



Instructions for use

WorkBeads 17 SEC and WorkBeads 17/100 SEC Pre-packed columns 8x500 mm

Ready to use pre-packed high performance columns for analytical and semi preparative purification of proteins.

Product Name	Column Size / Volume	Article Number
WorkBeads™ 17 SEC	Pre-Packed Column – 25 ml (8x500mm)	17 300 102
WorkBeads™ 17/100 SEC	Pre-Packed Column – 25 ml (8x500mm)	17 350 102

UNPACKING AND INSPECTION

Unpack the column as soon as it arrives and inspect it for damage.

Minor “bursts or cracks” might be seen in the packed bed upon arrival. These are quite harmless and will not influence on the performance of the column. They will vanish during the washing procedure of the column. Promptly report any damage or discrepancies to your local supplier or directly to Bio-Works Co. Ltd.

HOW TO USE THE COLUMNS

1 Installation of the column

- Connect the column to your chromatography system using the appropriate finger tight PEEK-connectors.

2 Washing the column

When the column is delivered it contains a storage solution of ~20% ethanol. This solution must be washed out. Once your column is connected, start preparing it by pumping distilled water through it.

Note! To avoid bacterial growth and poor column performance, use only freshly prepared and filtered buffers.

- Wash with distilled water at a flow rate of 0.5 ml/min for 60 minutes (minimum two column volumes).

NOTE! The ethanol solution will give rise to a pressure drop over the column of 0.8 -1.4 MPa at a flow rate of 0.5 ml/min. The corresponding pressure drop, when using distilled water or a buffer at the same flow rate, is 0.2-0.4 MPa. The difference will be noticed during the washing procedure.

WARNING! Do not exceed the maximum pressure of 2 Mpa (20 bar, 290 psi) or 1.8 mL/min

3 Equilibrating the column

- Equilibrate the column with 5 column volumes of your buffer.

4 Sample preparation and application

- Filter the sample through a 0.2-0.45 µm filter.
- Inject the filtered sample using a suitable injector.
- Optimal flow rate is 0.1-1.0 ml/min, which corresponds to 0.2-2.0 cm/min.

Note! The flow rate is dependent on the sample composition and purity.

5 Two (or more) columns in series

Resolution can be further enhanced in size exclusion chromatography by connecting two or more columns in series. This is very easy to do with the optional 1/4"-28 to 1/4"-28 union. The design of the column minimizes the dead volume. Columns connected in series require no special attention with respect to flow rates or back-pressure.

MAINTENANCE OF THE COLUMN

- Cleaning procedure

After every 25 injections you should clean your column to maintain its performance.

 - Wash the column with 5 column volumes of distilled water at a flow rate of 0.1-1.0 ml/min (the exact rate depends on the back-pressure, which should not exceed 4 MPa).
 - Wash with 1 column volume of 0.5 mol/l NaOH at a flow rate of 0.5 ml/min.
 - Wash with 5 column volumes of distilled water at a flow rate of up to 3 ml/min.
 - Re-equilibrate with your buffer at a flow rate of up to 1.8 ml/min.
- If the column runs dry

There is no reason for alarm if the column runs dry. If it happens:

 - Wash the column with 3 column volumes of distilled water at a flow rate of 0.5 ml/min.

If air bubbles are trapped in the gel:

 - Wash the column with 1-2 column volumes of ethanol at a flow rate of 0.75 ml/min.
Note! Ethanol will increase the pressure drop over the column.
 - Wash the column with 5 column volumes of distilled water at a flow rate of 1 ml/min.
 - Re-equilibrate your column with 5 column volumes of your buffer at a flow rate of up to 1.8 ml/min.
- Storage

To prevent bacterial growth in the column, you must store it correctly.

 - Short term storage

When you are using the column every day, you can store it in freshly prepared buffer. Keep the column installed in the system.
 - Long-term storage

Wash the column with distilled water and then fill it with a storage solvent of 20% ethanol.
- Cleaning the filter

The column adaptors have titanium filters to ensure biocompatibility and optimize performance. If the back pressure is becoming abnormal, you can remove both the top and bottom adaptors for cleaning. The top adaptor is likely to be the one that is clogged.

 - Remove the adaptor(s) according to the procedure under: HOW TO REMOVE THE TOP ADAPTOR
 - Clean the adaptor with the titanium filter for 5 minutes in an ultrasonic bath containing distilled water.

Note! Never try to remove the filter from the adaptor, because this will destroy the sealing!

 - Reinstall the adaptor(s) and assemble your column.
- Evaluation of column efficiency

The column packing may be evaluated to ensure the efficiency of the column to be according to specification. Run a low molecular weight solute e. g. acetone, at 0.5 ml/min and calculate the number of plates per meter (N) from $N = 5.54 \times (v_{\text{acetone}}/w_h)^2 / L$, where w_h is the peak width at half peak height, v_{acetone} is the elution volume of acetone and L is the length of the column (0.5 m).

HOW TO REMOVE THE TOP ADAPTOR

- Disassembling the column
 - Unwind and remove the End cap.
 - Unwind the holder for the adjustable adaptor. With the locking clip in the correct position (locking tag pointing downwards), the adjustable adaptor will be pulled up.
 - When you have completely unwound the holder, remove the locking clip and the holder.
 - Gently pull the adjustable adaptor all the way up without turning it.
 - Grip the the top and bottom endpieces firmly with your hands, and unwind them.

Depending on how much friction there is, either endpiece may loosen. If the bottom end piece loosens, take it off (the fixed adaptor will not move) and remove the cover tube.

 - Loosen the top endpiece from the glass tube. Remove it, and then replace and retighten the bottom endpiece. Replace the cover tube.

- Reassembling the column
 - Reassemble your column in the reverse order from disassembling it.
 - To prevent the sealing O-ring from breaking, the adaptors must be gently pushed into the glasstube.
NOTE! Do not push the adjustable adaptor too far into the chromatographic tube. If the adjustable adaptor, when winding on the holder, comes to the very down position in the tube - it will be very difficult to move it.
 - Moisten the O-ring with water. Never overtighten the end pieces. Your glass tube may crack.
Warning! Never operate the column without having the cover tube in place. A hazardous situation could arise if the glass tube should break.