Rev. CO073003

Packed Columns for Aqueous
Size Exclusion Chromatography

TSKgel SuperMultiporePW-N
TSKgel SuperMultiporePW-M
TSKgel SuperMultiporePW-H
TSKgel SuperOligoPW

INSTRUCTION MANUAL

TOSOH

TOSOH CORPORATION

Safety Precautions

To help protect you and/or your property from potential damage and ensure personal safety, please read this manual thoroughly before using the product.

[Notational Conventions]

Notation	Explanation
⚠WARNING	Indicates a hazard with a medium level of risk which, if not avoided, could result in death or serious injury.
⚠ CAUTION	Indicates a hazard with a low level of risk which, if not avoided, could result in minor or moderate injury.

⚠ WARNING

■**Keep away from fire**

Not taking proper precautions when using flammable solvents could result in fire, explosion, or poisoning.

⚠ CAUTION

■**Use only in well-ventilated areas**

In case of insufficient ventilation, flammable and toxic solvents can cause fire, explosion, or poisoning.

■**Do not spill solvents**

Spillage and leakage can cause fire, electric shock, poisoning, injury, or corrosion.
Wear appropriate protective gear when cleaning up a spill.

■**Wear protective eye gear and gloves**

Organic solvents and acids should not come into direct contact with the skin.

■**Handle the package with care**

Inappropriate handling may cause rupturing and/or splattering of the product.

■**Only use this product for its intended use**

This product is intended for the separation and purification. Do not use it for any other purpose.

■**Make sure compounds are safe**

Check that the target compounds and solutions after separation and purification are safe.

■**Proper disposal**

Dispose in accordance with local laws and regulations.

NOTE

Keep this manual with the product for future reference.

Precautions: Packing Material

First Aid	Inhalation	・Move the person to an area with fresh air and rinse mouth with plenty of water.
	Skin exposure	・Wash the exposed area with plenty of soap and water.
	Eye exposure	・Open the eyes as wide as possible and rinse with clean water for at least 15 minutes. ・Call immediately for medical attention.
	Ingestion	・Rinse mouth with plenty of water. ・Call immediately for medical attention.
Handling and Storage	Ventilation	・Provide adequate air ventilation to keep organic vapor concentrations below approved level.
	Container handling	・Container may break if not handled with care.
	Wear appropriate protective equipment	・Use solvent-resistant gloves and protective eye gear when using this product. Use of a gas mask, additional protective clothing or rubber boots may be appropriate when handling this product.
	Hazardous substance storage	・If any flammable solvents are used for shipping or storage of this product, keep away from fire or open heat sources.
	Fire precautions	・Do not expose this chromatographic resin to fire or open heat sources.
Waste Disposal	Disposal methods	・Dispose in accordance with local laws and regulations.
	General considerations	・Please pay attention to all safety precautions with respect to the handling and storage of this product.
	Disposal precautions	・This product can be safely incinerated. ・Appropriate nitrogen oxides exhaust emission precautions should be taken specifically for this product.

☐ TSKgel SuperMultiporePW Series and TSKgel SuperOligoPW column products contain combustible chromatographic media based on a co-polymer of vinyl compounds.

☐ Shipping solvent : distilled water
(note storage temperature as solvent may freeze near 0 ℃)

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1. General Information

TSKgel SuperMultiporePW Series and TSKgel SuperOligoPW columns are high performance and semi-micro SEC-packed columns in which a small particle sized hydrophilic synthetic polymer is used as a packing material.

Please read this INSTRUCTION MANUAL carefully and use the column as recommended in order to make effective use of its high performance.

2. Unpacking

Check that there is no visible damage to the outer package or the column.

Figure 1. Appearance of the Package

Check that the following documents are shipped with the column.

1) INSTRUCTION MANUAL 1 copy
2) INSPECTION DATA 1 copy

3. Column Parts

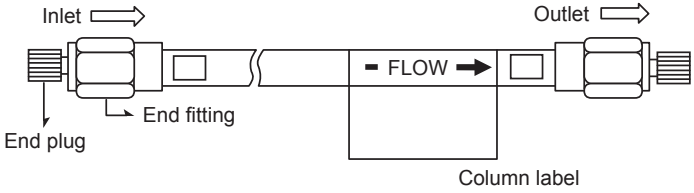

Figure 2. Column Parts

4. Column Installation

4-1 Connection of Column Parts

Confirm the correct product name is listed on the column label.

