

## INSTRUCTION

# GoBio Prep 16x100 affimAb GoBio Prep 26x100 affimAb

GoBio™ Prep 16x100 affimAb and GoBio Prep 26x100 affimAb are prepacked columns with WorkBeads™ affimAb, resin for fast and easy purifications of monoclonal and polyclonal antibodies. This resin has a superior base matrix in combination with an optimized alkaline-stable protein A ligand. This results in high dynamic binding capacity also at short residence times, and stable capacity over multiple purification cycles with cleaning-in-place with 0.5 M NaOH.

- Prepacked, ready-to-use columns for fast and easy purification of mAbs
- Reliable and reproducible results
- Easy scale-up



## Intended use

WorkBeads resins are developed and supported for both research and production-scale chromatography. WorkBeads resins are produced according to ISO 9001:2015, and Regulatory Support Files (RSF) are available to assist the process validation and submissions to regulatory authorities.

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 Sheets (SDS) for WorkBeads affimAb 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](mailto:complaints@bio-works.com)

## Principle

### Affinity chromatography

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. Feeds with low target expression can be purified using WorkBeads affimAb, provided that the loading exceeds 50% of the resin's dynamic binding capacity (DBC) to ensure optimal recovery. Elution is performed at a lower pH, yielding a purified and concentrated antibody. The pH should then be neutralized to avoid affecting the antibody's bioactivity.

## Product description

### GoBio Prep column characteristics

Make sure when using GoBio Prep columns that the connectors are tightened to prevent leakage. The pressure over the packed bed varies depending on parameters such as the resin characteristics, sample/buffer viscosities and the tubing used. Make sure that the flow through the column is in the direction of the arrow on the column.

These columns should not be opened and refilled.

**Note!** GoBio Prep column hardware is compatible with most aqueous chemicals, but NOT with concentrated alcohol. Maximal alcohol concentration is 20%.

**Table 1.** GoBio Prep 16x100 and GoBio Prep 26x100 columns characteristics.

Column characteristics	
Column hardware	Acrylic
Top and bottom plugs	Polypropylene
Top and bottom filters	Polyamide
Connections	1/16" female thread in both ends
Column volumes	20 mL (GoBio Prep 16x100) 53 mL (GoBio Prep 26x100)
Column dimensions	16 × 100 mm (GoBio Prep 16x100) 26 × 100 mm (GoBio Prep 26x100)
Maximal column hardware pressure	5 bar, 0.5 MPa, 70 psi

## Resins characteristics

WorkBeads affimAb resin is an alkali-stable resin prepacked in GoBio Prep 16x100 affimAb and GoBio Prep 26x100 affimAb. The alkali-stable recombinant protein A attached to the optimized base matrix is produced in *E. coli* under conditions free of components of animal origin and purified to high purity before coupling. This combination gives both high dynamic binding capacities for antibodies and the possibility for efficient cleaning-in-place with 0.5 M NaOH.

The specificity of the recombinant protein A for the Fc region of IgG provides excellent purification. Each batch of protein A is tested according to stringent requirements. The high capacity, chemical stability and the optimized agarose matrix make WorkBeads affimAb ideal for purification of monoclonal antibodies (mAb) as well as polyclonal antibodies.

The characteristics of GoBio Prep 16x100 affimAb and GoBio Prep 26x100 affimAb are listed in section "Product Description".

## Unpacking and connecting GoBio Prep 16x100 and GoBio Prep 26x100 columns to a chromatography system

Each packed column is sealed with a pressure syringe on the **bottom** of the column. It is then placed in a sealed plastic bag.

1. Cut the plastic bag and remove the column with care.
2. Follow the flow direction (indicated by an arrow on the column label) to clamp the column onto the chromatography system or to a vertical stand.
3. Prepare the chromatography system for connecting the column. The GoBio Prep 16x100 and GoBio Prep 26x100 columns are compatible with 1/16" male connectors with narrow heads. The length of the connector thread must be at least 7 mm to avoid leakage.

**Note!** It is recommended to use the two red connectors attached to the transport syringe when connecting the column to a chromatography system. One red connector should be used in each end of the column.

4. Gently unhook the springs from the shaft top of the transport syringe using even force.
5. Remove the syringe and keep it for further use during storage.
6. Unscrew the top plug, some liquid may come out. Connect the column to the chromatography system using one of the red connectors "drop-to-drop" avoiding introducing air into the packed column.
7. Connect the bottom of the column to the chromatography system using the second red connector.

