Bio-Prep SE-100/17
Bio-Prep SE-1000/17
Columns

Instruction Manual

Catalog Numbers
732-1501
732-1502

BIO-RAD

Bio-Prep SE Size Exclusion Columns

Introduction

Bio-Prep SE prepacked columns for size exclusion chromatography are for rapid and reproducible high-resolution separations of biomolecules. Each column contains a unique, high performance, size-exclusion media based on spherical agarose beads cross-linked by a patented process to produce a very rigid beaded support.

Bio-Prep SE gel is available with different exclusion limits and particle sizes. For analytical and method development purposes, the SE-100/17 and SE-1000/17 pre-packed columns contain a 17 µm bead which produces excellent resolution of biomolecules at low back-pressures. SE-100/17 is ideal for separating biomolecules up to 100,000 Da and SE-1000/17 is used for larger biomolecules up to 1,000,000 Da.

For scaling-up a separation with an identical selectivity performance, bottles of Bio-Prep SE SE-100/40 and SE-1000/40 media are available. The nominal bead size is 40 µm. Refer to the Product Information section for ordering information.

Table 1. Bio-Prep SE Column Characteristics

Media and column properties	Prepacked SE-100/17	Prepacked SE-1000/17
Linear separation range	5,000–100,000 Da	10,000–1,000,000 Da
Exclusion limit	~200,000 Da	~1,500,000 Da
Nominal particle size	17 ± 2 µm	17 ± 2 µm
Recommended flow rates	0.1–1.0 ml/min	0.1–1.0 ml/min
Maximum flow rate	2.0 ml/min (*i.e.* for buffer exchange)	2.0 ml/min (*i.e.* for buffer exchange)
Maximum operating pressure	580 psi (4 MPa, 40 bar)	435 psi (3 MPa, 30 bar)
Maximum recommended sample volume	0.3 ml	0.3 ml
Column bed dimensions	8 x 300 mm	8 x 300 mm
Volume of gel	15 ml	15 ml
Efficiency[1]	> 20,000 plates/m	> 20,000 plates/m
Asymmetry factor[1]	0.85–1.15	0.85–1.15
Working pH range	3–12 long term	3–12 long term
	1–14 short term	1–14 short term
Operating temperatures	4–40 °C	4–40 °C

1. Test solute; acetone. Eluant; water. Flow rate; 0.5 ml/min.

Unpacking and Inspection

Unpack the column and inspect it for damage. Check the delivery against the packing list.

The column is shipped with pressure-compliant tubing (black tubing). Before attaching the column to your system, remove the black tubing and the luer-1/4 x 28 adaptor from the outlet 1/4 x 28 unions.

Note: minor cracks may be observed in the gel bed after shipping. This will not affect the performance of the column and will disappear during the washing procedure of the column.

Connection to Chromatography Systems

Each Bio-Prep SE column is assembled with a male 1/4-28 fitting at each end.

- Use the two white 1/4-28 x 1/4-28 unions (included), to attach the column to a BioLogic System.
- For connection to systems using metric fittings, use the two yellow 1/4-28 x M6 unions (included).
- To connect to systems using 10-32 fittings, order 1/4-28 x 10-32 unions (catalog number 750-0564).

Note: Bio-Rad Laboratories does not recommend or warrant the use of the Bio-Prep SE columns with solvent delivery systems containing stainless steel parts when used with corrosive eluants containing *e.g.* halide salts. We recommend the use of inert, biocompatible (ceramic, PEEK, titanium) solvent delivery systems for maximum column life and recovery of sample biological activity.

Running the Column for the First Time

The column is supplied in 20% ethanol. To remove the storage buffer, pump deionized, filtered water at a flow rate of 0.5 ml/min for at least 60 minutes (equivalent to 30 ml or two bed volumes). The back pressure will decrease as the ethanol is washed from the column. **Warning: Do not exceed the maximum pressure of 435 psi for SE-1000/17 (3 MPa, 30 bar) and 580 psi for SE-100/17 (4 mPa, 40 bar).**

Equilibrating and Running the Column

1. Always use filtered (0.22–0.45 µm filter) and degassed buffers. This will prolong the life of your column. To avoid bacterial growth, contamination, and poor column performance, use only freshly prepared buffers.
2. Residual ionic charges on the Bio-Prep SE media are negligible. However, a running buffer ionic strength of at least 0.05 M is recommended.
3. The buffer should contain any necessary cofactors or protease inhibitors previously identified as being essential to maintain enzyme activity.
4. Equilibrate the column with at least 5 bed volumes (75 ml) of the buffer.
5. It is recommended to calibrate the column using the gel filtration standard.

