WorkBeads 40S
WorkBeads 40Q
WorkBeads 40 DEAE

WorkBeads™ 40S, WorkBeads 40Q and WorkBeads 40 DEAE resins for ion exchange chromatography are designed for research and industrial scale purification of proteins, peptides and oligonucleotides by utilizing the difference in their surface charge. WorkBeads 40S resin is a strong cation exchanger with sulfonate ligands. WorkBeads 40Q resin is a strong anion exchanger with quaternary amine ligands. WorkBeads 40 DEAE is a weak anion exchanger with tertiary amine ligands. The property of high-resolution separation while giving low backpressure allow both capture and polishing purification applications in standard bioprocess columns.

- High throughput, binding capacity and purity
- Reliable and reproducible results
- High chemical stability for easy cleaning-in-place

Short protocol

This short protocol is for column packing of WorkBeads 40S, WorkBeads 40Q and WorkBeads 40 DEAE and protein purification using ion exchange chromatography. Detailed instructions and recommendations for optimization are given later in this document. Recommended buffers are listed in Table 1. WorkBeads 40S resin is suitable for basic proteins (i.e., protein with high isoelectric point (pI)) and WorkBeads 40Q and WorkBeads 40 DEAE resins are suitable for purification of acidic proteins (i.e., proteins with low pI).

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., 20 - 30 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. For WorkBeads 40S it is recommended to also add 0.2 M sodium acetate in the storage solution.

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 40S is a strong cation exchanger and will bind positively charged substances. WorkBeads 40Q is a strong anion exchanger and will bind negatively charged substances. WorkBeads 40 DEAE is a weak 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.
WorkBeads 40S and WorkBeads 40Q which are strong ion exchangers can be used over a broad pH range of 3 - 13. WorkBeads 40 DEAE which is a weak ion exchanger can be used in the pH range of 3 - 9. The density of positive charges in WorkBeads 40 DEAE will decrease gradually when the pH is increased above pH 6. This change may change the selectivity of the resin. Optimizing the pH may thus allow improved purity. Notice that reducing the density of charged groups may decrease the binding capacity of the target substance.

We recommend using WorkBeads 40Q as the first choice of anion exchanger, and to use WorkBeads 40 DEAE as an alternative when there is a need to modify the selectivity. The structures of the ligands in WorkBeads 40S, WorkBeads 40Q and WorkBeads 40 DEAE are shown in Figure 1.

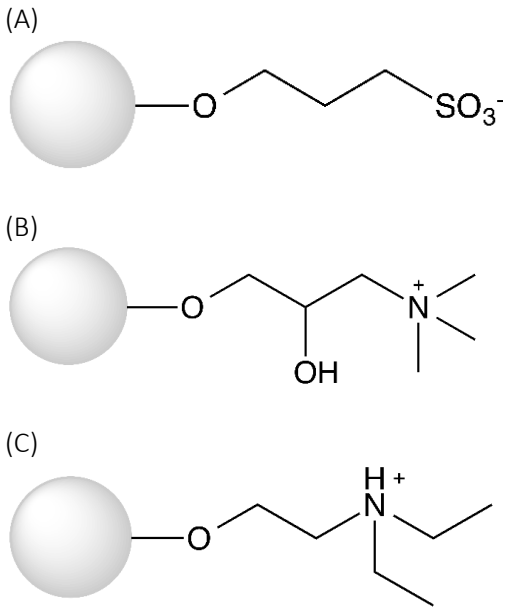

Figure 1. Structure of the ligand used in WorkBeads 40S (A), WorkBeads 40Q (B) and WorkBeads 40 DEAE (C).

The charges available on the surface of a protein depend on the pH of its environment. When the pH is equal to the isoelectric point (pI) of the protein the net charge is zero. At pH values below the pI the net charge will be positive, and at a pH greater than the pI the net charge will be negative. It should be noted that the interaction of the protein 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 of binding to either the cation or the anion exchange resin will increase the further the pH moves away from the pI.

Ion exchange chromatography begins with equilibration of the column in order to establish the correct 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. Avoid using buffer substances that have a charge opposite the charge of the resin to avoid uncontrollable negative effects on the separation. On applying the sample 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 usefully be used in samples and buffers for IEX. 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. As an example, purification of peptides can be done in the presence of up to 30 % acetonitrile or ethanol if required to keep the peptide soluble and to facilitate a good separation.