## 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 – see Table 2.

**Table 2.** Recommended buffers for purification. Other buffers can possibly be used.

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

## Purification

**Note!** Do not exceed the maximum recommended flow rate and back pressure for the column, see "Product description".

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. 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."

1. Wash out the storage solution with 1-2 column volumes (CV) deionized/distilled water if there is a risk that the binding buffer salts may precipitate upon exposure to ethanol. Use a reduced flow rate, 50% of the maximum flow rate when washing out the storage solution. This step can be omitted if precipitation is not likely to be a problem.
2. Equilibrate with 3-5 CV binding buffer.
3. Apply a clarified sample under neutral conditions.
4. Wash using 5-10 CV binding buffer.

5. Elute with 3-5 CV elution buffer. Include 100  $\mu$ L 1 M Tris-HCl, pH 9 per 1 mL collected fraction, to prevent degradation of eluted target protein.
6. Re-equilibrate with 5-10CV binding buffer.
7. Equilibrate with 5 CV 20% ethanol for storage.
8. Make sure that the stop plugs are tight to prevent leakage.  
For prolonged storage, connect the included transport syringe filled with storage solution to the bottom end of the column.

## Optimization

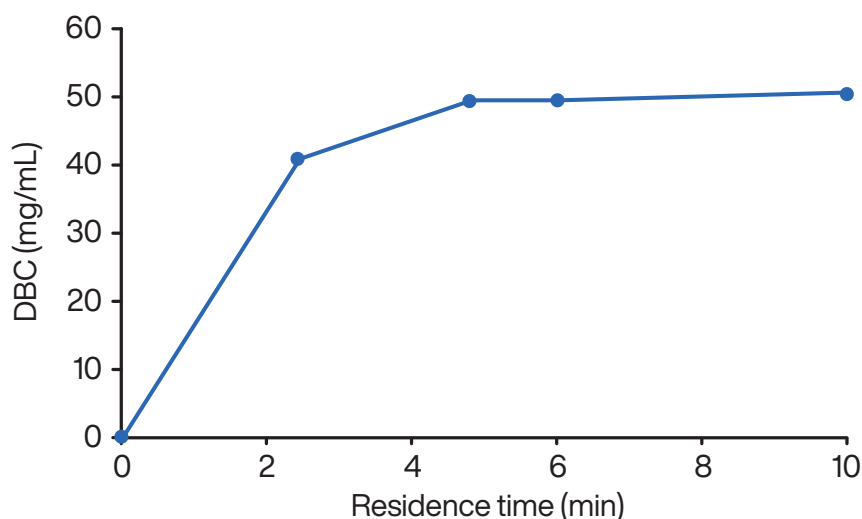
### Selection of column size

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 1 shows an example of DBC at different flow rates (or residence times). At a low flow rate, the capacity is higher. At increasing flow rate the binding capacity usually decreases.

To obtain the highest possible recovery of the target protein, we recommend the loading of 50-80% 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. 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 and subclasses. For WorkBeads affimAb, the binding capacity is more than 40 mg human polyclonal IgG/mL resin at 2.5 minutes residence time in a 6.6 x 100 mm column, which corresponds to a linear flow rate of 240 cm/min. Normally the binding capacity decreases with increased flow rate, see Figure 1.



**Figure 1.** Dependency of dynamic binding capacity to residence time. Frontal analysis using 1 mg/mL human polyclonal IgG in PBS, pH 7.4 in a 6.6 x 100 mm glass column.

## Optimization of binding

Human IgG and IgG from several other species bind to WorkBeads affimAb 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 or 50 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, 3 M NaCl, pH 9.

## Extra wash step

To remove weakly adsorbed impurities, it may be useful to add an extra washing step 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. Alternatively, collect the target protein and perform buffer exchange using a prepacked GoBio Prep Dsalt column equilibrated with a neutral buffer or pack your own column using WorkBeads Dsalt, see “Related products”.

The purity can sometimes be increased by applying a pH gradient for elution. This reduces the risk of elution of impurities adsorbed to the resin and denaturation of the target antibody.

For example, 100 mM sodium citrate, pH 6.0 to 100 mM sodium citrate, pH 3.0 over 20 CV can be applied. Desorption will occur when the pH is low enough, while avoiding too low pH. 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 use it in step elution in for scale-up runs.