Sample Size

1. Always filter (0.22–0.45 µm filter) or centrifuge your sample to remove any particulate matter.
2. In size exclusion chromatography, mass loading is less critical than the volume of the sample. As a general rule, to obtain maximum performance, the sample volume should not exceed 2% of the bed volume (*i.e.* 0.3 ml for a 15 ml bed volume) and the sample concentration should not exceed 20 mg protein/ml. Larger volumes may be applied but resolution may decrease. Never exceed 5% of the total bed volume unless a simple buffer exchange procedure is required.

Flow Rates for Sample Elution

The flow rate for optimal resolution is dependent on the sample composition and purity. In general, lower flow rates provides greater performance. Optimal flow rates for both SE-100/17 and SE-1000/17 columns are 0.1 to 0.5 ml/min (corresponding to linear flow rates of 12 to 60 cm/hr). For very pure samples, a flow rate of 1.0 ml/min may be used. Never exceed 2.0 ml/min.

Two (or more) Columns in Series

Resolution can be enhanced in size exclusion chromatography by connecting two or more columns in series. A white 1/4-28 x 1/4-28 union is required. Columns connected in series require no special attention with respect to flow rates or back-pressure but the maximum back pressure allowed for a single column should not be exceeded.

Chemical Stability of the Bio-Prep SE Columns

Bio-Prep SE columns are completely biocompatible and have excellent chemical inertness. The materials in contact with the sample, media and eluants are inert—glass, PVDF, PEEK, and titanium. The columns are stable over the pH range 3–12 (long term) and 1–14 (short term) and may be used with the following eluants:

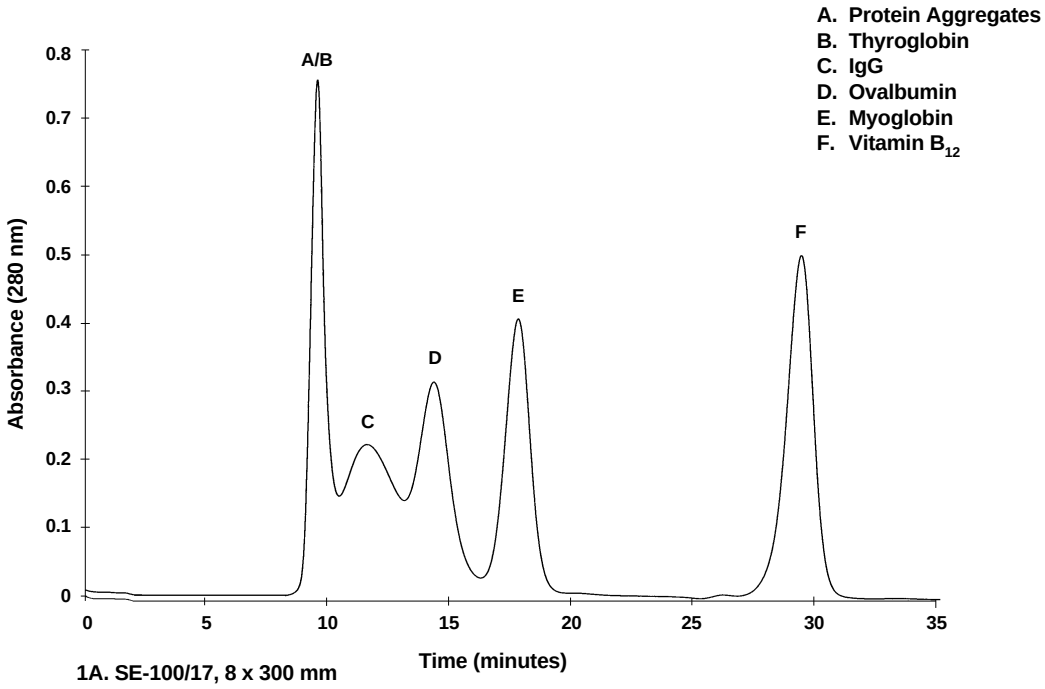
8 M urea, 6 M guanidinium HCl, 30% acetonitrile, 70% formic acid, 1 M NaOH, 0.1 M HCl, 5% SDS, 5% 2-mercaptoethanol, 30% acetic acid, 0.1% TFA.

