

INSTRUCTION

GoBio Prep SEC

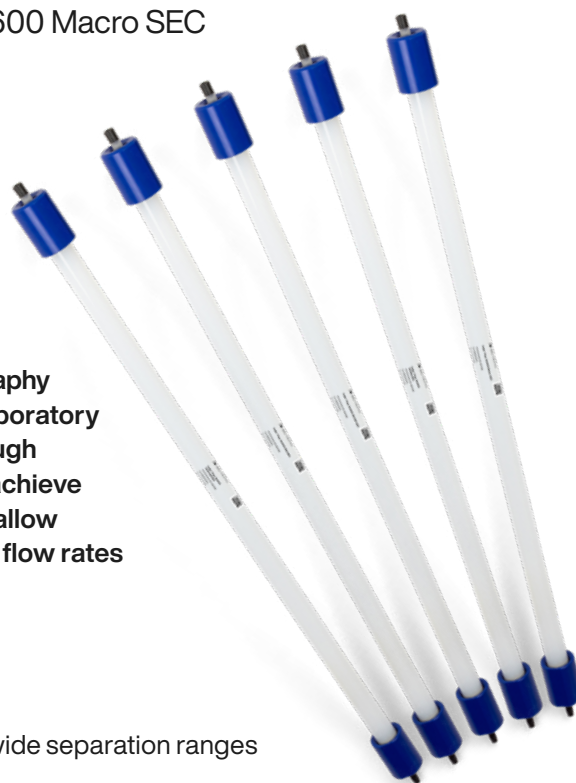
GoBio Prep 16x600 40/100 SEC, GoBio Prep 16x600 40/1000 SEC,
GoBio Prep 16x600 40/10 000 SEC and GoBio Prep 16x600 Macro SEC

GoBio Prep 26x600 40/100 SEC, GoBio Prep 26x600 40/1000 SEC,
GoBio Prep 26x600 40/10 000 SEC and GoBio Prep 26x600 Macro SEC

GoBio™ Prep 16x600 and GoBio Prep 26x600 are prepacked ready-to-use columns available with five different size exclusion chromatography resins, WorkBeads™ 40/100 SEC, WorkBeads 40/1000 SEC, WorkBeads 40/10 000 SEC and WorkBeads Macro SEC. The column volumes are 120 mL and 320 mL, respectively.

These resins are used for preparative size exclusion chromatography (SEC) purification of proteins, viruses, and other biomolecules in laboratory and process scale, by utilizing the differences in their size. Although the general recommendation for SEC is to use low flow rates to achieve optimal purification, the rigidity and tight particle size distribution allow purification of viruses and other large substances even at higher flow rates for fast processing and high yields.

- GoBio prepacked columns for fast and reproducible results
- No need for column packing skills
- WorkBeads SEC resins with different porosities give robust and wide separation ranges



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 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 associated Safety Data Sheets (SDS) for 40/100 SEC, WorkBeads 40/1000 SEC, WorkBeads 40/10 000 SEC, WorkBeads Macro SEC 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

Principle

In SEC, large substances in the applied sample will elute earlier than small molecules, since large substances do not enter the pores of the bead, or they can only enter a fraction of the pores. Smaller substances can enter a larger fraction of the pores, and they will therefore elute later. Intermediate size substances will enter the bead pores to different degrees, thus being eluted at different elution volumes. The eluent (buffer) that is used for equilibration of the column will enter completely into the pores of the beads. This means that the substances to be separated will be eluted in the eluent used for equilibration, thus allowing buffer exchange or salt removal (or addition). In addition to size, other characteristics e.g., shape, charge, hydrophobicity and interactions between substances or substances and resin, may affect the elution volume, thus the separation.

Optimization of chromatography conditions (buffer composition, flow, and sample volume) may be needed to obtain the required purification. SEC is usually applied as the last purification step (polishing step) since it is limited in flow and sample volume, and since it allows removal of salts and aggregates of the target substance as well as buffer exchange.

