Bio-Works

INSTRUCTION

WorkBeads Protein A

WorkBeads™ Protein A resin is designed for purification of monoclonal and polyclonal antibodies using affinity chromatography. For small scale purification and initial screening in process development we recommend GoBio™ Mini A 1 mL and 5 mL columns prepacked with WorkBeads Protein A resin. WorkBeads Protein A resin can also be used for applications in other formats, such as test tube batch adsorption, spin columns, gravity columns or multi-well filter plates. The resin can be used for immunoprecipitation experiments.

- High dynamic binding capacity for monoclonal and polyclonal antibodies, with excellent recovery and purity
- Improved coupling chemistry results in high pH stability nd low protein A leakage
- Reliable, reproducible and efficient

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.

The GoBio prepacked column family has been developed for convenient, reproducible, and rapid results and can be used for small scale purification and all the way up to process development and full-scale manufacturing.

Safety

Please read the Safety Data Sheet (SDS) for WorkBeads Protein A 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 of WorkBeads Protein A and protein purification. Detailed instructions and recommendations for optimization are given later in this instruction. Recommended buffers are listed in Table 1.

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. Elute the target protein with elution buffer.
8. Wash the column with deionized water.
9. Equilibrate the column with 20% ethanol for storage.

Principle

Affinity chromatography is a useful technique for the separation of proteins by exploiting the reversible interaction between the target protein and the immobilized ligand. The interaction can be bio-specific, for example antibodies binding to protein A, or not bio-specific, for example histidine-tagged proteins binding to metal ions.

This chromatography technique provides high selectivity, resolution and capacity. High purity is often achieved in a single step. Large sample volumes can be applied under conditions that favour specific binding to the ligand. Elution is often carried out under gentle conditions which helps to preserve bioactivity. The target protein is eluted, in a purified and concentrated form, by modification of pH, ionic strength, or by introducing a competitive agent..

Column packing

WorkBeads resins are cross-linked using a proprietary method that results in a very rigid resin, which 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:

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

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 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 300 cm/h for columns up to 26 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 225 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.

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 on next page shows a UV trace for acetone in a typical test chromatogram from which the HETP and A_s values are calculated.

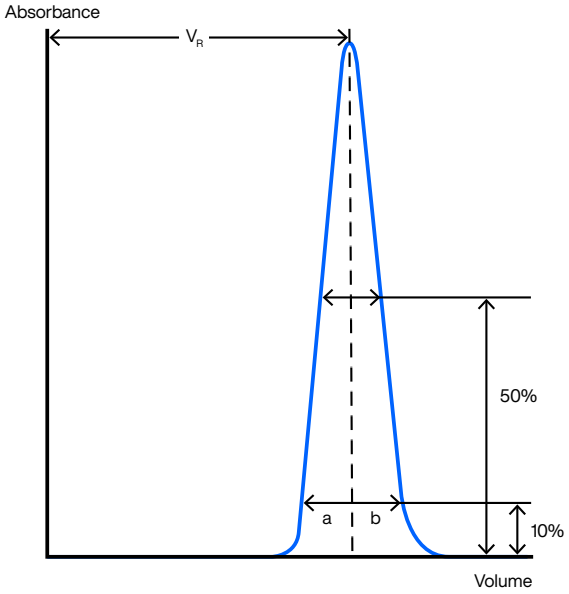

Fig 1. A typical test chromatogram showing the parameters used for HETP and A_s calculations.

Purification

The following brief instruction gives general conditions for purification using a column packed with WorkBeads Protein A. Recommended buffers are listed in Table 1 and recommendations for optimization are mentioned below.

Table 1. Recommended buffers for purification of antibodies.

Buffer	Composition
Binding buffer	20 mM sodium phosphate, 150 mM NaCl, pH 7.4 (PBS)
Elution buffer	100 mM sodium citrate, pH 3.0

Sample preparation

Clarify the sample by centrifugation at 10 000 – 20 000 × g for 15 – 30 minutes. It is recommended to also pass the sample through a 0.22 – 0.45 µm filter to remove any remaining particles. If the sample contains only small amounts of particles, it may be enough to only carry out filtration. Make sure that the sample has a pH between 5 and 8. Preferably, the sample should have the same pH and ionic strength as the binding buffer.

Standard purification

1. Equilibrate the column using 10 column volumes (CV) binding buffer.
2. Apply a clarified sample under neutral conditions.
3. Wash using 10 – 20 CV binding buffer.
4. Elute with 5 CV elution buffer. Include 100 µl 1 M Tris-HCl, pH 9 per 1 mL collected fraction, to prevent degradation of eluted target protein.
5. Re-equilibrate with 10 CV binding buffer.
6. Equilibrate with 10 CV 20% ethanol for storage.

