Instructions for use

WorkBeads 40 Q and WorkBeads 40 S Prepacked columns 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 Q	**Pre-Packed Column - 4.3 ml**	**40 100 102**
WorkBeads 40 Q	**Bulk Media - 200 ml**	**40 100 002**
WorkBeads 40 Q	**Bulk Media – 1 Litre**	**40 100 010**
WorkBeads 40 Q	**Bulk Media – 5 Litre**	**40 100 050**
WorkBeads 40 S	**Pre-Packed Column - 4.3 ml**	**40 200 102**
WorkBeads 40 S	**Bulk Media - 200 ml**	**40 200 002**
WorkBeads 40 S	**Bulk Media – 1 Litre**	**40 200 010**
WorkBeads 40 S	**Bulk Media – 5 Litre**	**40 200 050**

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 (20 bar, 290 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 $N = 5.54 \times (v_{acetone}/w_h)^2 / L$, 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 MEDIA

The ion exchange groups 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 manufacture'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 that 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.

EXPERIMENTAL CONDITIONS

It is important to note that running conditions should be optimised for each experimental situation. There are other agarose ion exchangers on the market and conditions optimised for those may have to be adjusted when WorkBeads 40 Q or S are used instead. WorkBeads 40 ion exchangers are cross linked with a method that gives higher rigidity and hence the influence from the matrix is slightly different. When scouting for the best conditions it is important to start with sufficiently low ionic strength.

As a hint on what to choose you can use the following guideline:
WorkBeads 40 Q: 0.01 M TRIS pH 8 and elution with a gradient of NaCl up to 0.5 M.
WorkBeads 40 S: 0.02 M Phosphate pH 7 and elution with a gradient of NaCl up to 0.5 M.
These conditions are suggestions and a guideline intended to give starting point for designing the experiments.

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.

HOW TO REMOVE THE TOP ADAPTOR

- Disassembling the column

- Unwind and remove the End cap (1).
- Unwind the holder for the adjustable adaptor (3). With the locking clip (2) in the correct position (locking tag pointing upwards), 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 (4) all the way up without turning it.
- Grip the the top (5) and bottom (6) 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.