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 16x600 and GoBio Prep 26x600 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	120 mL (GoBio Prep 16x600) 320 mL (GoBio Prep 26x600)
Column dimensions	16 ×600 mm (GoBio Prep 16x600) 26 ×600 mm (GoBio Prep 26x600)
Theoretical plates	Approx. 4 000 m ⁻¹
Maximal column hardware pressure	5 bar, 0.5 MPa, 70 psi

Resins characteristics

WorkBeads 40 SEC products are based on agarose, which is excellent for separation of biomolecules. They have different pore size distribution, which give them different size separation ranges, see Table 2. WorkBeads resins are cross-linked using a proprietary method that results in a very rigid structure.

The characteristics of GoBio Prep16x600 and GoBio Prep 26x600 columns prepacked with the different WorkBeads SEC resins are listed in section “Product description”.

Table 2. Comparison of WorkBeads SEC resins.

	Average bead size, μm	Separation range, kD	Exclusion limit, kD	Separation range, D				
				10^4	10^5	10^6	10^7	10^8
WorkBeads 40/100 SEC	45	10 – 150	150					
WorkBeads 40/1000 SEC	45	10 – 1200	1200					
WorkBeads 40/10 000 SEC	45	10 – 10 000	10 000					
WorkBeads Macro SEC	45	10 – 30 000	30 000					

Purification planning

SEC purifications can often be done under standard recommended conditions, see recommended buffer conditions in Table 3. Although this may be a good starting point for testing, optimization may be needed to obtain desired results.

The most important parameters that affects resolution is the sample volume and the flow rate (especially for large proteins). The goal for most separations is to achieve the required resolution in the shortest possible time. If the analysis shows low resolution between protein peaks, the first action should be to set a lower flow rate for the run. Generally, a lower flow rate will allow time for molecules to diffuse in and out of the chromatography matrix and improve resolution. The effect is most pronounced for large molecules. Decreasing the flow rate can, on the other hand, have a negative impact on resolution for very small molecules.

Recommended sample volumes for optimal separation are presented in Table 4.

Several other factors, such as sample complexity, resin separation range, column bed height and packing quality are also influencing the resolution.

Unpacking and connecting GoBio Prep 16x600 and GoBio Prep 26x600 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 16x600 and GoBio Prep 26x600 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.

- Remove the syringe and keep it for further use during storage.
- 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.
- Connect the bottom of the column to the chromatography system using the second red connector.

Buffer preparation

The buffer species and buffer concentration are important for robust and reproducible methods. Choose a suitable pH and buffer for keeping the target protein in an active form, see some examples in Table 3.

Table 3. Recommended buffers for purification of His-tagged proteins.

Buffer
20 mM sodium phosphate, 150 mM NaCl, pH 7.4 (PBS)
50 mM sodium phosphate buffer, pH 7.0
20 mM Tris-HCl, 100 mM NaCl, pH 8.0

¹ The imidazole concentration may have to be optimized. A too high concentration may elute the target during washing. An imidazole concentration just below where the target proteins is still bound will prevent impurities to bind. This is an ideal washing buffer.

Sample preparation

If necessary, clarify the sample by passing it through a 0.22 - 0.45 µm filter, e.g., a syringe filter, to avoid transferring any remaining contaminants onto the column or do a centrifugation at 10 000 - 20 000 × g for 10-15 minutes. Application of a sample that has not been properly clarified may reduce the performance and lifetime of the column.

The sample concentration should not exceed 70 mg/mL for proteins and 5 mg/mL for dextran.

Note: We recommend **not** to use Blue Dextran as a molecular weight marker due to it may give unspecific binding to the resin.

Sample volumes of 0.5% to 4% of the total column volume is recommended.

Table 4. Recommended sample volumes and flow rates.

	GoBio Prep 16x600	GoBio Prep 26x600
Sample volume	0.6 – 4.8 mL (0.5% - 4% of CV)	1.6 – 12.8 mL (0.5% - 4% of CV)
Recommended flow rate ¹	0.5 – 1.78 mL/min	1.3 – 4.4 mL/min

¹ At room temperature in H₂O.

