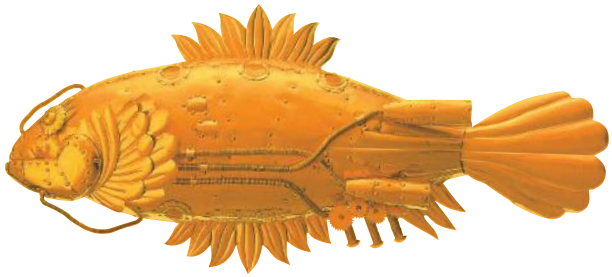
Shodex™



HPLC Columns

MANUAL

PROTEIN KW-2000

SHOWA DENKO EUROPE

Columns manufactured by Showa Denko K.K Japan
Made in Japan

Shodex HPLC Columns
Europe, Middle East, Africa, Russia

For technical support please use contact details shown below:

SHOWA DENKO EUROPE GmbH
Shodex Business
Konrad-Zuse-Platz 3
81829 Munich, Germany

E-mail: support@shodex.de
Phone: +49 (0)89 93 99 62 37
www.shodex.de

Operation Manual

Shodex™ PAK PROTEIN KW series

(Please read this manual carefully before using the column to ensure performance and life.)

1. Introduction

The packed columns of Shodex PROTEIN KW -2000 series are designed to be used in high performance gel filtration chromatography (GFC) for separation of proteins, enzymes and polysaccharides.

The packing material is totally porous spherical silica gel covered with hydrophillic hydroxy groups. Since the base material is silica gel, its swell and shrinkage are very small, enabling use of various kinds of buffer solutions or polar organic solvents as the eluent.

2. Specifications

Nomenclature	Column size (I.D. x length)	Exclusion limit	Theoretical plates	Solvent packed
Shodex PROTEIN KW-2002.5	20mm x 300mm	5×10^4	16,000 min.	H_2O
Shodex PROTEIN KW-2003	20mm x 300mm	1.5×10^5	16,000 min.	H_2O
Shodex PROTEIN KW-2004	20mm x 300mm	6×10^5	10,000 min.	H_2O
Shodex PROTEIN KW-LG	8mm x 50mm	Guard column	Guard column	H_2O

Note: Exclusion limits are the molecular weight of pullulan.

Endfitting: Internally-threaded type, No. 10 32 UNF.

Column material: SUS 316.

Packing material: Porous silica gel covered with chemically-bonded hydroxyl groups.

Max. temperature: 45 °C.

Max. pressure: 5.0 MPa/cm^2)

Max. flow rate: For the analysis:

5.0ml/min.

For the replacement of eluent:

1.5ml/min.

Caution!

1) Do not abruptly change the column pressure or the flow rate while the liquid chromatograph is in operation.

Use a damper- equipped or pulseless pump to maintain the performance of the column at the designed level for a long period of time.

2) Check the column pressure from time to time and never allow the pressure to go above 5.0MPa/cm².

3) The temperature of the column should generally be between 10°C and 45°C. Avoid a temperature above 45°C.

4) Do not impact or bend the column.

5) Do not remove the endfittings of the column under any circumstances; otherwise, its performance will deteriorate.

6) Install guard column immediately upstream of the main column to protect it from contamination by the sample.

The guard column is intended to maintain the column performance as designed for a long period of time and not to improve its resolving power.

3. Eluent

1) The following solutions are generally used as the eluent.

① Phosphate buffer solution.

② Tris hydrochloric buffer solution.

③ Acetate buffer solution.

2) Salts such as Na_2SO_4, K_2SO_4 and $(NH_4)_2SO_4$ are usually added to the above mentioned buffer solutions. The suitable salt concentration is 0.1 to 0.3M.

Caution! ① The pH range of the eluent should be 3.0 to 7.5 range.

② Use of salts containing chlorine ions should preferably be avoided, as chlorine ions are corrosive to the column or piping of the instrument. If such a salt has to be used, keep its pH above 6.0.

3) Use of urea or guanidine hydrochloride.

Urea of 6M guanidine hydrochloride solutions, which are often used as modifier of proteins, can be used as the eluent.

Caution! ③ Since those eluents have high viscosity, keep the flow rate at 1.5ml/min. maximum.

Since the replacement of the eluent takes a fairly long time, it is recommended to use one column specifically for such a eluent.

4) Use of surface active agent

Aqueous solutions of surface active agent such as SDS and Brij can also be used.

5) Use of polar organic solvent

Polar organic solvents, such as acetonitrile, methanol and ethanol, can also be used as the eluent.

Caution! Replacement of the solvent should be carried out at a maximum flow rate of 1.5ml/min.

