Packed Columns for Aqueous
High Performance GFC
TSKgel PW Type

INSTRUCTION MANUAL

TOSOH

TOSOH CORPORATION

Safety Precautions

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

[Notational Conventions]

Notation	Explanation
⚠WARNING	Alerts the user to the potential for serious injury or death.
⚠ CAUTION	Alerts the user to the potential for damage to hardware or bodily harm.

⚠ WARNING

■**Keep away from fire**
Take proper precautions when using flammable solvents. There is the potential for 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, and corrosion.
When cleaning up a spill, wear appropriate protective gear.

■**Wear eye protection and protective gloves**
Organic solvents and acids are harmful and shuld not come in direct contact with the skin.

■**Handle package with care**
Inappropriate handling may cause rupturing and spattering.

■**Only use this product as intended**
This product is for separation and purification, do not use for any other purpose.

■**Confirm compounds are safe**
Check that obtained compounds and solutions after separation and purification are safe.

■**Proper disposal**
Dispose of in accordance with local laws and regulations.

NOTE

Keep this manual with the product for future reference.

Table of Contents

1. Introduction

The TSKgel PW type columns are high performance packed columns developed by TOSOH Co.,Ltd. for use in aqueous high performance GFC for various water-soluble substances. These columns were designed for analytical and preparative separation of synthesized water-soluble polymers, oligomers and biological substances such as polysaccharides, nucliec acids, proteins, peptides, etc.

Please read this Instruction Manual carefully for correct and effective use of these high performance columns.

Table 1 Application

Application	Suitable columns
Water-soluble synthetic polymers Water-soluble biopolymers Polysaccharides	G4000PW(XL), G5000PW(XL), G6000PW(XL), GMPW(XL)
Water-soluble oligomers Peptides	G2500PW(XL), G3000PW(XL)
Nonionic oligomers (oligosaccharides)	G-Oligo-PW, G2000PW
Nucleic acids	G-DNA-PW

Note: TSKgel G-Oligo-PW and G2000PW absorb ionic samples over a wide range of pH, so restrict their use to the measurement of nonionic oligomers.

2. Unpacking

Check that nothing is wrong with the package or appearance of the column.

Fig.1 Appearance of the Package

Confirm that the following documents are attached to the column:

1 copy Instruction Manual

1 copy Inspection Data

3. Column Parts

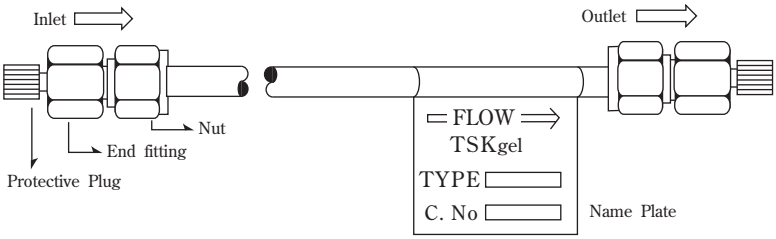

Fig.2 Column parts

4. Installation

4-1 Connections

All connections are of the swage lock type and scaled to inch measurements.

4-2 Flow Direction

Use the column in the direction shown with an arrow on the tag in Fig. 2.
Flow in the reverse direction for a long time will cause degradation of the column performance.

4-3 Prevention of Bubble Entrance into Column

Be careful not to admit any bubble into the column at the time of its installation or removal from the equipment. Install the column only after removing bubbles from all piping. Admitting bubbles into the column will cause degradation of its performance through occurrence of channeling, etc.

4-4 Installation

When the solvent oozes from the end fitting at the time of removing the cap on the inlet side of the column, connect the column to the equipment carefully as mentioned above so that no bubble will be brought into the column.

When no solvent oozes from the inlet side of the column, connect the outlet side to the equipment and send the solvent with the feed pump in order to expel air. (Feed the solvent slowly in this case, since rapid pressurization or solvent feeding may cause degradation of column Performance.) After confirming solvent leakage without any bubble at the inlet side of the column, arrange the column in the direction of normal flow and connect the inlet side to the injector.

4-5 Connection of Columns in Series

When a number of columns are connected in series, connect them as described above in sequence of descending pore size in order to separate first the higher molecules which tend to cause overloading effect. Interconnecting tubing should be inserted fully into the compressor fittings before tightening in order to minimize dead volume. Finally, connect the outlet end of the last column to the detector.

