

**Packed Column for Aqueous  
High Performance GFC  
TSK<sub>gel</sub> SW Type**

# **INSTRUCTION MANUAL**



**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
 <b>WARNING</b>	Alerts the user to the potential for serious injury or death.
 <b>CAUTION</b>	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 should 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.

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## 1. Introduction

A packed column TSKgel SW is for aqueous high performance GFC.

This column has been designed for analytical and preparative separation of various water soluble substances such as proteins, enzymes and nucleic acids.

We have both Steel column and Glass column.

Especially Glass column is biocompatible type which consists of a high precise glass tube with plastic end fittings.

This Instruction Manual contains crucial information on how to care for and use these columns in the proper manner, so as to make most effective use of their high performance capabilities.

## 2. Unpacking

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



Fig.1 Appearance of the package

Then check that the following documents are attached to the column:

- 1 copy Instruction Manual
- 1 copy Inspection Data

### 3. Column Parts

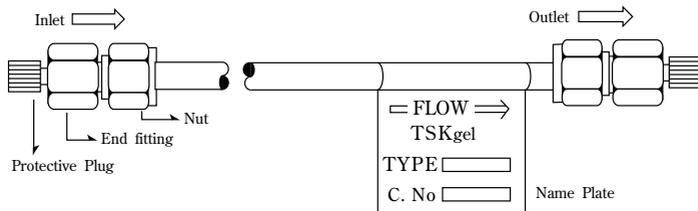


Fig.2 Steel Column

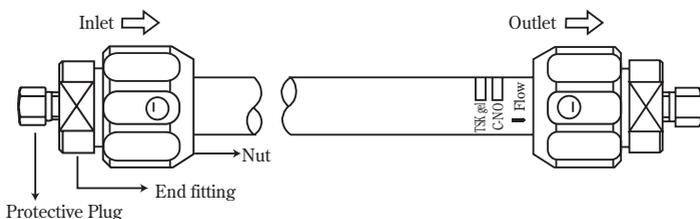


Fig.3 Glass Column

## 4. Installation and Safety Considerations

### 4.1 Connections

The connections of Steel columns are of the swage lock type and that of Glass columns are 1/4-28 UNF flange type joints.

### 4.2 Flow Direction

Use the column in the direction shown by the arrow on the tag in Fig.2 & 3. Operating the column with the flow in the reverse direction for a long time will cause degradation of column performance.

### 4.3 Prevention of Bubbles

Be careful not to admit any bubbles into the column during its installation or removal from the equipment. Always remove all bubbles from all pipings before installing the column.

Admitting bubbles into the column will cause degradation of its performance through the occurrence of channeling, etc.

#### 4.4 Installation

If solvent leaks from the end fitting when the cap on the inlet side of the column is removed, connect the column to the equipment carefully, as mentioned above, so that no bubbles will be introduced into the column. If no solvent leaks from the inlet side of the column, connect the outlet side to the equipment and feed solvent through the column in the reverse direction with the feed pump in order to expel the air.

Feed the solvent slowly (i.e. below the flow rate shown in Table 1) in this case, since rapid pressurization or solvent feeding may cause degradation of column performance.

After confirming bubble-free solvent leakage 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 molecular weight molecules which tend to cause overloading.

For a glass column, do not use the column at the condition that the pressure at the top end is above 3.0 MPa to prevent the damage of the column.

Interconnecting tubing should be inserted fully into the end fitting before tightening in order to minimize dead volume.

Finally, connect the outlet end of the column to the detector.

#### 4.6 Prior to Measurement

After installation of the column, measurement can begin. As mentioned above, rapid pressurization or solvent feeding must be avoided, since these may cause degradation of column performance.

Be especially careful when using a feed pump with a rapid pressure rise.

#### 4.7 Prevention of Pulsatory flow

This type of column is easily affected by pulsatory flow of the solvent.

Preferably, a pump with no fluctuation should be used.

If a pump with pulsation must be used, connect a pulse damper (accumulator) to the outlet side of the pump in order to compensate for the pulsation.

The damper must be highly resistant to corrosion.

#### 4.8 Daily Routine Use

If the column is being used daily, you need not remove the column from the

equipment and the buffer may be left in the column overnight.

#### 4.9 Short-Term Storage (Several Days)

When the columns will be used in several days, remove the column from the equipment and seal the ends of column with the protective screws.

#### 4.10 Long-Term Storage

When the columns are not to be used again soon the column treatment mentioned above is unsatisfactory, since growth of microorganism and/or corrosion by a corrosive buffer may result in column deterioration.

For long-term storage, replace the solvent in the columns with an aqueous solution or buffer containing 0.05% sodium azide at the flow rate described in Table 1.