## Scale-up

If it is a need for scaling up, this is easily done from a GoBio Prep 16x100 (20 mL) to a GoBio Prep 26x100 (53 mL) and furthermore to GoBio Prod 80x200 (1 L). The GoBio Prod prepacked column family is available in column sizes up to 21.4 L. Bulk WorkBeads resins can also be packed into other column formats of choice.

Large-scale purification is often carried out in columns with bed heights of 200 - 300 mm. The column diameter is selected based on the required column volume.

## 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 scaling-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 phase, 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 as 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 height is kept constant during scale-up, the linear flow rate should be kept constant (as well as the residence time).

**Table 3.** Example of scale-up parameters.

Column dimension	Residence time (minutes)	Linear flow rate (cm/h)	Volumetric flow rate (mL/min)
16x100	4	150	5.0
26x100	4	150	13.3
80x200	8	150	126
130x200	8	150	332
200x200	8	150	785
240x200	8	150	1131
330x250	10	150	2138

## Additional purification

Antibody purification on WorkBeads affimAb frequently gives high purity in a single step. For very high purity requirements, it may be necessary to add a second purification step. The additional purification step is used to remove traces of leached protein A ligand, host cell proteins and nucleic acids, aggregates of the target antibody, 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 and potential aggregates of the target protein. Size exclusion chromatography can be done using e.g. prepacked GoBio Prep16x600 40/100 SEC or GoBio Prep 26x600 40/100 SEC columns. Several different WorkBeads SEC resins are available both in GoBio prepacked formats and as WorkBeads SEC bulk resins for packing column of choice.

Ion exchange chromatography is suitable for both research scale purification and industrial purification. WorkBeads TREN, WorkBeads 40S, WorkBeads 40Q and WorkBeads 40 DEAE resins provide different selectivities for ion exchange chromatography. These resins are also available as ready-to-use GoBio Mini 1 mL and 5 mL, as well as GoBio Prep 16x100, GoBio Prep 26x100 and GoBio Prod starting from 1 L.

The polishing purification step can be based on several chromatographic techniques depending on the target molecule and the contaminants.

To find out more about Bio-Works' chromatography products visit [www.bio-works.com](http://www.bio-works.com)

### Size exclusion chromatography

Size exclusion chromatography (SEC) can be used for the separation of monomeric antibodies from dimeric antibodies and aggregates as well as complexes of leached protein A and antibody. SEC separates proteins and other biomolecules according to size, hence the monomeric antibodies will elute after antibody dimers, aggregates and complexes of leached protein A and antibody. This technique is simple to run. It is carried out under neutral conditions and is recommended for high purity demands in lab-scale purification, (e.g. using WorkBeads 40/100 SEC). Optimization is often not required for this technique, although it may sometimes be worthwhile. SEC is not recommended for bioprocess scale applications due to dilution effects, low capacity and that it is time consuming.

### Cation exchange chromatography

Cation exchange chromatography is commonly used as a polishing step in antibody purification processes. Many antibodies are weakly basic at neutral pH and will bind to a cation exchange chromatography resin, (e.g., WorkBeads 40S). Conversely, protein A does not bind to a cation exchange resin under the same conditions. Dissociation between antibodies and potential leached protein A can therefore be carried out by cation exchange chromatography technique under neutral pH. This technique usually requires optimization for each specific antibody to be purified.

### Anion exchange chromatography

Anion exchange chromatography technique is often used in a negative chromatography mode, during the polishing antibody purification. Potential leakage of protein A as well as complexes between protein A and the antibody tend to bind to an anion exchange chromatography resin (e.g., WorkBeads 40Q) at neutral pH, whereas the antibody itself usually does not bind and will elute in the flow through. Also, the use of this technique as a polishing step, usually require optimization for optimal antibody purification.

### Multimodal anion exchange chromatography

WorkBeads 40 TREN is another type of anion exchanger with higher salt tolerance which offers a unique separation possibility with the immobilized TREN ligand. This resin is useful as a “guard” column before loading the crude antibody sample directly on the protein A resin to prevent fouling of the protein A resin increasing the lifetime of the protein A resin. Several of the host cell proteins as well as chromatin (large DNA-protein complexes) will bind to WorkBeads 40 TREN.