Use of strong oxidizing agents should be avoided.

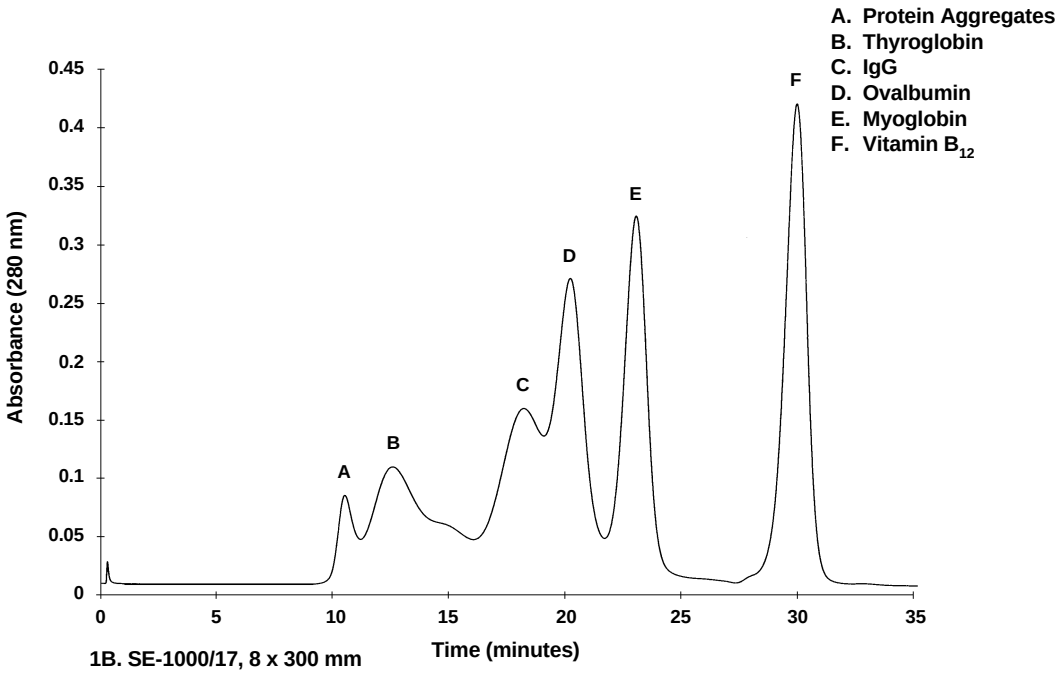
The typical detergents used for protein solubilization may be used with the Bio-Prep SE columns. No special precautions are needed other than complete equilibration. However, it is suggested that a column is clearly designated and reserved for such use.

Column Calibration

Use the Bio-Rad Size Exclusion Standards, catalog number 151-1901. One vial contains 18 mg of a lyophilized mixture of thyroglobulin (M_r 670,000), bovine gamma-globulin (M_r 158,000), chicken ovalbumin (M_r 44,000), equine myoglobin (M_r 17,000), vitamin B-12 (M_r 1,350). **Important note: Do not use Blue Dextran as a void volume marker. Use calf thymus DNA instead.** Figures 1a and 1b are example separations using the above standard on the Bio-Prep SE-100/17 and SE-1000/17 columns in 0.05 M sodium phosphate, 0.15 M sodium chloride pH 6.8 at 0.5 ml/min.



1A. SE-100/17, 8 x 300 mm



1B. SE-1000/17, 8 x 300 mm

Column Maintenance

- **Routine maintenance.**

To maintain performance, the column should be cleaned after every 25 injections using the following protocol.

1. Wash the column with 75 ml of deionized water at a flow rate of 0.1–1.0 ml/min. The back pressure should not exceed 450 psi (3 MPa) for SE-1000/17 and 580 psi (4 MPa) for SE-100/17.
2. Wash with 15 ml of 0.5 M NaOH at a flow rate not to exceed 0.5 ml/min.
3. Wash the column with 75 ml of deionized water at a flow rate not to exceed 2.0 ml/min.
4. Re-equilibrate the column with the desired buffer.

- **If the column runs dry.**

Wash the column with at least 45 ml of deionized water or buffer at a flow rate of 0.5 ml/min. Performance should be restored.