For a Steel columns, no corrosive solvent should be used.

**Table 1 Recommended Flow Rate for Solvent Exchange**

Column Types	Column Sizes mm (ID×cm (L))	Flow Rate (ml/min)
Steel Column SuperSW	4.6×30, 4.6×3.5	0.2
Steel Column SW <sub>XL</sub>	7.8×30, 6.0×4.0	0.5
Steel Column SW	7.5×30, 7.5×60, 7.5×7.5	0.5
Steel Column SW	21.5×30, 21.5×60, 21.5×7.5	3.0
Glass Column SW	8.0×30	0.5
Glass Column SW	20.0×30	5.0

## 5. Storage of Column

### 5.1 Storage Method

Refer to Section 4.9 & 4.10

### 5.2 Storage Temperature

Store the column preferably at constant temperature in the range of 4°C to 30°C. Do not Store the column below 4°C not to freeze the solvent in the column.

### 5.3 Exposure to Direct Sunlight

Avoid exposing the column to direct sunlight.

#### 5.4 Corrosive Gases

Store the column in a place safe from corrosive gases.

## 6. Solvents

### 6.1 Replacement of solvents

SW type columns are filled with 0.1 mol/L phosphate buffer (pH6.7) containing 0.05% sodium azide and 0.1 mol/L Na<sub>2</sub>SO<sub>4</sub> for shipment.

Replace this solvent with an appropriate solvent, using an appropriate flow rate shown in Table 1.

Since frequent solvent replacement accelerate degradation of column efficiency, use the same solvent as long as possible.

### 6.2 Solvent Selection

In selecting the solvent composition certain considerations such as column stability, sample solubility and elimination of the interaction between sample and gel, are crucial.

#### 6.2.1 pH Range

SW type gel will gradually dissolve under high pH conditions, since silicagel is employed as the base material.

The use of low pH solvents is limited by the stability of the gel itself and of steel columns.

Therefore, use a solvent with a pH between 2.5 and 7.5.

#### 6.2.2 Aqueous Salt Solutions and Buffer Solutions

SW type gel is stable in an aqueous salt solution or buffer solution.

Ideally, in GFC, sample are separated only according to molecular size, with no interaction between the sample and the gel.

Since ionic materials are usually present in samples, measurements are ordinarily performed using aqueous salt solutions or buffer solutions in order to suppress the ionic interactions between the samples and the gel.

**Table 2 Typical Buffer & Aqueous Solvents**

Phosphate buffer, Tris-acetate buffer (Alkali) Citrate buffer (Alkali) Acetate buffer Sodium sulfate aqueous solution, Ammonium acetate aqueous solution, Potassium dihydrogenphosphate aqueous solution, Ammonium formate aqueous solution.
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The salt concentration of aqueous solutions should generally be kept below 0.5 mol/L in order to avoid a rise in viscosity (due to addition of salts) and in order to avoid precipitation (due to temperature changes).

Furthermore, for a steel column halogen ions should be avoided as far as possible, in order to prolong the lifetime of the columns.

### 6.2.3 Organic Solvents

SW type columns are used not only with aqueous solutions, such as those described above, but also with water-soluble organic solvents such as methanol or acetonitrile, and their aqueous solutions.

However, solvent viscosity must be taken sufficiently into consideration as an important factor in determining the flow rate.

To replace the solvent used in shipment (a phosphate-salt buffer) with an organic solvent to be used in separation, first replace it with distilled water, and then replace the water with the organic solvent.

To change the concentration of an organic solvent in a solution, reduce the flow rate and preferably apply a gradient so that the organic solvent concentration change gradually.

A rapid change in organic solvent concentration may cause degradation of column efficiency. Also be careful to avoid salt precipitation when adding salts to aqueous solutions containing organic solvent.

### 6.2.4 Solubilizing Agents and Protein Denaturants

SW type column can be used with aqueous solutions containing solubilizing agents or protein denaturants, such as SDS, guanidine hydrochloride, or urea.

These solutions can be very effective in the measurement of sample such as proteins which have poor solubility in an aqueous solution.

However, there is a tendency toward shorter column life with such systems than with standard aqueous solution.

Avoid using a column that has been used with such a system or later with other systems.

Furthermore, be careful in selecting the column grade (pore size) for the

measurement of denatured proteins since their molecular size is larger than that of their native forms.

### 6.3 Elimination of Insoluble Matter

The presence of insoluble matter in the solvent can cause problems such as a raise in pressure (due to clogging of the column filter) or top-off (the phenomenon in which a gap is generated at the inlet side of the column).