To find out more about Bio-Works' chromatography products visit [www.bio-works.com](http://www.bio-works.com)

### Desalting and buffer exchange

Buffer exchange or desalting of a sample can be used before analysis and/or after purification with for example ion exchange chromatography. 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) columns depending on sample volumes, see “Related products”. These columns are also very useful alternatives to dialysis or when samples need to be processed rapidly to avoid degradation. For larger sample volumes prepacked GoBio Prod columns starting from 1 L are available or diafiltration can be used.

## Maintenance

### Cleaning using NaOH

During purification 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 packed column, and the pre-treatment of the sample. The bound 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 contamination, and retains the capacity, resolution and flow properties of the column. Cleaning the resin using 0.5 M NaOH gives efficient cleaning.

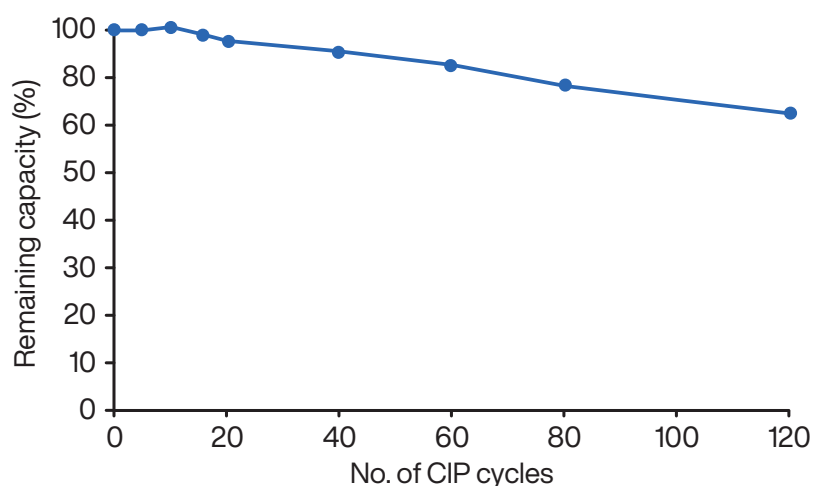


Sanitization (reduction of microorganisms) can be done using combinations of NaOH and ethanol, e.g., incubation with a mixture of 0.5 M NaOH and 40% ethanol for 3 hours. The sanitization procedure and its effectiveness will depend on the microorganisms to be removed and needs to be evaluated for each case. Prolonged wash with alkaline conditions will reduce the functionality of the column and must therefore be kept to a minimum (see Figure 2).

### Regeneration of WorkBeads affimAb

After purification using WorkBeads affimAb 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 5-10 CV 0.5 M NaOH over 15-30 minutes.  
For increased efficiency, before the NaOH wash, include a passage of 10 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.



**Figure 2.** DBC for polyclonal human IgG on WorkBeads affimAb determined by frontal analysis at 2.5 minutes residence time after 120 CIP cycles with 0.5 M NaOH at 15 minutes contact time.

### Storage

Store the resin at 2 to 8°C in 20% ethanol.

For prolonged storage, connect the included transport syringe filled with storage solution to the bottom end of the column.

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



## Product descriptions

	GoBio Prep 16x100 affimAb GoBio Prep 26x100 affimAb
Target substance	Antibodies (IgG), bound via the F <sub>c</sub> -region
Resin	WorkBeads affimAb
Matrix	Rigid, highly cross-linked agarose
Average particle size <sup>1</sup> (D <sub>v50</sub> )	50 µm
Ligand	Recombinant protein A expressed in <i>E. coli</i> using animal-free medium
Dynamic binding capacity <sup>2</sup> (DBC)	>4.0 mg human IgG/mL resin
Sample load	> 50% of DBC <sup>3</sup>
Column volumes	20 mL (16x100) 53 mL (26x100)
Column dimensions	16 x 100 mm 26 x 100 mm
Recommended flow rates	4-6 mL/min, 120-180 cm/h (16x100) 10-15 mL/min, 115-170 cm/h (26x100)
Maximum flow rate <sup>4</sup>	8 mL/min, 240 cm/h (16x100) 20 mL/min, 230 cm/h (26x100)
Maximum back pressure <sup>5</sup>	5 bar, 0.5 MPa, 70 psi
Chemical stability	Compatible with all standard aqueous buffers used for protein purification, 10 mM HCl (pH 2), 0.5 M NaOH (pH 12), 0.1 M sodium citrate buffer (pH 3), 6 M guanidine-HCl, 20% ethanol. Should not be stored at low pH for prolonged time.
pH stability	3 to 12
Storage	2 to 8 °C in 20% ethanol

<sup>1</sup> The median particle size of the cumulative volume distribution.

