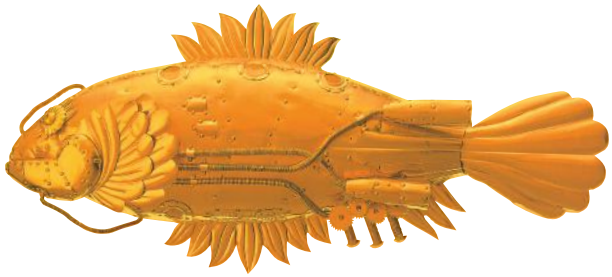
Shodex™



# HPLC Columns

## MANUAL

## OHpak LB-800

SHOWA DENKO EUROPE

**Shodex HPLC Columns**
Europe, Middle East, Africa, Russia

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# Operation Manual
# Shodex™ OHpak™ LB-800 series

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

## 1. Introduction

Shodex OHpak LB-800 series is Gel Filtration Chromatography columns which is appropriate for seprarating aqueous polymer, protein,enzyme,oligomer etc. Each column type has different pore size of packing material which are appropriate for each molecular weight range. When the column is used with Light Scattering Detector, it is possible to measure molecular weight exactly due to improving baseling noise.

## 2. Handling Instructions <Important>

**CAUTION!** *Please consult the MSDS for the reagents and solvents used with the columns for health concerns caused by acute exposure due to leakage from the column or adjoining tubing.

**ATTENTION!** *Use the column within the regular range of flow rate, pressure and temperature. The column performance may deteriorate when it is handled beyond the permissible range even for a short time.

## 3. Specifications

Packing material: Poly hydroxymethacrylate

Column material: SUS 316

Screw type: Internally-threaded type
No. 10-32 UNF

Shipping solvent: $H_2O$

* estimated value

** rough molecular range, not linear range

***measured with 0.1M $NaNO_3$ as eluent

## 4. Usable conditions

| Product code | Column type | Exclusion limit (Pullulan) | Plate number (TP/per column) | Exclusion limit (PEG) (Pullulan) | Plate number (TP/per column) |
|---|---|---|---|---|---|
| F6429211 | LB-802.5 | 1 x 105 | >16,000 | 1 x 105 | >12,000 |
| F6429201 | LB-803 HQ | 1 x 105 | >16,000 | 1 x 105 | >12,000 |
| F6429212 | LB-804 HQ | 1 x 106 | >16,000 | 1 x 106 | >12,000 |
| F6429213 | LB-805 HQ | 4 x 106 | >12,000 | 4 x 106 | >12,000 |
| F6429214 | LB-806 HQ | (2 x 107) | >12,000 | (2 x 107) | >12,000 |
| F6429202 | LB-806M HQ | (2 x 107) | >12,000 | (2 x 107) | >12,000 |
| F6709434 | OHpak LB-G 6B | Guard column | | Guard column | |

| Product Name | Flow rate (mL/min) | | | Max Pressure (MPa) | pH range | Temperature range (℃) |
|---|---|---|---|---|---|---|
| | Normal | Max | Compatible solvents | | | |
| LB-802.5 | 0.5-1.0 | 1.2 | ≤0.5 | 5.5 | 3-10 | 4-70 |
| LB-803 | 0.5-1.0 | 1.2 | ≤0.5 | 5.5 | 3-10 | 4-70 |
| LB-804 | 0.5-1.0 | 1.2 | ≤0.5 | 3.5 | 3-10 | 4-70 |
| LB-805 | 0.5-1.0 | 1.2 | ≤0.5 | 3.5 | 3-10 | 4-70 |
| LB-806 | 0.5-1.0 | 1.2 | ≤0.5 | 3.5 | 3-10 | 4-70 |
| LB-806M | 0.5-1.0 | 1.2 | ≤0.5 | 3.5 | 3-10 | 4-70 |
| LB-G 6B | 0.5-1.0 | 1.2 | ≤0.5 | - | 3-10 | 4-70 |

**ATTENTION!** *High-temperature operation may result in the generation of an air bubble, necessitating degassing. Low-temperature operation may require reduced flow rates, because of increased eluent viscosity.

