WorkBeads 100S
WorkBeads 100Q

The WorkBeads™ 100S and WorkBeads 100Q resins for ion exchange chromatography are designed for industrial purification that requires high flow rate and low backpressure. The products are intended for use in the purification of proteins, peptides and oligonucleotides by utilizing the difference in surface charge. WorkBeads 100S is a strong cation exchanger with sulfonate ligands. WorkBeads 100Q is a strong anion exchanger with quaternary amine ligands.

- High throughput and scalability
- Reliable and reproducible results
- High chemical stability for easy cleaning-in-place

Short protocol

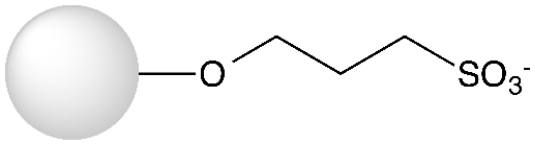
This protocol describes column packing and protein purification using WorkBeads 100S and WorkBeads 100Q. Detailed instructions and recommendations for optimisation are given later in this document. Recommended buffers are listed in Table 1. The WorkBeads 100S resin is in general suitable for basic proteins, i.e., with high isoelectric point (pI), that tend to have positive surface net charge in a broad range of buffer pH. WorkBeads 100Q is suitable for acidic proteins, i.e., with low pI, that tend to have negative net charge. However, it is often useful to combine these resins in two separate steps in a purification process, since the difference in selectivity can be dramatic. Oligonucleotides can be purified on WorkBeads 40Q.

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. Equilibrate the column with binding buffer.
6. Apply sample.
7. After sample application, remove unbound material by washing with, e.g., 10 - 20 CV (column volumes) washing buffer.
8. Elute the target protein with elution buffer.
9. Wash the column with deionized water.
10. Equilibrate the column with 20% ethanol for storage.

Principle

Ion exchange chromatography (IEX) can be used for the purification of biomolecules, such as proteins, peptides and oligonucleotides, by utilizing the difference in their surface charge. The biomolecules interact with the immobilized ion exchange groups on the chromatography resin with opposite charge. WorkBeads 100S is a strong cation exchanger and will bind positively charged substances. WorkBeads 100Q is a strong anion exchanger and will bind negatively charged substances. The strength of the binding will depend on the number of charges involved in the interaction, and the distribution of the charges on the surface of the biomolecule. Charges on the biomolecule that is same as on the resin may reduce the interaction by repulsion. The structures of the ligands in WorkBeads 100S and WorkBeads 100Q are shown in Figure 1.

(A)



(B)

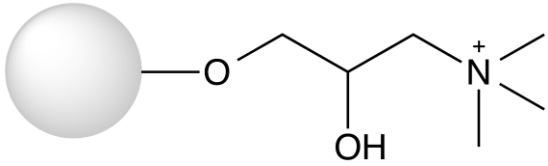


Figure 1. Structure of the ligand used in WorkBeads 100S (A) and WorkBeads 100Q (B).

The charges available on the surface of a protein depend on the pH of its environment. The isoelectric point (pI) of a protein is defined as the pH value where the protein net charge is zero. At pH values below the pI the net charge will be positive, and at pH values greater than the pI the net charge will be negative. It should be noted that the interaction of the protein with the resin depends on the presence and distribution of both positive and negative charged groups on the surface. A protein may therefore interact with an ion exchange resin also at its isoelectric point. The likelihood and strength of the binding to either the cation or the anion exchange resin will increase when moving the buffer pH away from the pI.

Ion exchange chromatography begins with equilibration of the column to establish the desired pH, and to charge the resin with suitable counter ions to the charged ligands on the resin. The negative sulfonate groups can interact with Na^+-ions, and the positive trimethyl amine groups (quaternary amine) can interact with Cl^--ions. It is common to use an equilibration buffer composed of a buffer substance to control the pH, and NaCl to include suitable counter ions. Other neutral salts (other counter ions) can be used to modulate the separation. Avoid using buffer substances that have a charge opposite to the charge of the resin to avoid uncontrollable pH effects that may destroy the separation. During the sample application, proteins with suitable charge will bind to the charged groups of the resin in the process displacing the counter ions. Desorption of the proteins (elution) is done by increasing the concentration of counter ion (salt gradient elution). A high enough concentration of the counter ion will displace the proteins. Various additives, e.g., enzyme inhibitors, non-ionic detergents, urea and low concentrations of organic solvent, can be added to the sample or the buffers to improve protein stability or purification results. Usually it is important that they do not strongly interact with the charged groups on the resin or the protein and interfere with the protein binding to the resin. An example of use of additives is the presence of up to 30% acetonitrile or ethanol to improve resolution during peptide purification. Where the additive reduces unspecific interactions of the target peptide or impurities with the resin.