4. Installation and start- up

1) Prior to connection of the column to the liquid chromatograph, replace the solvent in the chromatograph with the solvent that is to be used as the eluent.

If the liquid chromatograph is equipped with a device in which complete replacement of the solvent is not possible, e.g., a Bourdon pressure gauge, disassemble the device and wash it with the solvent that is to be used as the eluent.

2) Pass the eluent through a 0.45μm membrane filter to remove extraneous and insoluble substances.

3) Thoroughly degas the solvent that is to be used as the eluent, by subjecting it and ultrasonic vibration and simultaneous heating or pressure reduction with an aspirator.

Use of sol vent degassing devices will facilitate the degassing work.

4) After replacing the solvent in the chromatograph, set the flow rate at 3.0ml/min.

Flow rate should not exceed 5.0ml/min.

5) Connect the column to the chromatograph as that the arrow mark on the column will face downstream. Do not let air get into the column while connecting the column to the chromatograph.

6) Upon completion of the connection, start the pump, watching for any sudden change in the column pressure or the flow rate.

5. Pre-treatment of sample

1) Dissolve the sample in the same solvent that is to be used as the eluent.

To make the blank peaks as small as possible when a detector such as a differential refractometer is used, it is recommended that the sample be dissolved in the eluent obtained from the reservoir.

2) Remove extraneous matter or gels from the dissolved sample by passing it through a 0.45µm filter.

Use of the disposable filter unit Shodex DT is recommended.

6. Safekeeping

1) After completing analysis, keep pumping the eluent at a flow rate of 1.0ml/min. until the column is cooled down to room temperature.

2) When the column is not to be used for two or three days while the column is connected to the chromatograph, replace the solvent in the column with purified water.

3) When the column is not to be used for more than a week, replace the solvent in the column with purified water and then dismount the column from the liquid chromatograph. First, disconnect one end and place a cap before disconnecting the other end. And then, disconnect the other end and place a cap to the end. The caps prevent the eluent from leaking out.

4) Package it as delivered from the manufacturer.

5) Store it in a room that has little temperature fluctuation.

7. Calibration

The column is calibrated by ensuring that the specified plate number is maintained.

Following are the conditions for calculation of the plate number:

1) Sample: 0.4% ethylene glycol aqueous solution.

2) Injection volume: 200 µl.

3) Eluent: Purified water.

4) Flow rate: 4.0 ml/min.

5) Detector: RI.

6) Column temperature: Room temperature.

7) Calculation formula: $N = 5.54 \times (t_R/W)^2$

where N: Theoretical plate number

t_R: Retention time

W: Peak half width

8. Warranty

1) Showa Denko K. K. warrants that the Shodex Column, at the time of delivery to the user, will conform to the specification of the attached Certificate of Analysis, if the Shodex Column is used in accordance with the operating manual. The foregoing warranty is exclusive and is in lieu of all other warranties with respect to the Shodex Column, whether written, oral, implied, statutory or otherwise. No warranties by Showa Denko K. K. are implied or otherwise created, including, but not limited to, the warranty of merchantability and fitness for particular purposes.

2) Any claim of inconformity to the specification must be notified to Showa Denko K.K. within ten (10) days after delivery to the user. User's exclusive remedy and Showa Denko K.K.'s exclusive liability for such claim are limited to the replacement of the Shodex Column in question. In no event is Showa Denko K.K. liable for any indirect, incidental or consequential damage arising out of in connection with the Shodex Instrument, whether or not such damage is allegedly based on breach of warranty, negligence or otherwise.

3) No warranty is made in any of the following cases:

(1) If the Shodex Column is not used in accordance with the operating manual.

(2) If the Shodex Column is remodeled by anyone other than person or firm designated by Showa Denko K.K.

(3) If the Shodex Column is resold by the user without giving prior written notice to Showa Denko K.K.

(4) If the performance of the Shodex Column is not conform to the specification of the attached Certificate of Analysis due to any of the reasons below:

a) Computer virus

b) Impurities contained in the sample, reagent, gas air or cooling water provided by the user

c) Breakdown or malfunction of equipment, apparatus or component used in combination with the Shodex Column

d) Force majeure such as fire, earthquake, flood, other natural disaster, rime, riot, act of terrorism, war or radioactive contamination

4) In no event is Showa Denko K.K. liable for (i) the results of analyses or preparations using the Shodex Column or any portion of the same, including, but not limited to, the reliability, accuracy, efficacy and safety of said results, and (ii) the occupational hazard in the use of the Shodex Column, whether or not such use is made in accordance with the attached Conditions for use.

5) The Shodex instrument is for laboratory use only. It must not be used for clinical diagnosis. Showa Denko K.K. is not liable for any use of the Shodex Instrument except laboratory use.