Each column is equipped with a union nut enabling a connection to a 1/16”O.D. capillary tubing. The union nut is designed for American standard compression plugs and ferrules.

4-2 Flow Direction

Confirm the flow direction on the column label or etched onto the column as shown in Figure 2. Solvent should flow only into the column from the inlet side. The columns are designed so that optimal resolution is obtained when the flow direction is as indicated on the column.

4-3 Prevention of air into the Column

Purge all air out of the tubing using the mobile phase. This helps to prevent any air from entering the column. Any air in the tubing causes serious deterioration of column efficiency.

4-4 Order of Columns

When both a sample column and a reference column are installed on the system, install the reference column first.

4-5 Connection of Sample Column

Make sure that the solvent is flowing freely out of the end of the tubing from the injector.

Remove the end plugs from the column and connect the inlet of the column to the tubing from the injector. Make sure that the tubing is fully inserted into the compression fittings before tightening in order to minimize dead volume. Always keep dead volume to the absolute minimum throughout the entire system.

After the solvent flows from the outlet of the column, connect the column to the detector.

Start pumping the solvent at a flow rate of 0.2 mL/min or less. Avoid a sudden pressure surge to the column.

4-6 Connection of Multiple Columns

Using the end fitting on the outlet side of the column connected as above connect

additional columns in sequence, first at the top and then at the bottom of the column (use 1/16 inch tubing for column-to-column connections). To reduce dead volume, use short sections of tubing and insert the tubing fully into the end fitting before tightening with ferrules. If there is space between the tubing and end fitting inside the connection, the flow of solvent will mix, and resolution will be decreased.

4-7 Before Measurement

The columns are very sensitive to pressure pulsing. A pulseless pumping system should be used.

4-8 Prevention of Pulsed Flow

This column is easily affected by pulsed flow. Use a pump with no pulsation. When a pump with pulsation is used, apply a pulse damper (accumulator) to the outlet side of the pump in order to eliminate pulsed flow.

5. Column Maintenance

5-1 After Measurement

5-1-1 Measurement higher than Room Temperature

Do not stop the pump immediately after measurement. Continue to pump the eluent solvent until the temperature of the column is decreased to 20℃ ~ 30℃. If the pump is stopped at a high temperature, air bubbles may be introduced into the column due to contraction of the eluent solvent.

5-1-2 When the Same Column Is Used on the Following Day

If the column is used in routine daily operation, it is permissible to leave the mobile phase in the column overnight. If the next measurement is 3 or more days later, store the column according to Section 5-2.

5-1-3 When Salt Solution Is Used As Solvent

Rinse the system at a flow rate of 0.2 mL/min or less with distilled or de-ionized water. The amount of water for rinsing should be more than the amount necessary for replacing the column volume and the entire tubing system.

5-1-4 Long-Term Storage (3 days or more)

When the column is not used for long period refer of section 5-2.

5-2 Storage of Column

5-2-1 Storage Method

Store the column after treating as follows.

Purge the system with distilled or ion-exchanged water. Remove the column from the system and keep the ends of the column tightly capped with the end plugs supplied with the column.

5-2-2 Storage Temperature

Store the column at a relatively constant temperature (20℃~30℃) in its original shipping container. Take care not to allow the column to freeze during storage.

5-2-3 Direct Sunlight

Avoid exposure to direct sunlight.

5-2-4 Corrosive Gas

Store the column in a safe place without corrosive gases.

6. Solvent Selection and Preparation

6-1 Solvent Replacement

The shipping solvent is distilled water for TSKgel SuperMultiporePW Series and TSKgel SuperOligoPW columns. Before using the column, the solvent should be replaced with an appropriate mobile phase for analysis at a flow rate of 0.2 mL/min or less.Note that a drastic change of solvent composition or frequent solvent replacements may shorten the lifetime of the column.

6-2 Selection of Solvent

Select the solvent composition while considering column stability, solubility of samples, elimination of non-specific interaction between sample and support, etc.

6-2-1 Available pH Range

TSKgel SuperMultiporePW Series and TSKgel SuperOligoPW gels can be operated over a relatively wide pH range of 2.0~12.0 at 20℃~ 30℃. They are excellent in stability at higher pH values compared with the SW type gels utilizing silica gel as the base material which only are able to be used in the pH range of 2.5~7.5.