Ion exchange chromatography is one of the most frequently used chromatography techniques because of its versatility and ability to separate proteins even with small differences in charge and because the eluted protein is usually concentrated. It is also one of the more cost-effective chromatography techniques and is therefore excellent for scale-up.

Column packing

WorkBeads are cross-linked using a proprietary method that results in very rigid resins that tolerate pressures of several bars, and consequently can run at high flow rates. Follow both this general advice when packing a column and the column manufacturer's specific instructions. The column should have an adjustable top adaptor.

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 contribute to the pressure inside the column and may reduce the maximum flow that can be used. Wear eye protection.

1. Wash the resin

The resin is provided in 20% ethanol. To avoid undue backpressure when packing, wash the desired amount of resin with several column volumes of deionized water before packing.

2. Make a slurry

Add deionized water to the washed resin to obtain a 40 - 60% 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 or pump the slurry into the column

Pour the slurry slowly down the side of the column to avoid formation of air bubbles when packing a lab-scale column (preferably use a packing reservoir). Process-scale columns can be filled using a slurry pump or built-in hydraulics to suck slurry into the column by moving the top adaptor.

4. Pack the bed

Pack the resin with a downward flow higher than the intended operational flow. Make sure the packing flow rate does not exceed the maximum pressure of the column hardware or the resin. The operational flow should not be more than 70 - 75% of the packing flow rate. For example, in a 10x100 mm column, we recommend 2000 cm/h as packing flow rate in water.

5. Close the column

When the bed height is constant mark the bed height on the column. Stop the flow, and if used, remove the packing reservoir. 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. Insert the adjustable adapter on top of the packed bed. Apply a small axial compression of less than 2% of the final bed height by lowering the adapter into the packed bed.

6. Apply a flow

Apply a flow of 70 - 75% of the packing flow rate and 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

Strong ion exchangers, such as WorkBeads 100S and WorkBeads 100Q, can be used within a broad pH range. The limitations in pH will be set by the protein stability. It is often possible to use either an anion exchange column or a cation exchange column to purify the same target protein. This can be carried out by altering the pH of the buffers below or above the protein pI to change its overall charge.

Sample preparation

After cell disruption or extraction, clarify the sample by centrifugation at 10 000 - 20 000 × *g* for 15 - 30 minutes. It is generally also recommended to pass the sample through a 0.22-µm or 0.45-µm filter, e.g., a syringe filter, to avoid transferring any remaining contaminating particles onto the column. Large sample volumes may be clarified by filtration through depth filters or by tangential flow filtration, which may be cheaper and more efficient than investing in a large-scale centrifuge. Application of a sample that has not been properly clarified may reduce the performance and lifetime of the column.

The sample should have a pH that confers a net charge to the target protein that is opposite to the charge of the column resin. The ionic strength should be low. The optimal binding conditions depends on the combination of the pH and the ionic strength of the sample. The sample solution may therefore need to be adjusted before applied to the column. The aim is to capture the target protein, and to avoid binding impurities. It is generally recommended that the sample should have a similar pH and conductivity as the binding buffer. Sample adjustments can be done by dilution using the binding buffer, by chromatographic desalting or diafiltration, or through adjusting the pH by addition of an acid or a base.

Binding

Choose a suitable pH value and buffer composition for the binding of the target protein. One pH unit below pI, for WorkBeads 100S or above pI, for WorkBeads 100Q is a good starting point. Usually, the binding conditions are optimized to achieve binding of the target protein, while minimizing the binding of impurities. When scouting for the best binding conditions it is important to start with sufficiently low ionic strength. Guideline for starting points for designing the experiment are given in Table 1.

Table 1. Typical buffer compositions for purification using WorkBeads 100S and WorkBeads 100Q.

Resin	Buffer composition
WorkBeads 100S	20 mM phosphate-buffer at pH 7 with a gradient elution from 0 to 500 mM NaCl over 20 column volumes (CV)
WorkBeads 100Q	20 mM Tris-buffer at pH 8 with a gradient elution from 0 to 500 mM NaCl over 20 CV

Elution

Elution can be carried out by applying a linear gradient of increasing concentration of NaCl, by gradually increasing the proportion of elution buffer (high salt). See Table 2 for buffer examples. A short step gradient to 1 or 2 M NaCl for 5 column volumes (CV) can be included after elution to desorb remaining substances from the column. When a suitable elution condition is known it is common to apply step gradient to elute the target protein. This will reduce process time, and is generally recommended for the Capture step when using high-flow large particle resins. A Cleaning-in-place (CIP) step using 5 CV 1 M NaOH is recommended between all runs, and must be followed by a careful re-equilibration before the next run.

