



Instructions for use

WorkBeads 40 SEC, pre-packed column and bulk

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

Product Name	Column Size / Volume	Article Number
WorkBeads™40 SEC	Pre-Packed Column - 15 ml (8 x 300mm)	40 300 100
WorkBeads™40 SEC	Bulk Media – 300 ml	40 300 003
WorkBeads™40 SEC	Bulk Media – 1 L	40 300 010
WorkBeads™40 SEC	Bulk Media – 5 L	40 300 050

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 COLUMN

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 ~22% 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 4 Mpa (40 bar, 580 psi)

3 Equilibrating the column

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

4 Sample preparation and application

- Filter the sample through a 0.22-0.45 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 (Cat. No. 10 13 06). 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.

Never exceed the maximum allowed pressure for your column.

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 3 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 1.0 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 3 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 22% ethanol.

Do not forget the stoppers at both ends!

- 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. If the filter is still blocked, you should order a new adaptor (see ORDERING INFORMATION).

- 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 (0.3 m).



PACKING OF BULK MATERIAL

The ligands are attached to WorkBeads 40 i.e. beads that have a mean bead size of 40 micrometer. The beads are cross-linked with a proprietary method that results in very rigid beads that can take pressure of several bars and run at high flow rates. Follow this general advice when packing a column as well as 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 used.

Make 50% slurry of the gel and pour into the column. Pack the media with a downward flow higher than the intended operational flow or maximum 10 cm/min linear flow rate. When the bed height is constant, stop the flow and place the adjustable adaptor on top of the packed bed and squeeze it down approximately 2 mm into the bed (axial compression).

Equilibrate the column with a few column volumes of buffer and the column is ready for use.

SANITATION (STERILIZATION)

Do not autoclave your prepacked column. If sterilization is needed it must be sterilized chemically.

- Wash the column with 1-0.5 M NaOH following the instructions under: MAINTENANCE or
 - Wash the column with 70% ethanol following the instructions under: MAINTENANCE.
- Note! that ethanol will increase the pressure drop over the column.