Before starting a purification run, it is recommended to make a blank run (with no sample applied) to remove any loosely bound ligands or impurities on the resin. Do this also for a newly packed resin. Although the above standard conditions usually give excellent results it may be worthwhile to optimize the purification protocol for highest purity of the target protein, see "Optimization".

Regeneration of WorkBeads Protein A

After purification using WorkBeads Protein A perform the following steps:

1. Unless elution was carried out at very low pH there may be a need for regeneration by cleaning the column with, for example, 10 CV 100 mM glycine-HCl, pH 2.7 or 100 mM sodium citrate, pH 3.
2. Wash the column with 5 CV deionized water.
3. Cleaning-in-place by passing 15 CV 15 mM NaOH over 15 minutes, or for harsher conditions 50 mM NaOH.

   For increased efficiency, before the NaOH wash, include a passage of 15 CV 100 mM 1-thioglycerol, pH 8.5, over 15 minutes to reduce any oxidized aggregates adsorbed to the column.
4. Wash with 10 CV neutral buffer. Make sure that neutral pH is restored in the column. Prolonged exposure to extreme pH may harm the resin.
5. Wash with 10 CV deionized water.
6. Wash with 10 CV 20% ethanol before storage.

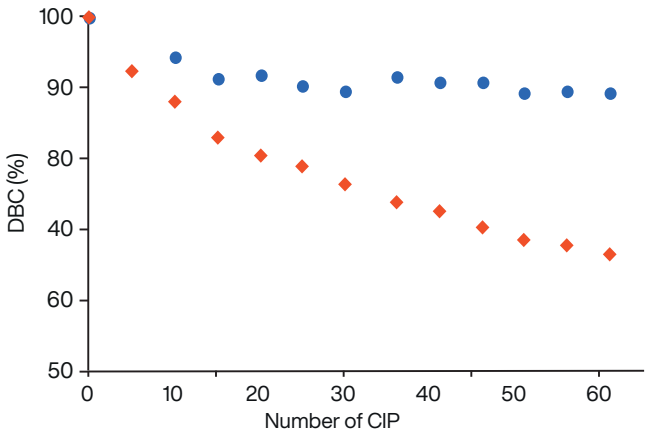

Fig 2. Alkaline stability of WorkBeads Protein A determined by frontal analysis using 1 mg/mL IgG in the presence of PBS, pH 7.4. CIP cycle: 100 mM 1-thioglycerol, pH 8.5, 15 minutes' incubation; followed by 15 mM NaOH (blue circles) or 100 mM NaOH (red diamonds), for 15 minutes.

Optimization

Selection of column size

The column size should be selected based on estimated amount of target protein in each run, and the dynamic binding capacity (DBC) of the resin. DBC is the capacity obtained under the chosen run conditions and is usually lower than the static binding capacity (total binding capacity). Figure 2 shows an example of the DBC at different flow rates (or residence times). At a low flow rate, the capacity is high. At increasing flow rate the binding capacity decreases.

To obtain the highest possible recovery of the target protein we recommend the application of no more sample than 80% of the capacity of the packed column at the selected flow rate. Consider using a larger column or dividing the sample into repeated purification runs if needed. If the amount of the antibody in the sample is not known, it can be determined by running a small sample on a GoBio Mini A 1 mL and determine the amount of eluted antibody. Alternatively, make the purification and collect the flow through material for subsequent analysis to determine whether the column was "over-saturated". If desired the collected flow through material can be reapplied on the packed column after proper regeneration, in a new purification run.

Binding capacity

Antibody binding capacity depends on the flow rate used for binding and may differ between different antibody species. For WorkBeads Protein A, the binding capacity is more than 40 mg human serum IgG/mL resin at 2.5 minutes residence time in a 6.6 × 100 mm column, which corresponds to a linear flow rate of 240 cm/min. The binding capacity decreases with increased flow rate, see Figure 3.