Purification

Note: Do not exceed the maximum recommended flow rate and back pressure for the column, see “Product description”.

- Wash out the storage solution with 1-2 column volumes (CV) deionized/distilled water if the buffer salts may precipitate upon exposure to ethanol. Use a reduced flow rate such as 50% of the recommended maximum flow rate.
- Equilibrate with 2-4 CV buffer.
- Apply the adjusted sample.
- Elute with 2 - 3 CV buffer until the UV trace of the effluent returns to near baseline.
- Wash with 2 CV elution buffer including 1 M NaCl to remove any remaining ionically bound material.

7. If required perform a cleaning-in-place (CIP), see page 6.
8. For storage wash the column with at least 3 CV 20% ethanol.
9. 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

The conditions used for SEC purifications may have to be optimized to give the optimal results. Below follow some recommendations.

Sample

Most sample compositions can be applied to the equilibrated column. The components of the sample will quickly move into the buffer that was used for equilibration, since the components to be separated move much faster than the buffer through the column. Very high viscosity of the sample may give zone broadening, thus reduced separation, e.g., protein concentrations of more than approx. 70 mg may negatively affect the separation. A recommendation is to slightly dilute the sample before loading but keep track of the sample volume.

Sample volume

Resolution, i.e., separation between the peaks, depends on the sample volume loaded on the column. Sample volumes of 0.5% - 4% of the total column volume is recommended. Smaller sample volumes may be used to further improve resolution. Notice that application of very small amounts of sample may compromise the sample yield due to inevitable adsorption of minute amounts of material to the resin during separation. In special applications where there is a large difference in elution volume between the sample components to be separated, the sample volumes can be as much as 25% of the column volume.

Flow rate

Optimization of the resolution can be carried out by lowering the flow rate. Optimum flow rate for proteins is often approx. 15 - 150 cm/h, a higher flow rate can decrease the resolution. The selection of flow rate in SEC is often a trade-off between resolution and purification time. For purifications of large substances that are eluted in the void volume, a higher flow rate can often be used.

Buffer composition

It is recommended to use buffers with slightly elevated ionic strength (or conductivity, or salt concentration) to mask electrostatic interactions between the resin and the substances to be separated. Addition of 150 mM NaCl to any buffer is a general recommendation and the NaCl concentration can be up to 0.5 M - 1 M. Separation can generally be done over a broad range of pH from 3 to 10. However, extreme pH and high salt concentrations may affect the structure of the proteins, or target molecules. Selection of buffer should be done within the stability window of the target substance, and to avoid any aggregation during separation.

Additives such as 1 - 10% glycerol, detergents, 0.5% arginine, or denaturants such as urea and guanidine-HCl are sometimes added to the buffer for stabilisation or to keep the target soluble during separation.

If the target molecules are known to be hydrophobic and tend to have retention times longer than 1 CV, up to 25% isopropanol may be added to prevent the non-specific hydrophobic interactions. Up to 0.3 M arginine instead of sodium chloride may be added to suppress both ionic- and hydrophobic non-specific binding and thus generate shorter retention times and smaller elution volumes. This is sometimes seen for e.g. monoclonal antibodies.

Scale-up

Bulk packages of 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 at least 600 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., 600 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 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:

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

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

If a smaller column has been used, the flow rate for the larger column can be calculated from the flow that was established on the small column, using the equation above by keeping the residence time of the small column the same 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. 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).

We recommend a purification strategy based on three phases: Capture, Enhance and Polish. In the capture phase, usually one purification step, it is common to use affinity chromatography or ion exchange chromatography, to remove bulk impurities and to concentrate and stabilize the target substance. The Enhance phase (one or several purifications steps) aims at further removing impurities. The polish phase aims at removing any final impurities, and when possible, adjusting the conditions of the product to be suitable for subsequent use. If the capture is performed using a sufficiently selective method, the enhance phase may be omitted and it may be enough with a polish step.