4-6 Prior to Measurement

After installation of the columns, mesurement can begin. Rapid pressurization or solvent feeding must be avoided as mentioned above, since they may cause degradation of the column performance. Be careful especially when a feed pump showing rapid pressure increase is applied.

4-7 Prevention of Pulsatory Flow

This type of column is easily affected by pulsatory flow of the solvent. A pump with no fluctuation must be applied. If applying a pump with pulsation, connect a pulse damper (accumulator) to the outlet side of the pump in order to compensate the pulsation. The damper must be highly resistant to corrosion.

5. Maintenance

5-1 Measurement at higher Temperature than Room Temperature

Do not stop the pump immediately after finishing the measurement. Continue to feed solvent until the column temperature lower to the room temperature. If the pump is stopped while the column is hot, air may be sucked into the column by contraction the solvent.

5-2 Routine Use

If the column is to be used in daily operation, it is permissible to leave buffer in the column overnight if the buffer is not corrosive. If halides are unavoidable, it is better to replace the buffer with distilled water even for one night.

5-3 Storage for a Short Period (Several Days)

When the columns will be used again after a short period, remove the columns from the equipment and seal them with the protective plugs providing that the solvent does not contain any corrosive ions. When the solvent contains corrosive ions like halogen, rinse the column with distilled water before storage.

5-4 Storage for a Long Period

When the columns will not be used for longer period, replace the solvent with distilled water. Then remove the columns from the equipment and seal both their ends with the protective plugs. Addition of sodium azide (0.05%) to the water is recommended for very long period storage (for example, more then 3 months).

5-5 Storage Temperature

Store the columns at room temperature. The columns may freeze and their efficiency may degrade if they are left at a temperature below 0℃.

5-6 Exposure to Direct Sunlight

Avoid exposure to direct sunlight.

5-7 Corrosive Gases

Store at a safty place from corrosive gases.

6. Solvents

6-1 Replace with solvent to be used

The TSKgel PW type columns are filled with distilled water on delivery. Replace this with the solvent to be used. Solvent replacement must be carried out with the flow rate below the value shown in table 2. Furthermore, since frequent solvent exchange accelerates degradation of column efficiency, use the same solvent as long as possible.

Table 2 Flow rates for Replacement of Solvents

Column	Column Size (mm×cm)	Max. flow rate
PW	21.5×30, 21.5×60	3.0 ml/min
PW	7.5×30, 7.5×60	0.5 ml/min
PW_{XL}	7.8×30	0.3 ml/min
G-Oligo-PW	7.8×30	0.3 ml/min
G-DNA-PW	7.8×30	0.3 ml/min

6-2 Selection of Solvent

It is necessary to select the solvent composition from the view points of column stability, solubility of samples, elimination of interaction between sample and support, etc.

6-2-1 Available pH Range

The PW type gels are usable over the relatively wide pH range of 2.0～12.0 at room temperature.

They are excellent in stability in the higher pH region, compared with SW type gels using silica gel as the base material which 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 measured with distilled water. it is general to carry out the measurement with salt aqueous solutions or buffer solutions, considering presence of ionic impurities, which interact with the support. The representative available solutions are shown below.

Available aqueous solutions.

Salt aqueous solutions: Sodium sulfate aqueous solution, sodium acetate aqueous solution, sodium dihydrogenphosphate aqueous solution, ammonium acetate aqueous solution, ammonium formate aqueous solution.

Buffer solutions : Phosphate buffer, tris hydrochloric acid buffer, tris acetate buffer, tris phosphate buffer, citrate buffer, actate buffer.

The salt concentration is generally adjusted to below 0.5 mol/L in order to avoid viscosity rise through salt addition and salt precipitation due to temperature

change, etc. Furthermore, avoid use of halogen ions as much as possible to prolong the stainless column life. If measurement has been carried out with such salt aqueous solutions, rinse the columns according to item 5-3 after finishing the measurement.

6-2-3 Organic Solvents

The PW type columns are compatible with aqueous solutions of water-soluble organic solvents such as methanol, ethanol and acetonitrile.

The PW type 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 and etc.

In related with the concentration of organic solvents, it is preferable to use the solvents of its concentration ratio under 20% in consideration of support swelling.

However, it is possible use 50% organic solbents by carrying out the changing of solvent carefully. When the concentration of the organic solvent in the eluent is changed, it should be changed gradually by reducing the flow rate (preferably with the gadient) as rapid change may cause degradation of column efficiency. In case of drastic change of the concentration, (ex. 0%→30～50%), it should be done by gradient method.