<sup>2</sup> DBC was determined at 10% breakthrough (Q<sub>B10%</sub>) by frontal analysis with 1 mg/mL human polyclonal IgG in PBS, pH 7.4 at 1.4 mL/min (245 cm/h, 2.5 minutes residence time) in a column packed with WorkBeads affimAb resin, column bed 6.6 x 100 mm.

<sup>3</sup> It is recommended to load 50-80% of resin's DBC to achieve maximum yield.

<sup>4</sup> Maximum flow rate for aqueous buffers at 20 °C. 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 for 20% ethanol). High flow rates will decrease DBC.

<sup>5</sup> The maximum pressure the packed bed can withstand depends on the sample/liquid viscosity and chromatography resin characteristics. The pressure also depends on the tubing used to connect the column and the system restrictions after the column outlet.

## 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.

## Related products

Product name	Pack size <sup>1</sup>	Article number
<b>Prepacked columns</b>		
GoBio Mini affimAb 1 mL	1 mL × 5	45 800 103
GoBio Mini affimAb 5 mL	5 mL × 5	45 800 107
GoBio Mini S 5 mL	5 mL × 5	45 200 107
GoBio Mini Q 5 mL	5 mL × 5	45 100 107
GoBio Mini TREN 5 mL	5 mL × 5	45 655 217
GoBio Mini Dsalt 5 mL	5 mL × 5	45 360 107
GoBio Screen 7x100 affimAb <sup>2</sup>	3.8 mL × 1	55 800 001
GoBio Prep 16x100 40S	20 mL × 1	55 420 021
GoBio Prep 16x100 40Q	20 mL × 1	55 410 021
GoBio Prep 16x100 40 TREN <sup>2</sup>	20 mL × 1	55 463 021
GoBio Prep 16x100 Dsalt <sup>2</sup>	20 mL × 1	55 700 021
GoBio Prep 26x100 40S <sup>2</sup>	53 mL × 1	55 420 031
GoBio Prep 26x100 40Q <sup>2</sup>	53 mL × 1	55 410 031
GoBio Prep 26x100 40 TREN <sup>2</sup>	53 mL × 1	55 463 031
GoBio Prep 26x100 Dsalt	53 mL × 1	55 700 031
GoBio Prod 80x200 affimAb <sup>2</sup>	1 L	55 800 042
GoBio Prod 130x200 affimAb <sup>2</sup>	2.7 L	55 800 062
GoBio Prod 200x200 affimAb <sup>2</sup>	6 L	55 800 072
GoBio Prod 240x200 affimAb <sup>2</sup>	9 L	55 800 082
GoBio Prod 330x250 affimAb <sup>2</sup>	21.4 L	55 800 093
<b>Bulk resins</b>		
WorkBeads affimAb	25 mL	40 800 001
	200 mL	40 800 002
	1 L	40 800 010
	5 L	40 800 050
	10 L	40 800 060
WorkBeads 40S	25 mL	40 200 001
	200 mL	40 200 002
	1 L	40 200 010
WorkBeads 40Q	25 mL	40 100 001
	200 mL	40 100 002
	1 L	40 100 010
WorkBeads 40 TREN	25 mL	40 603 001
	150 mL	40 603 003
	1 L	40 603 010
WorkBeads Dsalt	300 mL	40 360 003
	1 L	40 360 010

<sup>1</sup> All different pack sizes are available on [www.bio-works.com](http://www.bio-works.com)

<sup>2</sup> Packed on request.

## Ordering information

Product name	Pack size	Article number
GoBio Prep 16x100 affimAb <sup>1</sup>	20 mL × 1	55 800 021
GoBio Prep 26x100 affimAb <sup>1</sup>	53 mL × 1	55 800 031

<sup>1</sup> Packed on request.

Orders: [sales@bio-works.com](mailto:sales@bio-works.com) or contact your local distributor.

For more information about local distributor and products visit [www.bio-works.com](http://www.bio-works.com) or contact us at [info@bio-works.com](mailto:info@bio-works.com)

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