The SEC technique is usually best applied as a polish step since it is limited in sample volume, and since it in addition to removal of impurities allows removal of co-purified aggregates of the target, and since it also may allow modification of the conditions (e.g., buffer exchange or salt removal). The technique can also be useful earlier in the purification scheme in certain applications, depending on sample and purification goals.

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

Maintenance of the resin

Cleaning

During purification, impurities such as cell debris, lipids, nucleic acids, and protein precipitates from the samples may gradually build up in the resin and cause fouling, even for well-clarified samples. The severity of this process depends on the composition of the sample applied to the column. These adsorbed impurities will reduce the performance of the packed column over time. Regular cleaning (Cleaning-in-place, CIP) keeps the resin clean, reduces the rate of further fouling, and maintains the capacity, resolution, and flow properties of the packed column. Cleaning of the packed column using 1 M NaOH applied at a low flow for 15–30 minutes is often sufficient. If possible, perform the CIP using reversed flow to release any particles derived from the sample that may have been collected on the top filter.

Sanitization (reduction of microorganisms) can be carried out using combinations of NaOH and ethanol. The sanitization procedure and its effectiveness will depend on the microorganisms to be removed and needs to be evaluated for each case.

Storage

Store at 2 to 25°C in 20% ethanol.

For prolonged storage of the prepacked GoBio columns 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 description

	GoBio Prep 16x600 40/100 SEC	GoBio Prep 16x600 40/1000 SEC	GoBio Prep 16x600 40/10 000 SEC	GoBio Prep 16x600 Macro SEC
Resin	WorkBeads 40/100 SEC	WorkBeads 40/1000 SEC	WorkBeads 40/10 000 SEC	WorkBeads Macro SEC
Matrix	Highly cross-linked agarose	Highly cross-linked agarose	Highly cross-linked agarose	Highly cross-linked agarose
Average particle size (D_{v50}) ¹	45 µm	45 µm	45 µm	45 µm
Separation range ²	10 - 150 kD	10 - 1200 kD	10 - 10 000 kD	10 - 30 000 kD
Exclusion limit	150 kD	1200 kD	10 000 kD	30 000 kD
Column volume	120 mL	120 mL	120 mL	120 mL
Column dimension	16 × 600 mm	16 × 600 mm	16 × 600 mm	16 × 600 mm
Recommended sample volume	≤ 4.8 mL	≤ 4.8 mL	≤ 4.8 mL	≤ 4.8 mL
Recommended flow rate ³	0.5 - 1.8 mL/min (15 - 50 cm/h)	0.5 - 1.8 mL/min (15 - 50 cm/h)	0.5 - 1.8 mL/min (15 - 50 cm/h)	0.5 - 1.8 mL/min (15 - 50 cm/h)
Maximum flow rate ⁴	5 mL/min (150 cm/h)	5 mL/min (150 cm/h)	5 mL/min (150 cm/h)	5 mL/min (150 cm/h)
Maximum back pressure ⁵	5 bar, 0.5 MPa, 70 psi	5 bar, 0.5 MPa, 70 psi	5 bar, 0.5 MPa, 70 psi	5 bar, 0.5 MPa, 70 psi
Chemical stability	Compatible with all standard aqueous buffers used for protein purification. Should not be stored at low pH for prolonged time.			
pH stability	2 - 13	2 - 13	2 - 13	2 - 13
Storage	2 to 25 °C in 20% ethanol	2 to 25 °C in 20% ethanol	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 Globular proteins.

³ The flow rate is important for the resolution and a lower flow rate often gives an increased resolution. A higher flow rate can be used during equilibration to speed up the separation. Optimal flow is depending on the sample. Note: 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.

⁴ Determined in water using a 25 × 200 mm column. 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).

⁵ **Note:** Make sure that the column hardware max pressure is not exceeded.