**ATTENTION!** *1) In case of high molecular weight, it might indicate lower value when the eluent is used over 1.2 mL/min due to shearing polymer chain. In addition, if the polymer chain is shered, it is impossible to measure correct molecular distribution.

2) 2-4 columns can be connect directly according to separation poupose or samples.

3) In case of connecting different exclusion limit columns, higer one is preferable to be on upstream side.

**5. System Clean up**

Clean up the LC system including the injector and the sample loop by switching the valve, and flow the eluent before column installation.

**ATTENTION!** *The previous eluent used for analyses in the system may damage the column, if it is not compatible with the column.

**ATTENTION!** *When replacing nonpolar solvent to water, replace first with methanol and then replace with water. When replacing buffer solution to acetonitrile/water, replace first with 100% water and then repalce with eluent. Substances adsorbed in the pump and tubing may not be compatible with the column.

**6. Column Installation**

Install and use the column with the flow through the column matching the flow direction arrow on

the column tag. Set the flow rate at 0.2 mL/min, and connect the column. Flow at the low rate until the column temperature increases to the setting temperature, and then increase the flow rate to the analytical condition.

**ATTENTION!** *The column should always be installed in the manner above, for safe and effective operation.

**7. Eluent**

Some nonionic samples can be analyzed with the use of deionised water as the eluent. However, it is a general practice to use eluents that are either an aqueous salt solution or buffer solution with or without a polar organic solvent added to it.

1) Ionic and nonionic hydrophilic samples.

Salt solutions or buffer solutions are generally used as the eluent, typical examples are:

| | |
|---|---|
| Aqueous salt solutions | Sodium chloride, Sodium nitrate, Sodium sulfate, Potassium sulfate, Ammonium sulfate aqueous solution |
| Buffer solutions | Phosphoric acid buffer, tris-hydrochroric acid buffer, acetic acid buffer, Citric acid buffer solution |

Baseline noise of Light Scattering Detectors is often improved when salt solutions or buffer solutions are used in comparison with water alone. Recommended eluent condition is 0.1M $NaNO_3$.

**ATTENTION!** *A salt concentration range between 0.05 M to 0.3 M is recommended. When the eluent contains salt, the flow rate should be less than 0.5 mL/min. When the eluent has a salt content over 0.2 M, the flow rate should be slower than 0.3 mL/min at the solvent replacement.

**ATTENTION!** *pH of the eluent should be in the range of 3.0 to 10.0.

**ATTENTION!** *pH of the eluent should be higher than 6.0 when the eluent contains chloride ions.

**ATTENTION!** *Boric acid buffer solution is not recommended because boric acid makes complexes with the diol groups of the packing material.

**ATTENTION!** *Baseline noise of Light Scettering Detector on Certificate of Analysis is mesuread with 0.1M $NaNO_3$. If the eluent condition is different, baseline noise might sometimes show worse.

2) Nonionic and ionic hydrophobic samples

In case of analysis for hydrophobic sample, addition of polar organic solvents to the eluent is recommended to decrease the hydrophobic adsorption. Furthermore, addition of salts to the eluent is also recommended, when the hydrophobic sample is ionic. The recommended concentration of polar organic solvents is below

| Column type | Methanol | Acetonitrile | DMF |
|---|---|---|---|
| LB-803 | 100% | 100% | 100% |
| LB-806M | 100% | 100% | 100% |
| LB-G 6B | 100% | 100% | 100% |

**ATTENTION!** * Flow rate should be less than 0.5 mL/min. when exchanging solvents from 100% aqueous to one containing polar organic solvent.

**ATTENTION!** *In case of solvent replacement, enough flow of intermediate solvent is applie at first, which is a 1:1 mixture of the current solvent and the final desired solvent. Next, the final desired solvent flow is applied.

**ATTENTION!** *Replacement of a solvent might cause deteriorationof the column performance. It is recommended that the same solvent be used wherever possible. (At your request, the solvent is replaced when you purchase a column.)