To prevent these kinds of problems, the solutions should be purified.

This can be accomplished by filtering the solution through a micro-pore filter (of eg. 0.45  $\mu$  m pore size) either before use or during use by attaching the filter to the suction or delivery side of the pump.

### 6.4 Degassing

Bubbles may be generated in the solvent during solvent replacement (especially when switching to a system containing an organic solvent).

The solvent should be thoroughly degassed before use to avoid the possibility of subsequent bubble formation.

## 7. Flow Rates

### 7.1 Choice of Flow Rates

Factors such as resolution, analytical time and column life should be carefully considered in selecting flow rates.

A higher flow rate results in a shorter analytical time.

Conversely a lower flow rate results in improved column efficiency, especially in the case of large molecules such as proteins.

Furthermore, a lower flow rate tends to extend column life and to decrease the occurrence of top-off.

### 7.2 Recommended Flow Rates

Recommend flow rates of SW columns are shown in Table 3.

Do not use these column with flow rates and pressure-drops over the maximums shown in Table 3.

#### Caution :

On the glass column's application in series, the pressure at the top of the column should be lower than 3.0 MPa to prevent the damage of the column and the leakage of the gel at the end fitting.

**Table 3 Recommended Flow Rates**

Column Types	Column Sizes mm (ID) ×cm (L)	Recommend Flow Rates (ml/min)	Max Flow Rates (ml/min)	Max. Pressure-drops /Column (MPa)	
				Column Length 30cm	60cm
〈Steel〉 SuperSW2000 SuperSW3000	4.6×30	0.10~0.35	0.40	12.0	
G2000SW <sub>XL</sub>				7.0	
G3000SW <sub>XL</sub>	7.8×30	0.5~1.0	1.2	7.0	
G4000SW <sub>XL</sub>				3.5	
G2000SW				2.0	4.0
G3000SW	7.5×60,60	0.5~1.0	1.2	2.5	5.0
G4000SW				1.5	3.0
G2000SW				1.0	2.0
G3000SW	21.5×30,60	3.0~6.0	8.0	1.5	3.0
G4000SW				1.0	2.0
〈Glass〉 G2000SW				2.0	
G3000SW	8.0×30	0.4~0.8	0.8	2.0	
G4000SW				2.0	
G3000SW	20.0×30	4.0~6.0	8.0	0.8	

Note: These flow rates are attainable with the buffers or aqueous solvents having approximately the same viscosity as the shipping solvent (0.1 mol/L phosphate buffer containing 0.05% sodium azide and 0.1 mol/L Na<sub>2</sub>SO<sub>4</sub>)

### 7.3 Solvent Flow Rate

With a less viscous of the solvent a higher flow rate can be used.

When using a higher viscosity solvent, keep the flow rate at a lower level.

Select a lower flow rate in the case of using a water-alcohol solvent, guanidine hydrochloride or aqueous urea system.

## 8. Operating and Storing Temperature

### 8.1 Operating Temperature

The optimal operating temperature for SW type columns, both analytical and preparative, is between 10°C and 30°C.

Below 10°C use a lower flow rate to protect the columns.

### 8.2 Storing Temperature

Store the column at room temperature. Never store the column below 4°C since the solvents in the columns may freeze.

## 9. Preparation of a Sample Solution

### 9.1 Sample Solution

Prepare the sample solution immediately before use by dissolving the sample in the solvent that is to be used as an eluent.

### 9.2 Elimination of Insoluble Matter

Always purify the sample solution either by centrifugation or preferably by micro-pore filtration (of eg. 0.45  $\mu$  m pore size).

Even if nothing can be seen in the sample solution, insoluble matter may be present.

### 9.3 Sample Solution Composition

Adjust the pH and concentrations of salt and organic solvent in a sample as closely as possible to those of the eluent.

This is especially essential when the injection volume of a sample is large. A sample solution should be applied if it will from insoluble matter when mixed with the eluent.

## 10. Column Efficiency

The number of theoretical plates (N), the asymmetry factor (As) and their chromatographic conditions are as shown in the Inspection Data.

### 10.1 Method of Calculating the Number of Theoretical Plates

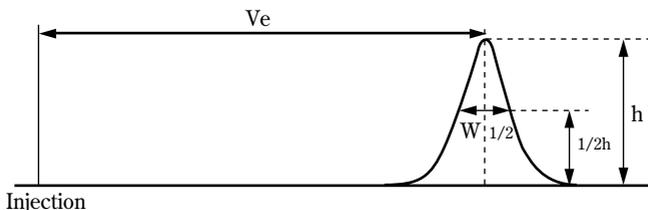


Fig.4 Method of Calculating the Number of Theoretical Plates

The number of theoretical plates of a column (N) is calculated by the half peak width method shown in Fig.4 and the following equation.