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 resins are cross-linked using a proprietary method that results in a very rigid resin 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. Preferably, use a column with an adjustable adaptor. 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 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% to 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 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 to extend the column volume to accommodate the entire slurry volume during packing. If no packing adaptor is available packing can be done by stepwise additions and packing. Although not recommended this will give acceptable results for most applications.

4. Pack the bed

Pack the resin with a downward flow higher than the intended operational flow. We recommend 600 cm/h for columns up to 25 mm i.d. and with 200 mm bed height. 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 75% of the packing flow rate.

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. 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 450 cm/h (taking account of section 4) 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 S and WorkBeads Q, can be used with a broad pH range.
A weak Ion exchanger such as WorkBeads 40 DEAE will gradually have lower charge density when the pH is increasing. This allows modulation of its selectivity. When decreasing the charge density the binding capacity may decrease. Because of the mentioned reason, WorkBeads 40 DEAE can be used in a pH range of 3 - 9.
The limitations in pH that can be used with an ion exchanger will also depend on 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 moving the pH of the buffers below or above the protein pI to change its overall charge.
Choose a suitable pH and buffer for the binding of the target protein. One pH unit below pI, for WorkBeads 40S or above pI, for WorkBeads 40Q is a good starting point. The binding conditions should be 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 40S, WorkBeads 40Q and WorkBeads 40 DEAE.

Resin	Buffer composition
WorkBeads 40S	20 mM phosphate-buffer at pH 7 with a gradient elution from 0 to 500 mM NaCl over 20 column volumes (CV)
WorkBeads 40Q	20 mM Tris-buffer at pH 8 with a gradient elution from 0 to 500 mM NaCl over 20 CV
WorkBeads 40 DEAE	20 mM Tris-buffer at pH 8 with a gradient elution from 0 to 500 mM NaCl over 20 CV

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 ensure desorption of all interacting proteins. When suitable elution conditions are known it is common to apply step gradient elution. This will reduce process time and is generally recommended for Capture step purification using high-flow large particle resins. A Cleaning-in-place (CIP) step using 5 CV 1 M NaOH is recommended between all runs and should be followed by a careful re-equilibration before the next run.
Some resins become yellowish during CIP with NaOH (0.5 M or 1 M), and they will become white again when washing with deionized water (recommended 3-5 column volumes) and after this apply the preferred equilibration buffer. From the product stability point of view our thorough work shows that for example WorkBeads 40S can tolerant to up to 1 M NaOH (one week at room temperature) without significantly decrease in ionic capacity and dynamic binding capacity or any significant change of pattern of selectivity. We conclude that this phenomenon is intrinsic to agarose products to a lesser or larger degree and will not change the functionality of the resin based on our measurements and analysis.

One explanation for the resin sometimes becoming yellowish during CIP with 0.5-1 M NaOH is that during the CIP we get a dehydration of the resin reducing the water content which makes the resin more compact resulting in a darker (yellowish) color of the resin.

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 - 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. The sample solution may therefore need to be adjusted before applied to the column. 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 base.

Optimization

The following paragraphs will give indications on some parameters that can be tuned to get the optimal conditions for purification of proteins, peptides and oligonucleotides using WorkBeads 40S WorkBeads 40Q and WorkBeads 40 DEAE columns.

Selection of buffer

Selecting a buffer with optimal binding and elution conditions for the target protein will improve the result of the purification. The buffer should be chosen with a good buffering capacity and with a pK_a-value within 0.5 units from the intended pH. Table 2 shows one example of buffers which can be used for ion exchange chromatography, however, the buffer choice will be depending on the target molecule and aim of the purification procedure. For other useful buffers and their pK_a-values at 25 °C see reference: Methods in Enzymology, Volume 463, pp 46-47, Burgess, R.R and Deutcher, M.P.

Table 2. Examples of buffers for model protein purification using WorkBeads 40S, WorkBeads 40Q and WorkBeads 40 DEAE. Other buffers can be used.