**ATTENTION!** *Store the column as filled with the eluant used.

**ATTENTION!** *In case of eluent replacement, baseline noise of Light Scattering Detector might sometimes show worse.

3) Protein samples

Urea or guanidine aqueous solutions, which are commonly used as protein modifiers, can be used as the eluent. An eluent containing surfactant, such as SDS or Brij-35, is recommended for samples such as membrane proteins when their solubility in water is poor.

**ATTENTION!** *Frequent exchanges of solvent from normal solvent to the above solvent will make the column life short. If such frequent use in the above solvent is required, it is recommended to dedicate one column specifically for that purpose.

## 8. Sample Preparation

1) To dissolve or dilute a solid sample ,the sample should be prepared with elution.

2) When fully dissolved, the sample solution should be filtered through a 0.45μm filter to remove

particulates.

3) To dissolve a sample with molecular weight of 1,000,000 minimum, first allow the sample to stand in the same solvent as eluant for a half or one day until it has been fully swollen. Next, slowly agitate the samples solution to completely dissolve the sample.
4) When samples including gel content and insoluble materials are measured, It is possible to prolong columns life with small injection.
5) For LB -800 series, an injection volume of 50 to 100 µL per column is recommended. The recommended sample concentration is 0.05 to 0.5 w/v%, but the optimal concentration varies depending upon the molecular weight and viscosity of the sample. The general relationship between molecular weight and optimal concentration is shown below:

| Linear range of MW | Optimal concentration (w/v%) |
|---|---|
| 5,000 | ≤1% |
| 5,000 - 25,000 | ≤0.5% |
| 25,000 - 200,000 | ≤0.25% |
| 200,000 - 2,000,000 | ≤0.1% |
| 2,000,000 | ≤0.05% |

For a sample with high molecular weight, if the concentration of a sample solution is too high, the accurate molecular weight distribution may not be obtained. Therefore, decrease the sample concentration to the possible extent and then inject the sample into the column.

**9. Column Cleaning**

Elution characteristics of a column may change considerably after long, repeated usage, due to the accumulation of pollution components on the packing material, for example metal ions from the LC system or the sample.

Clean the guard column and analytical column separately by flowing eluent at 50% of ordinary flow rate in the opposite direction of the arrow on the column tag.

**ATTENTION!** *After cleaning the column, there are cases that the theoretical plate number becomes higher than the initial plate number. In such cases, the plate number will get back to the initial level gradually by running the eluent or water.

**ATTENTION!** *Complete the washing procedure steps continuously. Do not store the colum with either acid or alkaline solution as it will advance the deterioration of the column.

**ATTENTION!** *Flow the alkaline waste into a separate bottle without flowing to the detector.

The strong alkaline solution may cause damage to the cell of the detector.

**10. Storage solvent**

Store the column as filled with eluent, when the eluent is neutral. If the column is stored with acidic or alkaline eluent, the column might be damaged. In such case, please store the column with appropriate neutral eluent.

**11. Column Inspection**

Column inspection method is described in Certificate of Analysis (CoA).

**ATTENTION!** *Analyse by inspection condition on the Certificate of Analysis and Confirm column performance after pucahsing and in each used.

**12. Attention**

1) Do not remove the end fittings of the column to prevent performance deterioration and for safety reasons.
2) Do not make a strong impact on the column: such as hitting or dropping on the floor.
3) Replace the solvent in the LC system with the eluent to be used before connecting the column.
4) Connect the column so that the flow direction corresponds to the arrow mark on the tag.
5) When the column is not used for two weeks or more, replace the in-column solvent with the shipping solvent, remove it from the LC system, close each end with a stopper, and store it at controlled room temperature.
6) Contact Shodex website (http://www.shodex.com/) or Shodex partners regarding product and analysis applications.

**13. Warranty** (Ver. 3)

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 attached 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 Column, 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:

3-1 If the Shodex Column is not used in accordance with the operating manual

3-2 If the Shodex Column is remodeled by anyone other than person or firm designated by Showa Denko K.K.

3-3 If the Shodex Column is disposed of

3-4 If the Shodex Column is resold by the user without giving prior written notice to Showa Denko K.K.

3-5 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, crime, riot, act of terrorism, war or radioactive contamination

4) In no event is Showa Denko K.K. liable for

4-1 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

4-2 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 Column 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 Column except laboratory use.