6-3 Purification

The solvent must be filtered with micropore-filter (0.5 μm) to prevent from clogging at the top of the column.

6-4 Degassing

There is a possibility of generating bubbles from the solvent at the time of replacing solvents (especially with an organic solvent-added system). Degas the solvent sufficiently to avoid such bubble generation.

7. Flow Rate

7-1 Determination of Flow Rate

The flow rate must be determined in consideration of resolution analytical time column life, etc. With a higher flow rate the time required for measurement becomes shorter, but resolution improves with a lower flow rate. This tendency

becomes remarkable with large molecules. From the viewpoint of column durability lower flow rate is preferable and has the advantage that top-off (the phenomenon of generating a gap on the inlet side of the column) does not readily take place.

7-2 Suitable Flow Rate

The suitable flow rate and the pressure loss in the measurement in an ordinary aqueous solution. Do not use the column at a flow rate and pressure above maximum shown in Table 3.

Table 3 Flow rates

Type	Column size (mm×cm)	Suitable flow rate (ml/min)	Max. flow rate (ml/min)	Max. pressure (MPa)
TSKgel G2000PW TSKgel G2500PW TSKgel G3000PW	7.5×30 7.5×60	0.5～1.0	1.2	2.0 (30-cm column) 4.0 (60-cm column)
TSKgel G4000PW TSKgel G5000PW TSKgel G6000PW TSKgel GMPW	7.5×30 7.5×60	0.5～1.0	1.2	1.0 (30-cm column) 2.0 (60-cm column)
TSKgel G2500PW$_{XL}$ TSKgel G3000PW$_{XL}$	7.8×30	0.5～0.8	1.0	4.0
TSKgel G4000PW$_{XL}$ TSKgel G5000PW$_{XL}$ TSKgel G6000PW$_{XL}$ TSKgel GMPW$_{XL}$	7.8×30	0.3～0.6	1.0	2.0
TSKgel G2000PW TSKgel G2500PW TSKgel G3000PW TSKgel G4000PW TSKgel G5000PW TSKgel G6000PW	21.5×60	1.0～6.0	8.0	2.0
TSKgel G-Oligo-PW	7.8×30	0.5～0.8	1.0	4.0
TSKgel G-DNA-PW	7.8×30	0.2～0.5	1.0	2.0

Note: These flow rate should be used for distiled water or aqueous solvents which have similar viscosity of distilled water.
If solvent of higher viscosity was used, the flow rate should be lowered.

8. Temperature

8-1 Temperature Range

Use the PW type columns at temperaure range 10℃ to 80℃ (neutral aqueous solutions).

8-2 Measurement at High Temperature

Use the solvent after sufficient degassing. After finishing the measurement at high temperature, follow the instructions given in item 5-1.

8-3 Advantages of Measurement at High Temperature

The advantages of measurement at high temperature consist of the following points:

i) The viscosity can be reduced by elevating the solvent temperature.
ii) The number of theorerical plates increases and resolution improves in comparison with measurement at room temperature.
iii) The adsorptivity can be reduced.

8-4 Measurement at Temperatures below Room Temperature

In this case disadvantages contrary to the advantages stated above appear. Furthermore, since the viscosity of a solvent or sample becomes higher, it is necessary to keep the flow rate lower than in operation at room temperature.

9. Sample Preparation

9-1 Preparation of Sample Solution

Prepare a sample solution by dissolving the sample into the solvent to used as an eluent. If the eluent cannot be used for sample resolution. adjust factors like pH, salt concentration, etc. To those of the eluent as far as possible in order to avoid unexpected precipitation of salt.

9-2 Filtration of Insoluble Particles

Filter the sample solution with a micropore-filter (0.5 μm). Even if nothing can be seen, insoluble particles exist in the sample solution in many cases.

10. Calculation of the Number of Theoretical Plates and Asymmetry Factor

The number of theoretical palates (N), Asymmetry factor (As) and their chromatographic conditions are as shown in the inspection Data.

10-1 Calcutating Method for Number of Theoretical plates

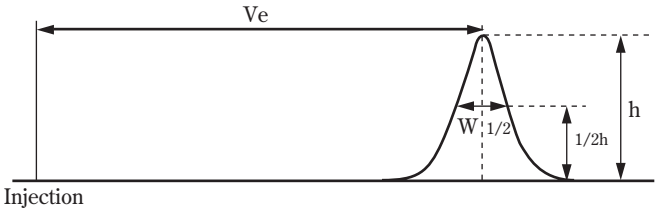

Fig. 3 Calculating method for number of theoretical plates

The number of theoretical plates of a column is calculated by the half peak width method as shown in Fig. 3 and the following equation.