Optimization

The following paragraphs describe briefly how key parameters can be tuned to get the optimal conditions for purification of proteins, peptides and oligonucleotides using WorkBeads 100S and WorkBeads 100Q columns.

Selection of buffer

Selecting a buffer with optimal binding and elution conditions for the target protein will improve the result of the purification. However, chromatographic conditions should be chosen so that the protein is stable under the conditions used for the purification. The buffering substance should be chosen to allow a good buffering capacity. This is obtained by selecting a buffering substance with a pK_a-value within 0.5 units from the intended pH value, and with high enough concentration. Table 2 shows one example of buffers which can be used for ion exchange chromatography. However, the choice of buffer composition depends on the target molecule and aim of the purification procedure. The buffers given in the table can be recommended for bioprocess purification, other buffers may be too expensive and/or be associated with problems of disposal. Other buffers mainly useful for lab-scale separations can be found in reference: Methods in Enzymology, Volume 463, pp 46-47, Burgess, R.R and Deutcher M.P..

Table 2. Example of buffers for purification using WorkBeads 100S and WorkBeads 100Q. Other buffers can be used. Buffer concentrations between 20-50 mM is common. For elution also other neutral salts can be used, as well as elution by pH change.

Product	Binding buffer	Elution buffer
WorkBeads 100S	50 mM Na-phosphate, pH 7.0	50 mM Na-phosphate, 1 M NaCl, pH 7.0
	50 mM HEPES, pH 7.4	50 mM HEPES, 1 M NaCl, pH 7.4
	50 mM Na-acteate, pH 5.0	50 mM Na-acteate, 1 M NaCl, pH 5.0
WorkBeads 100Q	50 mM Tris-HCl, pH 7.4	50 mM Tris-HCl, 1 M NaCl, pH 7.4
	50 mM Tris-HCl, pH 8.0	50 mM Tris-HCl, 1 M NaCl, pH 8.0
	50 mM Na-carbonate, pH 9.2	50 mM Na-carbonate, 1 M NaCl, pH 9.2

The buffer substance should be selected to have the same charge as the resin. A buffer with opposite charge will interact with the charged groups in the resin and may cause local pH disturbances that destroy the separation. However, sometimes it is not possible to follow this recommendation, and, e.g., phosphate buffer is used with good results in anion exchange chromatography following proper testing. Usually, low conductivity in the binding buffer is preferred, but optimization of the combination of pH and conductivity can improve binding capacity. An increase in ionic strength may decrease the ability of impurities to bind while the target protein remains bound.

Optimization of binding conditions

The key conditions to be optimized is usually pH and conductivity (by addition of NaCl or other salts, or by dilution). Conditions are usually selected to achieve binding of the target while avoiding the binding of impurities to maximize purity and yield of the target protein. The conditions must also be selected to keep the protein in its native state. Note that IEX can also be run in a “non-binding” mode, i.e., the impurities are bound to the resin and the target protein is found in the flow-through fraction.

The flow rate during sample loading may affect the binding capacity. A low flow rate during sample application promotes binding capacity since more time is allowed for mass transport of the target substance into the pores of the resin. A small substance, e.g., a peptide, that has a high diffusion rate will have fast mass transport into the resin and can thus be adsorbed efficiently also at high flow rates. A large target substance (e.g., a large protein) has a lower diffusion rate and is more hindered by the walls in the pores giving it slow mass transport. A high binding capacity of this substance may require a reduced flow rate. If only a part of the binding capacity of the column is used the sample application can be done at a higher flow rate without loss of the target substance.

The residence time can be defined as the time between entering and exiting the column of specific part of the sample or buffer. The residence time depends on the flow rate and the dimensions of the column, and is typically 1 to 5 minutes in IEX. Typical linear flow rates are 150 - 300 cm/h. See further discussion about flow in the section *Scale-up*.

Optimization of washing

A continuously decreasing UV signal is an indication of unbound material still being washed out. The washing should continue until the UV signal is stable and the same as in the washing buffer, or at least not more than 20 mAU. The washing buffer can be the same as the binding buffer, but it may be useful to add an additional step with a dedicated washing buffer to improve purification. Note that too stringent washing conditions may leach out or elute the target protein.