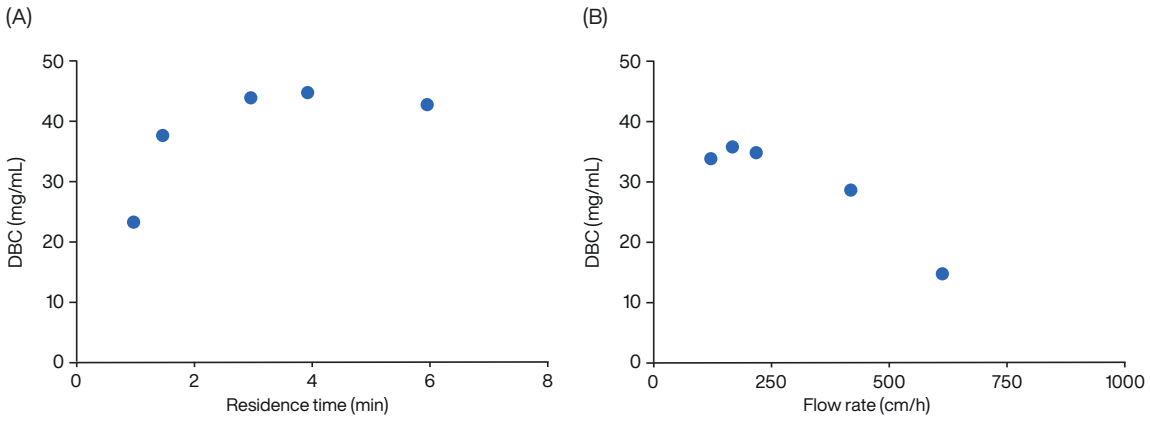

Fig 3. DBC at 10% (QB10%) for human serum IgG at different flow rates determined by frontal analysis. Sample: 1 mg/mL IgG in PBS, pH 7.4 in a 6.6 × 100 mm packed bed with WorkBeads Protein A. (A) DBC for WorkBeads Protein A versus residence time in minutes. (B) DBC for WorkBeads Protein A versus flow rate in cm/h.

Optimization of binding

Human IgG and IgG from several other species bind to WorkBeads Protein A under neutral pH at moderate salt concentrations. Apart from the recommended binding buffer in Table 1, other buffers can be used. For example, 50 mM sodium phosphate, pH 7.4 or50 mM sodium borate, pH 9. However, IgG with weaker affinity (e.g., mouse IgG1) may need a binding buffer with a combination of high pH and ionic strength to be able to bind. For example, 50 mM sodium borate, 4 M NaCl, pH 9.

Extra wash step

To remove weakly adsorbed impurities, it may be useful to add an extra washing step, with the binding buffer, after the standard wash. This can be done using a buffer with slightly increased ionic strength compared to the binding buffer or by a small decrease in pH that do not elute the target protein, see "Optimization of elution".

Optimization of elution

Apart from the recommended elution buffer in Table 1, other buffers can be used. For example, 100 mM glycine-HCl, pH 2.7. IgG can be sensitive to low pH. To avoid denaturation after elution with low pH, the pH can be neutralized by adding 100 µl of 1 M Tris-HCl, pH 9 per mL collected fraction to each fractionation tube before starting the purification or immediately after completed elution. Collect the target protein and perform buffer exchange using a GoBio Mini Dsalt column equilibrated with a neutral buffer, see “Related products”.

Perform gradient elution using a gradient from 100 mM Na-citrate, pH 6.0 to 100 mM sodium citrate, pH 3.0 over 10 – 20 CV. Desorption will occur when the pH is low enough, while avoiding too low pH. Run a test gradient elution (as above) with a small amount of sample to determine at what pH the target protein is eluting. The pH measured at the tail of the peak can be selected for elution. Prepare a 100 mM sodium citrate buffer with the selected elution pH and make the scale-up using this elution buffer.

Scale-up

After developing a chromatographic procedure in a small-scale column, e.g., 7 (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:

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

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 it is common to use a small column, e.g., 7 × 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 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 2 for examples. If the column bed heights are constant during scale-up the linear flow rate should be also constant (as well as the residence time).

Table 2. 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

Additional purification

Antibody purification on WorkBeads Protein A frequently gives high purity in a single step. For very high purity requirements, it may thought be necessary to add a second purification step. The additional purification step is used to remove traces of leaked protein A ligand, DNA, aggregates and other remaining impurities from the sample. In research-scale purification, size exclusion chromatography (gel filtration) is often a good polishing step since it removes impurities, the imidazole used for elution and potential aggregates of the target protein. Size exclusion chromatography can be done using e.g., prepacked GoBio Prep 16x600 40/100 SEC or GoBio Prep 26x600 40/100 SEC columns.

Ion exchange chromatography is suitable for both research scale purification and process scale. WorkBeads 40S, WorkBeads 40Q, WorkBeads 40 DEAE and WorkBeads 40 TREN resins provide different selectivities for ion exchange chromatography.