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

V<sub>e</sub> : Elution volume (min)

W<sub>1/2</sub> : Half width value of peak (min)

h : Peak height

### 10.2 Method of Calculating the Asymmetry Factor

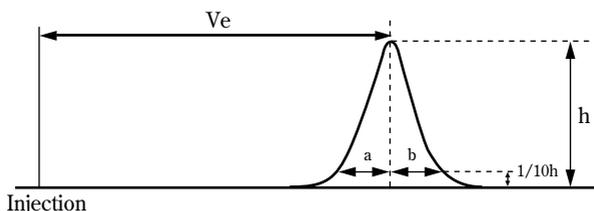


Fig.5 Method of Calculating the Asymmetry Factor

The asymmetry factor of a column (As) is calculated by the 1/10h method.

$$As = b/a$$

### 10.3 Dead Volume

If the dead volume of the equipment or the injection volume of a sample solution becomes too large, the number of theoretical plates may decrease.

## 11. Guard Column

Fundamental keys problem prevention have been outlined in Section 4 to 9. But when impurities that tend to be adsorbed by the packing material are present in a sample, 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 the original column efficiency can be maintained by connecting a guard column before the column and replacing it if efficiency becomes degraded by the adsorption of material to the guard column.

For maximum insurance against such problems, a guard column should be used as much as possible.

However, a guard column should not be used for analysis.

No improvement in resolution can be expected by connecting a guard column.

Utilize the guard column only to prevent problem

### 11.1 Effect of Guard Column Installation

- 1) Prevention of contamination of the main column by removal of adsorptive material.
- 2) Protection of the main column by removal of insoluble matter.
- 3) Prevention of top-off due to pump pulsation.

### 11.2 Type and Selection of Guard Columns

Guard columns specifications are shown in Table 4.

Table 4 Types and Guard Columns

Cat. No.	Types	Column Sizes mm(ID)×cm(L)	Applied Columns mm(ID)×cm(L)
08543	TSKguardcolumn SW <sub>XL</sub>	6.0×4.0	SUS Column SWXL series (7.8×30)
05371	TSKguardcolumn SW	7.5×7.5	SUS Column SW series (7.5×30,7.5×60)
05758	TSKguardcolumn SW	21.5×21.5	SUS Column SW series (21.5×30,21.5×60)
18762	TSKguardcolumn SuperSW	4.6×3.5	SUS Column SuperSW series (4.6×30)
08805	TSKguardcolumn SW Glass	8.0×4.0	Glass Column SW series (8.0×30)
14465	TSKguardcolumn SW Glass	20.0×4.0	Glass Column SW series (20.0×30)

### 11.3 Guard Column Replacement

Since the guard column has a limited adsorbing capacity, it has a finite lifetime. The guard column must be replaced before contamination extends to the main column.

Replacement frequency can not be standardized because it depends on various factors such as application (analysis or preparative separation), sample properties (properties of principal components, properties and concentrations of impurities, etc.), sample loading, solvents, flow rate, etc.

Since a pressure rise during operation means 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 risen to some extent.

In general, when some change in measured data is observed, the guard column should be replaced immediately.

## 12. Troubleshooting

When using TSKgel column, most problems can be avoided by following these instructions.

But some problems (such as those due to column life, adsorptive materials, production of air bubbles, dried gel, or frozen solvent) can not be corrected once they occur, care should be taken in handling these columns.

### 12.1 Clogging of the End Fitting

In case the pressure-drop increases or the flow rate decreases, the end fitting should be cleaned by reversing the flow through the column.

If the clog can not be removed; the end fitting must be replaced by a new one.

### 12.2 Replacement of End Fitting

Prepare a new end fitting and remove the old end fitting from the column. being very careful not to loose any of the packed gel underneath.

If some gel is lost from the column head, the void should be filled with a slurry of TSKtop-off gel following the instructions in Section 12.3.

Then expel air from the inlet side referring to Section 4.4.

### 12.3 Top-off (Void at a column head)

Top-off can be caused by rapidly increasing the pressure, using a flow rate faster than the defined limit, a pulsatory flow, frequent solvent exchange, etc. Top-off decreases drastically the number of theoretical plates (N)

(such columns typically have less than 70% as many as a normal column) and

tends to cause tailing of peaks.

If these phenomena are observed, remove the end fitting from the inlet side of the column, and if a void is found, fill it with TSKgel top-off gel.

On this occasion, especially for the glass column, the end fitting must be tightened at the force of 2.9 N · m by a torque-wrench.