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

Ve : Elution time (min)

$W_{1/2}$: Half width value of peak (min)

h : Peak height

N : Number of theoretical plates/column

10-2 Calculation Method for Asymmetry Factor

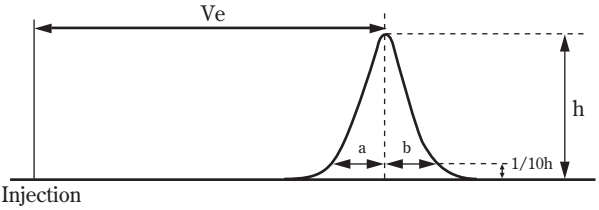

Fig. 4 Calculating method for Asymmetry factor

The Asymmetry factor of a columm (As) is calculated by 1/10h method.

As = b/a

10-3 Dead Volume

N and As should be measured with an instrument of sufficiently small dead volume. Indication of a lower number of theoretical plates than the standard value may be caused by too large a dead volume or increased injection volume.

11. Guard Column

Fundamental keys for preventing trouble are covered in items 4 to 9. But when impurities tending to be adsorbed by the packing material are present in a sample solution, they are adsorbed on the inlet side of the column and accumulate gradually, causing reduction of the number of theoretical plates and degradation of column efficiency. In such cases it is possible to restore the original column efficiency by connecting a guard column before the column and replacing it in the case of efficiency degradation by adsorbed material. Utilize a guard column as far as possible in order to prevent possible trouble more surely.

However, the guard column is not for analysis. No improvement of resolution can be expected by connecting a guard column. Utilize it only for preventing trouble.

11-1 Effects of Guard Column Installation

1) Prevention of top-off due to pump pulsation, abnormal flow rate and pressure rise.
2) Prevention of contamination of the main column by cutting off adsorptive material.
3) Protection of the main column by cutting off insoluble substances.

11-2 Kinds and Selection of Guard Column

Table 4 gives specifications of guard columns. They differ depending on sized and types of the PW columns. Select them correctly as shown in Table 4 in order to obtain the best performance from your column system.

Table 4 Kinds of Guard Column

Part No.	Type	size (mmID×cm)	Solvent	Applicable column
06763	TSK guardcolumn PWL	7.5×7.5	H_2O	for G2000PW(Analytical)
06762	TSK guardcolumn PWH	7.5×7.5		for G2500PW~G6000PW, GMPW(Analytical)
06757	TSK guardcolumn PWL	21.5×7.5		for G2000PW(Preparative)
06758	TSK guardcolumn PWH	21.5×7.5		for G2500PW~G6000PW(Preparative)
08033	TSK guardcolumn PW_{XL}	6.0×4.0		for G2500PW_{XL}~G6000PW_{XL}, GMPW_{XL}
08034	TSK guardcolumn Oligo	6.0×4.0		for G~Oligo~PW

11-3 Guard Column Replacement

Since the guard column has a limit of corresponding adsorbing capacity, it has a definite life. The guard column must be replaced before contamination extends to the main column.

The replacement frequency can not be standardised because it depends on various factors such as application (analysis or preparative separation), sample properties (properties of principlal components, properties and concentrations of impurities, etc.), sample loading amount, solvents, flow rates, etc.

Since pressure rise during the operation means clogging at the end fitting of the guard column or contamination of the gel, it is good to replace the guard column when the pressure rises to some extent. In general, when some change on meassured data is observed, replace the guard column immediately.

11-4 Guard Column Regeneration

The guard column can be regenerated by flushing adsorbed material with at least one of the following solvents.

1) Buffer of high salt concentration (0.5mol/L～1.0mol/L)
2) Buffer of low pH (pH2～3) or high pH (pH9～12)
3) Buffer containing an organic modifier such as methanol, or acetonitrile.
4) Buffer containing a solubilizer such as urea, SDS or other surfactants.

The guard column can be regenerated and used repeatedly.

12. Troubleshooting

Most trouble can be avoided by careful operation according to the instructions in items 4～9 and 11. Particularly, apporopriate utilization of a guardcolumn is

very effective. However, if trouble happens, follow the procedure described below.