Optimization of elution conditions

Elution can be carried out using a high salt concentration or by altering the pH to change the charge of the adsorbed protein. A stronger binding may require higher salt concentration for elution. The optimal salt concentration is dependent on the purity and recovery requirements as well as the properties of the target protein and the sample. Applying gradient elution gives better purity than step elution, but step elution may be desired to obtain the highest possible concentration of the target protein and is also preferred when working in process scale. In order to optimize the salt concentration for step elution an initial gradient test run can be carried out to obtain suitable step elution conditions for purification of the sample, see Figure 2.

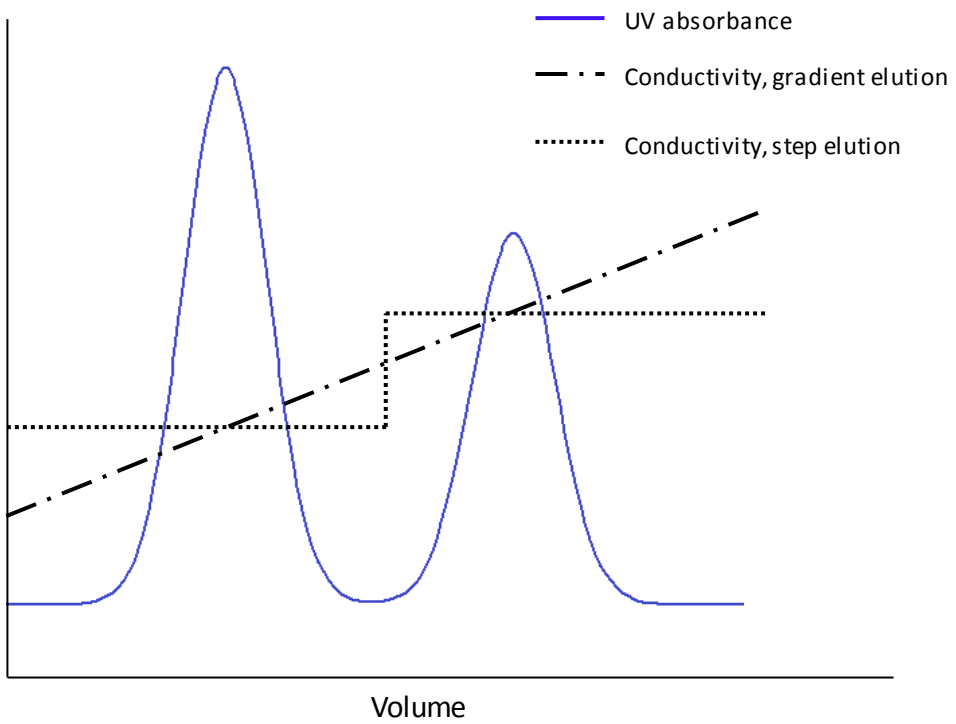


Figure 2. Optimization of step elution with salt. A test run with linear gradient elution gives information about suitable salt concentrations to be used in step elution. **Note:** Remember to take the system dead volume into account when comparing the print out of the gradient and the trace.

Scale-up

After developing a chromatographic procedure in a small-scale column, e.g., 10 (i.d.) × 100 mm (bed height), WorkBeads resins can be packed into larger columns for scale-up. Large-scale purification is often carried out in columns with bed heights of 200 - 300 mm.

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 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 are:

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

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

In the initial process development work, it is common to use a small column, e.g., 10x100 mm, to save sample, buffers and time while optimization the running conditions. This column often has a shorter bed height than the final column which may have a bed height of 200 mm or more. 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). Because of the low backpressure of WorkBeads 100 resins it is possible to run at higher linear flow rates than for smaller beads. This means that the column bed height can often be increase significantly without exceeding the pressure limit of the column, for example from 200-mm height to 400-mm height. This allows applying large sample volumes in shorter time, and make washing more efficient.

Table 3. Example of scale-up parameters

Column dimension	Column volume (ml)	Residence time (minutes)	Linear flow rate (cm/h)	Volumetric flow rate (ml/min)
25x200	98	4	300	24
50x200	390	4	300	98
250x200	9800	4	300	2450
450x200	31800	4	300	7950
25x400	195	4	600	49
50x400	785	4	600	195
250x400	19600	4	600	4900
450x400	63600	4	600	15900

Additional purification steps

Optimisation of the purification process by tuning the binding, washing and/or elution conditions of the IEX purification step may not be enough to obtain the required purity. Combining two or more purification steps based on additional chromatography techniques is then recommended. For example, cation exchange chromatography and anion exchange chromatography can be combined in a purification process. Other techniques, such as size exclusion chromatography (gel filtration) and hydrophobic interaction chromatography (HIC) are commonly used alternatives. Each purification step should be thoroughly optimized, and preferably in the context of the other steps applied on the overall process.