6-2-2 Salt Aqueous Solutions and Buffer Solutions

Although some non-ionic compounds can be measured with distilled water, it is generally recommended to carry out the measurement with aqueous salt solutions or buffered solutions, while considering the presence of ionic impurities which interact with the support. Representative mobile phases are shown below.

Aqueous solutions.

Aqueous salt solutions: Sodium sulfate aqueous solution, sodium acetate aqueous solution, sodium dihydrogen phosphate aqueous solution, ammonium acetate aqueous solution, ammonium formate aqueous solution.

Buffered solutions : Phosphate buffer, tris hydrochloric acid buffer, tris acetate buffer, citrate buffer, acetate buffer.

The salt concentration is generally adjusted to below 0.5 mol/L in order to avoid a viscosity rise through salt addition and salt precipitation due to temperature changes, etc. Furthermore, avoid the use of halogen salts as much as possible to prolong the stainless steel column life. If measurement has been carried out with such salt solutions, rinse the columns according to section 5-1-3 after finishing the measurement.

6-2-3 Organic Solvents

TSKgel SuperMultiporePW Series columns are compatible with aqueous solutions of water-soluble organic solvents such as methanol, ethanol and acetonitrile.

TSKgel SuperMultiporePW Series gels are physically and chemically stable in ordinary water-soluble organic solvents and capable of measurement in aqueous solutions of methanol, ethanol, acetonitrile, formic acid, dimethylsulfoxide, etc. In relation with the concentration of organic solvents, it is preferable to use solvents with concentration ratios under 20 % to minimize support swelling. However, it is possible to use 50 % organic solvents by carefully following the procedure for changing solvents. When the concentration of the organic solvent in the eluent is changed, it should be changed gradually using reduced flow rates (preferably using a linear gradient) as rapid change may cause degradation of column efficiency. A significant change of the concentration, (e.g., 0 % → 30 % ~ 50 %) should be done using a gradient method.

6-3 Degassing

Solvents should be degassed to ensure optimal flow through the system.

7. Flow Rate

Flow rates used for TSKgel SuperMultiporePW Series and TSKgel SuperOligoPW are shown in Table 1. Suitable flow rates and pressures for measurement with aqueous solutions vary depending upon the mobile phase. Never use flow rates or pressures higher than those listed for each column (Table 1).

Table 1 Maximum flow rates and pressures

Column Type	Column Size mm(I.D.)× cm(L)	Suitable Flow Rate (mL/min)	Maximum Flow Rate (mL/min)	Maximum Pressure (per column) (MPa)
TSKgel SuperMultiporePW-N	6.0×1.5	0.3~0.6	0.6	4.5
TSKgel SuperMultiporePW-M				2.7
TSKgel SuperMultiporePW-H				0.9
TSKgel SuperOligoPW				5.0

8. Temperature

8-1 Temperature for Use

Use all TSKgel SuperMultiporePW Series and TSKgel SuperOligoPW columns between 10℃ ~ 80℃.

8-2 Measurement at Elevated Temperatures

Degas all solvents before use. After measurement at elevated temperatures, always follow the procedure described in Section 5-1 in order to protect the column.

8-3 Advantages of Measurement at Elevated Temperatures

The main advantages are as follows.

(1) Viscosity is reduced at elevated temperatures.

(2) The number of theoretical plates is higher at elevated temperatures than that at 20℃ ~ 30℃ and resolution is improved.

8-4 Measurement at Temperatures Lower than Room Temperature

There are various disadvantages associated with measurement at low temperatures. These are directly opposite of the advantages listed above for measurement at elevated temperatures. In addition, it is necessary to lower the flow rate at decreased temperatures when compared to that used at 20℃ ~ 30℃ as solvent and sample viscosities will be higher.

9. Sample Preparation

9-1 When There Is Insoluble or Gelatinous Material in the Sample

Centrifuge or filter with a micropore filter (e.g., 0.45 μm) the sample prior to analysis. Even when nothing can be seen with the naked eye, it is possible that insoluble material is present. TOSOH recommends filtering the sample with a micropore filter.