Product	Buffer	Buffer composition
WorkBeads 40S	Binding buffer	50 mM sodium phosphate, pH 7.0
	Elution buffer	50 mM sodium phosphate, 1 M NaCl, pH 7.0
WorkBeads 40Q	Binding buffer	50 mM Tris-HCl, pH 8.0
	Elution buffer	50 mM Tris-HCl, 1 M NaCl, pH 8.0
WorkBeads 40 DEAE	Binding buffer	50 mM Tris-HCl, pH 7.4
	Elution buffer	50 mM Tris-HCl, 1 M NaCl, pH 7.4

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 destroys the separation. Usually, low conductivity in the binding buffer is preferred but optimization on pH and conductivity can improve binding capacity. An increase in ionic strength may decrease the ability of contaminants to bind

while the target protein remains bound. However, chromatographic conditions should be chosen so that the protein is stable during purification.

Optimization of binding conditions

The key conditions to be optimized is usually pH and conductivity (by addition of NaCl or other salts, or dilution). Conditions should be 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.

The flow rate during sample loading affects the binding capacity and resolution during the elution. 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 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 lowered flow rate. If only a part of the binding capacity of the column is used the sample application can be 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 depend 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.

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. 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.

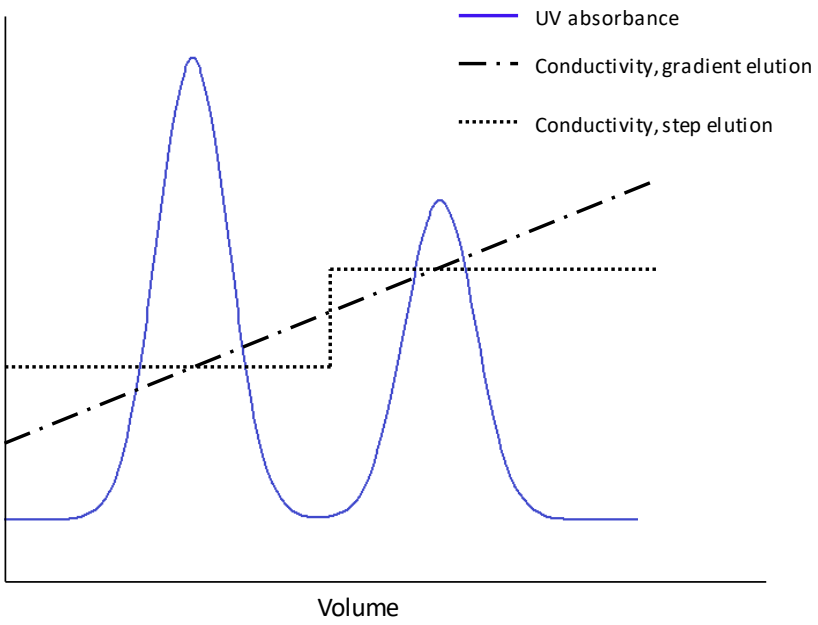

Figure 2. Optimization of step elution with salt. A test run with linear gradient elution gives information about suitable salt concentrations to be used in step elution. Note: Remember to take the system dead volume into account when comparing the gradient and the trace.

Scale-up

After developing a chromatographic procedure in 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 is 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:

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

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

In the initial process development work it is common to use a small column, e.g., 10 × 100 mm, to save sample, buffers and time. This column 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).

Table 3. Example of scale-up parameters

Column dimension	Residence time (minutes)	Linear flow rate (cm/h)	Volumetric flow rate (ml/min)
10 × 100	4	150	2
25 × 200	4	300	24
50 × 200	4	300	98

Additional purification steps

Optimization 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 step 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 ml 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 chromatography resins for additional purification visit www.bio-works.com

Maintenance

Unpacking and inspection

Unpack the shipment as soon as it arrives and inspect it for damage. Promptly 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 the build-up of contaminants in the resin, or 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.

Some resins become yellowish during CIP with NaOH (0.5 M or 1 M), and they will become white again when washing with deionized water (recommended 3-5 column volumes) and after this apply the preferred equilibration buffer.

From the product stability point of view our thorough work shows that for example WorkBeads 40S can tolerant to up to 1 M NaOH (one week at room temperature) without significantly decrease in ionic capacity and dynamic

binding capacity or any significant change of pattern of selectivity. We conclude that this phenomenon is intrinsic to agarose products to a lesser or larger degree and will not change the functionality of the resin based on our measurements and analysis.
One explanation for the resin sometimes becoming yellowish during CIP with 0.5-1 M NaOH is that during the CIP we get a dehydration of the resin reducing the water content which makes the resin more compact resulting in a darker (yellowish) color of the resin.