Desalting and buffer exchange

Buffer exchange or desalting of a sample can be used before analysis and/or after purification. This can be carried out quickly and easily in lab-scale using GoBio Mini Dsalt 1 mL, GoBio Mini Dsalt 5 mL, GoBio Prep 16x100 Dsalt (20 mL) and GoBio Prep 26x100 Dsalt (53 mL) prepacked columns depending on sample volumes. GoBio Prod prepacked columns starting from 1 L are available for larger sample volumes, see “Related products”.

These columns are very useful alternatives to dialysis for larger sample volumes or when samples need to be processed rapidly to avoid degradation.

Pre-swollen WorkBeads Dsalt is also available in bulk for packing column format of choice.

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

Maintenance of the resin

Cleaning using NaOH

During purification, cell-derived impurities such as cell debris, lipids, nucleic acids and protein precipitates, or synthesis-derived impurities such as failure sequences and counter ions from the samples may gradually build-up in the resin and cause 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 coating the resin may reduce the performance of the column over time. Regular cleaning (cleaning-in-place, CIP) reduces the rate of further fouling and prolongs the capacity, resolution and flow properties of the column. Cleaning using 10 mM NaOH applied at a low flow for 15 – 30 min will improve the resin’s performance. Do not use elevated temperature during the CIP treatment, since this may reduce the lifetime of the resin.

Storage

Store at 2 to 8°C in 20% ethanol.

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

Product description

	WorkBeads Protein A
Target substance	Antibodies (IgG), bound via the F_c-region
Matrix	Rigid, highly cross-linked agarose
Average particle size[1] (D_{v50})	45 µm
Ligand	Recombinant protein A expressed in *E. coli* using animal-free medium
Dynamic Binding Capacity[2] (DBC)	> 40 mg human IgG/mL resin
Maximum recommended flow rate[3]	300 cm/h
Chemical stability	Compatible with all standard aqueous buffers used for protein purification, 10 mM HCl (pH 2), 10 mM NaOH (pH 12), 0.1 M sodium citrate-HCl (pH 3), 6 M guanidine-HCl, 20% ethanol Should not be stored at low pH for prolonged time.
pH stability	3 – 10 (short term) 2 – 12 (cleaning)
Storage	2 to 8 °C in 20 % ethanol

1 The median particle size of the cumulative volume distribution.
2 DBC was determined at 10% breakthrough ($QB_{10\%}$) by frontal analysis with 1 mg/mL human polyclonal IgG in PBS, pH 7.4 at 1.4 mL/min (240 cm/h) in a column packed with WorkBeads Protein A resin, column bed 6.6 × 100 mm and 2.5 minutes residence time.
3 Maximum recommended flow rate at 20 °C using aqueous buffers. 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 when operating at 4 °C), or by additives (e.g., 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 A 1 mL	1 mL × 1 1 mL × 5 1 mL × 10	45 605 101 45 605 103 45 605 104
GoBio Mini A 5 mL	5 mL × 1 5 mL × 5 5 mL × 10	45 605 105 45 605 107 45 605 108
GoBio Mini affimAb 1 mL	1 mL × 5	45 800 103
GoBio Mini affimAb 5 mL	5 mL × 5	45 800 107
GoBio Mini Dsalt 1 mL	1 mL × 5	45 360 003
GoBio Mini Dsalt 5 mL	5 mL × 5	45 360 107
GoBio Prep 16x100 Dsalt[2]	20 mLx 1	55 700 021
GoBio Prep 26x100 Dsalt	53 mLx 1	55 700 031
GoBio Prep 16x600 40/100 SEC	120 mL × 1	55 434 026
GoBio Prep 16x600 40/100 SEC	320 mL × 1	55 434 036
GoBio Prep 16x600 40/1000 SEC	120 mL × 1	55 430 026
GoBio Prep 16x600 40/1000 SEC	320 mL × 1	55 430 036
GoBio Prep 16x600 40/10 000 SEC[2]	120 mL × 1	55 435 026
GoBio Prep 16x600 40/10 000 SEC[2]	320 mL × 1	55 435 036

[1] All different pack sizes are available on www.bio-works.com
[2] Packed on request.

Ordering information

Product name	Pack size	Article number
WorkBeads Protein A	10 mL 100 mL 1 L	40 605 003 40 605 004 40 605 005

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

bio-works.com

Bio-Works, Virdings allé 18, 754 50 Uppsala, Sweden. For local office contact information, visit bio-works.com/contact.

IN 40 605 010 BA

Bio-Works