12-1 Clogging of Filter

This is evidenced by a decline in pressure or flow rate. In this case, clean the fitting by reversing flow through the column (the flow must be kept below the value shown in Table 2). If the clogging can not be removed, change the end fitting as follows.

a) Prepare a new end fitting and carefully remove the clogged end fitting from the column.
b) Be careful not to loose the gel. Transfer the gel remaind in the old end fitting into the new one.
c) Attach the new end fitting to the colume.
b) Expel air from the inlet side by connecting the column reversely to the pumping system (Refer to the item 4-4.).
e) Connect the column in the normal direction and test the efficiency by measuring the number of theoretical plates and asymmetry factor.

12-2 Contamination

Prolonged operation with complex mixtures may lead to the gradual accumulation of strongly ionic compounds or hydrophobic compounds. this is evidenced by changes in chromatographic behavior and loss of resolution. Adsorbed material may be stripped from the column by flushing with at least one of the solvents described in item 11-4.

12-3 Void in the Inlet Side End of Column

There is a case of forming a void in the inlet side end of column if the application of pressure is abrupt. the flow rate exceeds the specified maximum. prevention against pulsation of the solvent flow described in 4-7 is not provided, or the solvent replacement has been frequent. In this case the number of theoretical plates will be greatly reduced (to 30% or less of the nomal number of plates of columns of all grades) and the shape of peaks in the chromatogram of monodispersed sample will show a good deal of tailing. Dismount the column and examine for the void and, if topped off, feed the top-off gel to the column top. This could recover the column performance and, if this remedy is not successful, the column can no longer be regenerated and

shall be replaced with a new column. As shown in Table 5, the topoff gel comes in two types, TSKtopoffgel PW_{XL} and TSKtopoffgel PW. Use the former on PW_{XL} type columns and the latter on PW type columns.

Table 5 Top off gel

Part No.	Type	Gel volume
08035	TSK topoffgel PW	1 ml
08036	TSK topoffgel PW_{XL}	1 ml

13. Quality Specifications and Warranty

13-1 Inspection Data

The inspection conditions and results are shown in the Inspection Data. The number of theoretical plates is expressed as that per column.

13-2 Quality Speciffcations

The PW type packed columns are delivered according to the following specifications. (See Table 6).

13-3 Warranty

Immediately after receipt, check the appearance of the column and test the performance according to the item 10. If the guaranteed specifications in Table 6 can not be obtained, contact your TOSOH Co.,Ltd. reperesentative within two weeks. Note that column life is not guaranteed.

Table 6 TSKgel PW type

Part No.	Type	column size (mmID×cm)	Number of theoretical plates (TP/Column)	Asymmetry factor	Solvent
05761	TSKgel G2000PW	7.5×30	5,000	0.7～1.6	H_2O
08028	TSKgel G2500PW		5,000		
05762	TSKgel G3000PW		5,000		
05763	TSKgel G4000PW		3,000		
05764	TSKgel G5000PW		3,000		
05765	TSKgel G6000PW		3,000		
08026	TSKgel GMPW		3,000		
05105	TSKgel G2000PW	7.5×60	10,000	0.7～1.6	H_2O
08029	TSKgel G2500PW		10,000		
05106	TSKgel G3000PW		10,000		
05107	TSKgel G4000PW		6,000		
05108	TSKgel G5000PW		6,000		
05109	TSKgel G6000PW		6,000		
08027	TSKgel GMPW		6,000		
05150	TSKgel G2000PW	21.5×60	10,000	0.7～1.6	H_2O
08030	TSKgel G2500PW		10,000		
05151	TSKgel G3000PW		10,000		
05152	TSKgel G4000PW		6,000		
05153	TSKgel G5000PW		6,000		
05154	TSKgel G6000PW		6,000		
08020	TSKgel G2500PW$_{XL}$	7.8×30	16,000	0.7～1.6	H_2O
08021	TSKgel G3000PW$_{XL}$		16,000		
08022	TSKgel G4000PW$_{XL}$		10,000		
08023	TSKgel G5000PW$_{XL}$		10,000		
08024	TSKgel G6000PW$_{XL}$		7,000		
08025	TSKgel GMPW$_{XL}$		7,000		
08031	TSKgel G-Oligo-PW	7.8×30	16,000	0.7～1.6	H_2O
08032	TSKgel G-DNA-PW	7.8×30	10,000	0.7～1.6	H_2O

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/

T1302-500