9-2 Composition of Sample Solutions

Adjust the salt concentration, pH and amount of organic solvent in the sample to those of the eluent.

When gradient elution is performed, match the sample solution to that of the initial eluent. If the sample contains high concentrations of salt, desalt before injection.

Do not inject a sample that forms insoluble material when mixed with the eluent.

10. Measurement of Number of Theoretical Plates and Asymmetry Factor

(1) Number of theoretical plates (N)

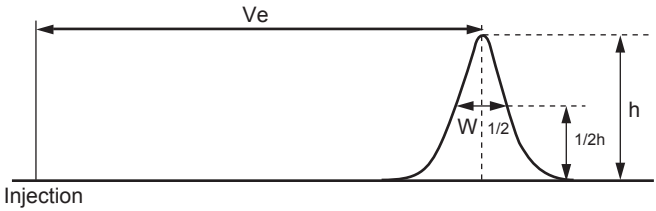

Figure 3 Calculation of Number of Theoretical Plates

The N is calculated using an unretained molecule by the half-peak width method as shown in Figure 3 and the following equation:

$$N=5.54(Ve/W_{1/2})^2$$

Where :

Ve : elution time (min)

$W_{1/2}$: half width of peak

h : peak height

N : number of theoretical plates per column

(2) Asymmetry factor (As)

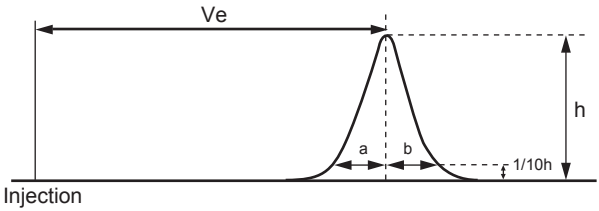

Figure 4 Calculation of Asymmetry Factor

The asymmetry factor is calculated according to Figure 4 and the following equation:

As=b/a

As: asymmetry factor

(3) The N and As should be measured with an instrument with small dead volume.

(4) The N and As are mentioned in the INSPECTION DATA sheet together with the experimental conditions.

11. Guard Column

Fundamental keys to prevent problems have been outlined in Section 4 to 9. When impurities that tend to adsorb onto the packing material are present in a sample, they are typically adsorbed at the inlet side of the column and gradually accumulate causing a reduction in the number of theoretical plates and a decrease in column performance.

In such cases the original column performance can be maintained by connecting a guard column between the injection valve and the analytical column. The guard column should be replaced when the performance deteriorates as a result of the adsorption of such a material to the guard column. A guard column can not be used in place of analytical column.

The use of a guard column will not improve the resolution obtained on the analytical column.

11-1 Effect of Guard Column Installation

(1) Contamination of the analytical column can be prevented by the removal of adsorptive or insoluble materials in the sample.

(2) Pressure shock, due to pump pulsation, to the analytical column should be avoided.

11-2 Type and Selection of Guard Columns

Guard columns specifications are shown in Table 2.

Table 2 Guard Column

Part No.	Type	Column size mm(I.D.)×cm(L)	Applied column
0022793	TSKguardcolumn SuperMP(PW)-N	4.6×3.5	TSKgel SuperMultiporePW-N
0022794	TSKguardcolumn SuperMP(PW)-M		TSKgel SuperMultiporePW-M
0022795	TSKguardcolumn SuperMP(PW)-H		TSKgel SuperMultiporePW-H
0022796	TSKguardcolumn SuperOligoPW		TSKgel SuperOligoPW

11-3 Guard Column Replacement

Since the guard column has limited adsorption capacity, it has a finite lifetime.

The guard column must be replaced before contamination extends to the main analytical column.

The frequency of the guard column replacement can not be standardized because it depends on various factors such as application, sample properties (properties of principal components, properties and concentrations of impurities, etc.), sample loading, solvents, flow rate, etc.

Since an increase in the system pressure during operation could indicate clogging at the end fitting of the guard column or contamination of the gel, it is a good idea to replace the guard column when the pressure has increased.

In general, when changes in the results are observed, the guard column should be replaced immediately.

12. Troubleshooting

If the following problems occur during the use of TSKgel SuperMultiporePW Series and TSKgel SuperOligoPW columns, follow the procedures described below and take appropriate measures. Resolution close to the original state may be

recovered if appropriate measures are taken. However, if the cause is due to column life, adsorbed materials, entry of air, drying, or freezing, the original resolution will not be recovered. Therefore, handle these columns with care.