Product description

	GoBio Prep 26x600 40/100 SEC	GoBio Prep 26x600 40/1000 SEC	GoBio Prep 26x600 40/10 000 SEC	GoBio Prep 26x600 Macro SEC
Resin	WorkBeads 4 0/100 SEC	WorkBeads 40/1000 SEC	WorkBeads 40/10 000 SEC	WorkBeads Macro SEC
Matrix	Highly cross-linked agarose	Highly cross-linked agarose	Highly cross-linked agarose	Highly cross-linked agarose
Average particle size (D_{v50}) ¹	45 µm	45 µm	45 µm	45 µm
Separation range ²	10 - 150 kD	10 - 1200 kD	10 - 10 000 kD	10 - 30 000 kD
Exclusion limit	150 kD	1200 kD	10 000 kD	30 000 kD
Column volume	320 mL	320 mL	320 mL	320 mL
Column dimension	26 × 600 mm	26 × 600 mm	26 × 600 mm	26 × 600 mm
Recommended sample volume	≤ 12.8 mL	≤ 12.8 mL	≤ 12.8 mL	≤ 12.8 mL
Recommended flow rate ³	1.3 - 4.4 mL/min (15 - 50 cm/h)	1.3 - 4.4 mL/min (15 - 50 cm/h)	1.3 - 4.4 mL/min (15 - 50 cm/h)	1.3 - 4.4 mL/min (15 - 50 cm/h)
Maximum flow rate ⁴	8 mL/min (90 cm/h)	8 mL/min (90 cm/h)	8 mL/min (90 cm/h)	8 mL/min (90 cm/h)
Maximum back pressure ⁵	5 bar, 0.5 MPa, 70 psi	5 bar, 0.5 MPa, 70 psi	5 bar, 0.5 MPa, 70 psi	5 bar, 0.5 MPa, 70 psi
Chemical stability	Compatible with all standard aqueous buffers used for protein purification. Should not be stored at low pH for prolonged time.			
pH stability	2 - 13	2 - 13	2 - 13	2 - 13
Storage	2 to 25 °C in 20% ethanol	2 to 25 °C in 20% ethanol	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 Globular proteins.

³ The flow rate is important for the resolution and a lower flow rate often gives an increased resolution. A higher flow rate can be used during equilibration to speed up the separation. Optimal flow is depending on the sample. Note: 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.

⁴ Determined in water using a 25 × 200 mm column. 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).

⁵ **Note:** Make sure that the column hardware max pressure is not exceeded.

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 Dsalt 1 mL	1 mL × 5	45 360 103
GoBio Mini Dsalt 5 mL	5 mL × 5	45 360 107
GoBio Prep 16x100 Dsalt ²	20 mL × 1	55 700 021
GoBio Prep 26x100 Dsalt	53 mL × 1	55 700 031
Bulk resins		
WorkBeads 40/100 SEC	300 mL	40 340 003
	1 L	40 340 010
	5 L	40 340 050
	10 L	40 340 060
WorkBeads 40/1000 SEC	300 mL	40 300 003
	1 L	40 300 010
	5 L	40 300 050
	10 L	40 300 060
WorkBeads 40/10 000 SEC	300 mL	40 350 003
	1 L	40 350 010
	5 L	40 350 050
	10 L	40 350 060
WorkBeads Macro SEC	300 mL	40 370 003
	1 L	40 370 010
	5 L	40 370 050
	10 L	40 370 060
WorkBeads Dsalt	300 mL	40 360 003
	1 L	40 360 010
	5 L	40 360 050
	10 L	40 360 060

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

² Packed on request.

Ordering information

Product name	Pack size	Article number
GoBio Prep 16x600 40/100 SEC	120 mL × 1	55 434 026
GoBio Prep 26x600 40/100 SEC	320 mL × 1	55 434 036
GoBio Prep 16x600 40/1000 SEC	120 mL × 1	55 430 026
GoBio Prep 26x600 40/1000 SEC	320 mL × 1	55 430 036
GoBio Prep 16x600 40/10 000 SEC ¹	120 mL × 1	55 435 026
GoBio Prep 26x600 40/10 000 SEC ¹	320 mL × 1	55 435 036
GoBio Prep 16x600 Macro SEC ¹	120 mL × 1	55 437 026
GoBio Prep 26x600 Macro SEC ¹	320 mL × 1	55 437 036

¹ Packed on request.

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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