- **If air bubbles are trapped in the column.**

1. Wash the column with 15–30 ml of 20% ethanol at 0.25 to 0.5 ml/min. Note that the back pressure will increase. Do not exceed 450 psi (3 MPa).
2. Wash the column with 75 ml of deionized water at a flow rate not to exceed 1.0 ml/min.
3. Re-equilibrate the column with the desired buffer.

- **Abnormally high back-pressure (cleaning the inlet filter).**

If the back pressure increases abnormally, it is likely that the titanium filter in the top adaptor is clogged. It should be removed using the method described below.

- **Disassembling the column (Refer to Figure 2).**

1. Unscrew and remove the end-cap (1).
2. Unscrew the holder (3) for the adjustable adaptor (4). With the locking clip (2) in the correct position (locking tag pointed upward), the adjustable adaptor may be pulled up.
3. When the holder is completely unscrewed, remove the locking clip and the holder.
4. Gently pull the adjustable adaptor (4) all the way up without turning it.
5. Grip the top (5) and bottom (15) end-pieces firmly and unscrew them.

   If the bottom endpiece loosens, remove it (the fixed adaptor will not move) and then remove the cover tube.
6. Loosen the top endpiece from the glass tube. Remove it and then replace and retighten the bottom end-piece.
7. Clean the top adaptor and titanium filter (7) with deionized water in an ultrasonic bath for at least 5 minutes.

   Note: Never try to remove the filter from the adaptor as this will irreversibly destroy the sealing.

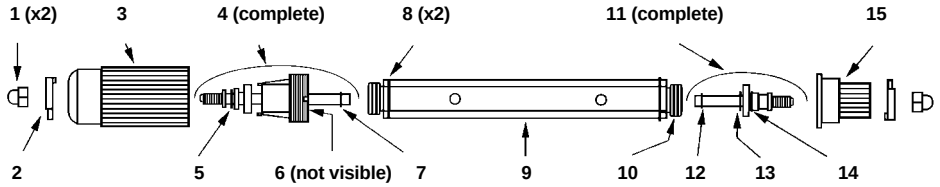


Fig. 2. Diagram of column hardware.

- **Reassembling the column.**

1. Reassemble the column in the reverse order from disassembling it.
2. Moisten the adaptor o-ring with water. To prevent the o-ring from breaking, the adaptors must be gently pushed into the glass tube. Avoid pushing the adjustable adaptor too far into the glass tube and then tightening the holder as the adaptor may become difficult to remove later. Never overtighten the end-pieces.

If column performance is not recovered using the above protocols, then run a protein standard to determine if the column is integrity compromised (See column calibration section). A new column should be purchased if the performance is not restored.

Sanitization (Sterilization)

Do not autoclave these prepacked columns. If sterilization is required the columns must be sterilized chemically. Wash the column with 0.5 M NaOH or wash the column with 70% ethanol. Note that ethanol will increase the pressure drop over the column.

Storage

To prevent bacterial growth in the column, it must be stored correctly. For short term storage (up to a few days), store it in freshly-prepared buffer. For long-term storage, wash the column with 30 ml of deionized water and then with a storage solution of 20% ethanol. Always store the column with plugs at each end.

Product Information

Catalog Number	Product Description
732-1501	**Bio-Prep SE100/17 Column,** 8 x 300 mm
732-1502	**Bio-Prep SE1000/17 Column,** 8 x 300 mm
160-0001	**Macro-Prep SE-100/40 bottle,** 50 ml
160-0002	**Macro-Prep SE-100/40 bottle,** 300 ml
160-0003	**Macro-Prep SE-100/40 bottle,** 1,000 ml
160-0010	**Macro-Prep SE-1000/40 bottle,** 50 ml
160-0011	**Macro-Prep SE-1000/40 bottle,** 300 ml
160-0012	**Macro-Prep SE-1000/40 bottle,** 1,000 ml
151-1901	**Gel Filtration Standard**, 6 vials
750-0516	**Union, 1/4 x 28 - M6,** 2
750-0565	**Union, 1/4 x 28 to 10–32,** 2
751-0099	**Bio-Scale Fittings Kit**, includes 2 Super Flangeless Nuts (1/4 x 28) and 6 ferrules, 2 Flangeless M6 nuts, 4 ferrules and Caps, 2 Fingertight II fittings (10–32). This kit allows the Bio-Prep SE column to be connected to almost any chromatography system.

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