12-1 If There Is a Drastic Decrease in Resolution

Measure the number of theoretical plates(N) in the column. If the presence of adsorbed materials is unlikely and the N is normal, the sample may be the cause. Prepare a new sample.

If the N is abnormal, deterioration in column performance may be the cause. Measure the number of theoretical plates in each column. Apply end plugs to end fittings so that air is not introduced into the respective columns.

Drastic decreases in the resolution of a column system may be due to any of the causes listed in Section 12-2.

12-2 Column Cleaning

Continuous column operation may lead to gradual accumulation of strongly ionic compounds or hydrophobic compounds.

This is demonstrated by changes in chromatographic behavior and loss of resolution. Adsorbed materials may be removed from the column by injections of solvent with different properties from the operating mobile phase.

Recommended column cleaning solutions are shown as follows.

Adsorption phenomena and flushing methods

(1) Ionic adsorption (removal of ionic materials)
    Increase salt concentration to obtain an appropriate ionic strength. (less than 0.5 mol/L)

(2) Hydrophobic adsorption (removal of adsorbed hydrophobic materials)
    Use an eluent containing a water-soluble organic solvent.

(3) Adsorption of basic materials
    Use an acidic aqueous solution (acetic acid buffer solution).

If all the above methods are used, deterioration of the column may occur due to frequent solvent changes. Examine the sample and use the most suitable flushing method.

13. Quality Specifications and Warranty

13-1 INSPECTION DATA

The inspection conditions and the results of each individual column are shown on the INSPECTION DATA sheet. The number of theoretical plates is expressed as the number per column.
The inspection results are different for each column.

13-2 Quality Specifications

The shipping specifications for TSKgel SuperMultiporePW Series and TSKgel SuperOligoPW columns are delivered according to the specifications as shown in Table 3.

13-3 Warranty

Upon receiving the column, check that the column is not damaged and test the performance according to Section 10. If the guaranteed specifications in Table 3 can not be obtained, contact a local TOSOH representative within two weeks. Note that the column lifetime is not guaranteed.

Table 3 Guaranteed Specifications

Part No.	Type	Column size mm(I.D.)×cm(L)	Number of theoretical plates (TP/Column)	Asymmetry factor
0022789	TSKgel SuperMultiporePW-N	6.0×15	16,000	0.7~1.6
0022790	TSKgel SuperMultiporePW-M		12,000	
0022791	TSKgel SuperMultiporePW-H		7,000	
0022792	TSKgel SuperOligoPW		16,000	

TOSOH CORPORATION

BIOSCIENCE DIVISION

Shiba-Koen First Bldg.
3-8-2 Shiba, Minato-ku, Tokyo 105-8623, Japan
Phone: +81-3-5427-5180 Fax: +81-3-5427-5220
Web site: http://www.separations.asia.tosohbioscience.com/
HPLC database: www2.tosoh.co.jp/hlc/hlcdb.nsf/StartE?OpenForm

TOSOH BIOSCIENCE LLC

3604 Horizon Drive Suite 100,
King of Prussia, PA 19406, USA
Phone: +1-800-366-4875 Fax: +1-610-272-3028
E-mail: info.tbl@tosoh.com
Web site: http://www.tosohbioscience.com/

TOSOH BIOSCIENCE GmbH

Zettachring 6, 70567 Stuttgart, Germany
Phone: +49-711-132570 Fax: +49-711-1325789
E-mail: info.tbg@tosoh.com
Web site: http://www.tosohbioscience.com/

TOSOH BIOSCIENCE SHANGHAI CO., LTD.

Room 301, Plaza B, No.1289 Yi Shan Road,
Xu Hui District, Shanghai 200233, China
Phone: +86-21-3461-0856 Fax: +86-21-3461-0858
E-mail: info@tosoh.com.cn
Web site: http://www.separations.asia.tosohbioscience.com/

TOSOH ASIA PTE. LTD.

63 Market Street #10-03 Singapore 048942
Phone: +65-6226-5106 Fax: +65-6226-5215
E-mail: info.tsas@tosoh.com
Web site: http://www.separations.asia.tosohbioscience.com/

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