Desalting and buffer exchange

Buffer exchange or desalting of a sample can be used before analysis and/or after purification by ion exchange chromatography. This can be carried out quickly and easily in lab-scale using BabyBio Dsalt 1 or 5 ml columns (see *Related products*). BabyBio Dsalt columns are also a useful alternative to dialysis for larger sample volumes or when samples need to be processed rapidly to avoid degradation. For large processes diafiltration is recommended.

To find out more about Bio-Works chromatography resins for additional purification, please visit www.bio-works.com

Maintenance

Unpacking and inspection

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

Cleaning and sanitization

During purification impurities such as cell debris, lipids, nucleic acids and protein precipitates from the samples may gradually build-up in the resin causing fouling. The severity of this process depends on the type of sample applied to the column, and the pre-treatment of the sample. The impurities covering the resin may reduce the performance of the column over time. Regular cleaning (Cleaning-in-place, CIP) keeps the resin clean, reduces the rate of further fouling, and prolongs the capacity, resolution and flow properties of the column. Cleaning of a column using 1 M NaOH applied by a low reversed flow for 2 hours or overnight is often sufficient.

Sanitisation (reduction of microorganisms) can be carried out using combinations of NaOH and ethanol, e.g., incubation with a mixture of 0.5 M NaOH and 40% ethanol for 3 hours. The sanitization procedure and its effectiveness will depend on the microorganisms to be removed, and needs to be evaluated for each case.

Storage

Store the resins at 2 to 25°C in 20% ethanol (WorkBeads 100Q) and in 20% ethanol containing 0.2 M sodium acetate (WorkBeads 100S).

Additional information

Product description

	WorkBeads 100S	WorkBeads 100Q
Target substances	Proteins and peptides	Proteins, peptides and oligonucleotides
Matrix	Rigid, highly cross-linked agarose	Rigid, highly cross-linked agarose
Average particle size[1] (D_{v50})	90 - 110 µm	90 - 110 µm
Ionic group (ligand)	Sulfonate ($-SO_3^-$)	Quaternary amine ($-N^+(CH_3)_3$)
Ionic capacity	180 - 250 µmol H^+/ml resin	140 - 200 µmol Cl^-/ml resin
Dynamic binding capacity (DBC)	>100 mg BSA/ml resin[2]	>40 mg BSA/ml resin[3]
Pressure flow characteristic	2 bar at 900 cm/h, 25 mm diameter column, 20 cm bed height	
Chemical stability	Compatible with all standard aqueous buffers used for protein purification, 1 M NaOH, 30% isopropanol or 70% ethanol. Should not be stored at < pH 3 for prolonged time	
pH stability	2 - 13	2 - 13
Storage	2 to 25°C in 20% ethanol containing 0.2 M sodium acetate	2 to 25°C in 20% ethanol

1. The median particle size of the cumulative volume distribution.
2. Dynamic binding capacity determined at 4-minutes residence time in the presence of 20 mM Na-citrate, pH 4.0.
3. Dynamic binding capacity determined at 4-minutes residence time in the presence of 50 mM Tris-HCl, 50 mM NaCl, pH 8.0.

Intended use

WorkBeads 100S and WorkBeads 100Q resins are intended for research, process development and industrial applications only.

Safety

Please read the Safety Data Sheets (SDS) for WorkBeads 100S and WorkBeads 100Q resins, and the safety instructions for any equipment to be used.

Related product

Product name	Pack size[1]	Article number
Prepacked columns		
BabyBio™ S 1 ml	1 ml x 5	45 200 103
BabyBio Q 1 ml	1 ml x 5	45 100 103
BabyBio Dsalt 5 ml	5 ml x 5	45 360 107
OptioBio™ 40S 10x100	7.9 ml x 1	55 420 011
OptioBio 40Q 10x100	7.9 ml x 1	55 410 011
Bulk resins		
WorkBeads 40S	25 ml	40 200 001
WorkBeads 40Q	25 ml	40 100 001

[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 100S	25 ml	10 200 001
	200 ml	10 200 002
	500 ml	10 200 005
	1 L	10 200 010
	5 L	10 200 050
	10 L	10 200 060
WorkBeads 100Q	25 ml	10 210 001
	200 ml	10 210 002
	500 ml	10 210 005
	1 L	10 210 010
	5 L	10 210 050
	10 L	10 210 060

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

Bio-Works
Virdings allé 18
754 50 Uppsala
Sweden