Sanitization (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 resin at 2 to 25°C in 20% ethanol.
For WorkBeads 40S it is recommended to include 0.2 M sodium acetate in the storage solution.

Additional information

Product description

	WorkBeads 40S	WorkBeads 40Q	WorkBeads 40 DEAE
Target substance	Proteins, peptides	Protein, peptides, viruses, oligonucleotides	Protein, peptides, oligonucleotides
Matrix	Rigid, highly cross-linked agarose	Rigid, highly cross-linked agarose	Rigid, highly cross-linked agarose
Average particle size[1] (D_{V50})	45 µm	45 µm	45 µm
Ionic group (ligand)	Sulfonate ($-SO_3^-$)	Quaternary amine ($-N^+(CH_3)_3$)	Diethylaminoethyl ($-CH_2CH_2N^+H(CH_2CH_3)_2$)
Ion capacity	180 - 250 µmol H^+/ml resin	180 - 250 µmol Cl^-/ml resin	110 - 160 µmol Cl^-/ml resin
Dynamic binding capacity	130 mg BSA/ml resin[2]	50 mg BSA/ml resin[3]	40 mg BSA/ml resin[3]
Max. flow rate[4] (20 cm bed height and 5 bar)	600 cm/h	600 cm/h	600 cm/h
Chemical stability	Compatible with all standard aqueous buffers used for protein purification, 1 M NaOH[5], 30% isopropanol and 70% ethanol. Should not be stored at low pH for prolonged time.		
pH stability	2 - 13	2 - 13	3 - 9 (recommended pH) 3 - 13
Storage	2 to 25 °C in 20% ethanol with 0.2 M sodium acetate	2 to 25 °C in 20% ethanol	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 20 mM sodium citrate, pH 4.0.
3. Dynamic binding capacity determined at 2.5-minutes residence time in 50 mM Tris-HCl, 50 mM NaCl, pH 8.0.
4. Optimal flow rate during binding is depending on the sample.
5. For more information, see pages 4 or 8.

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 40S and WorkBeads 40Q resins, and the safety instructions for any equipment to be used.

Related products

Product name	Pack size[1]	Article number
Prepacked columns		
BabyBio IEX Screening Kit[2]	1 ml x 4	45 900 001
BabyBio Peptide Purification Kit[3]	1 ml x 2	45 300 102
BabyBio S 1 ml	1 ml x 5	45 200 103
BabyBio S 5 ml	5 ml x 5	45 200 107
BabyBio Q 1 ml	1 ml x 5	45 100 103
BabyBio Q 5 ml	5 ml x 5	45 100 107
BabyBio DEAE 1 ml	1 ml x 5	45 150 103
BabyBio DEAE 5 ml	5 ml x 5	45 150 107
Baby Bio Dsalt 1 ml	1 ml x 5	45 360 103
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 100S	25 ml	10 200 001
	200 ml	10 200 002
WorkBeads 100Q	25 ml	10 100 001
	200 ml	10 100 002
WorkBeads Dsalt	300 ml	40 360 003

1. All different pack sizes are available on www.bio-works.com
2. BabyBio IEX Screening Kit includes one of each: BabyBio S 1 ml, BabyBio Q 1 ml, BabyBio DEAE 1 ml and BabyBio TREN 1 ml.
3. BabyBio Peptide Purification Kit is a bundle of: BabyBio S 1 ml x 1 and BabyBio Q 1 ml x 1.

Ordering information

Product name	Pack size	Article number
WorkBeads 40S	25 ml	40 200 001
	200 ml	40 200 002
	1 L	40 200 010
	5 L	40 200 050
	10 L	40 200 060
WorkBeads 40Q	25 ml	40 100 001
	200 ml	40 100 002
	1 L	40 100 010
	5 L	40 100 050
	10 L	40 100 060
WorkBeads 40 DEAE	25 ml	40 150 001
	200 ml	40 150 002
	1 L	40 150 010
	5 L	40 150 050
	10 L	40 150 060

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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