The higher resolution should be recovered. If, by some unfortunate circumstance, the resolution can not be recovered by this procedure, please exchange the column with a new one.

**Table 5 TSKgel top-off gel Specifications**

Cat.No.	Types	Gel Volume
08544	TSKtop-off gel SWXL	1 ml
06819	TSKtop-off gel SW	1 ml

**Note :** The solvent used in shipment is an 0.05% aqueous solution of sodium azide

#### 12.4 Never Eluted or Eluted Late

After repeated and long-term use of the column, the elution time is occasionally changed extremely. In this case cleaning the column with a different kind of solvent is effective.

The following are some typical adsorptions with their cleaning methods.

1) Ionic adsorption (eliminate ionic substance)

Increase the salt concentration of the solvent, and adjust the ionic strength appropriately.

2) Hydrophobic adsorption (Eliminate hydrophobic substances)

Use aqueous organic solvents such as :

0.05% phosphate buffer +30% acetonitrile solution

0.05% phosphate buffer +methanol solution

0.05% phosphate buffer +dioxane solution

3) Hydrogen bond adsorption

(Eliminate the poorly soluble protein or other material)

Add urea to the solvent.

4) Adsorption of alkaline substances

Use an acidic aqueous solution such as phosphate buffer (pH 2.5).

**Note :** These cleaning methods are very severe for the columns and the frequent solvent exchanges may result in column deterioration.

So the solvent exchange should be restricted to essential cases.

## 13. Quality Specifications and Warranty

### 13.1 Inspection Data

The results of each column inspection are described in the Inspection Data enclosed the column package.

In the Inspection Data, N is expressed as that per column.

The conditions used in determining the Inspection Data are follows :

#### 13.1.1 Solvent used for the inspection and shipping

0.1 mol/L phosphate buffer + 0.1 mol/L Na<sub>2</sub>SO<sub>4</sub> + 0.05% NaN<sub>3</sub>(pH 6.7)

#### 13.1.2 Flow Rates vs Column's ID

Column Types	Flow Rates (ml/min)	Column's ID (mm)
Steel Column	0.35	4.6
	1.0	7.5 & 7.8
	6.0	21.5
Glass Column	0.8	8.0
	6.0	20.0

#### 13.1.3 Samples and their Concentrations for Inspection

Table 6 Samples for Inspection

Types		Concentrations
TSKgel G2000SW(XL), SuperSW2000	Thyroglobulin(Bovine Type I )	0.2 g/L
	Albumin(Bovine Serum)	1.0
	Ribonuclease-A(Bovine Pancreas)	1.0
	p-Aminobenzoic Acid	0.01
TSKgel G3000SW(XL), SuperSW3000	Thyroglobulin(Bovine Type I )	0.5 g/L
	$\gamma$ -Globulin(Bovine Cohn Fraction II)	1.0
	Ovalbumin	1.0
	Ribonuclease-A(Bovine Pancreas)	1.5
	p-Aminobenzoic Acid	0.01
TSKgel G4000SW(XL)	Thyroglobulin(Bovine Type I )	1.0 g/L
	Ferritin(Horse Spleen)	2.0
	Ovalbumin	1.0
	p-Aminobenzoic Acid	0.01

### 13.1.4 Sample Volume vs Column's ID

Column Types	Sample Volume( $\mu$ l)	Column's ID(mm)
Steel Column	5	4.6
	20	7.5&7.8
	200	21.5
Glass Column	20	8.0
	200	20.0

### 13.1.5 Detector : UV-8000, UV-8020(for SuperSW),(made by TOSOH)

Wave Length (280 nm)

### 13.1.6 Measuring Temperature : Room Temperature

### 13.2 Quality Specifications

The shipping specifications of SW type columns are shown in Table 7

Table 7

Types	Cat.No.	Column. Sizes mm(ID)×cm (L)	N/Column	As
〈Steel Column〉				
TSKgel SuperSW2000	18674	4.6×30	30,000	0.7~1.6
TSKgel SuperSW3000	18675		30,000	
TSKgel G2000SW <sub>XL</sub>	08540	7.8×30	≥20,000	0.7~1.6
TSKgel G3000SW <sub>XL</sub>	08541		≥20,000	
TSKgel G4000SW <sub>XL</sub>	08542		≥16,000	
TSKgel G2000SW	05788	7.5×30	≥10,000	0.7~1.6
TSKgel G3000SW	05789		≥10,000	
TSKgel G4000SW	05790		≥8,000	
TSKgel G2000SW	05102	7.5×60	≥20,000	0.7~1.6
TSKgel G3000SW	05103		≥20,000	
TSKgel G4000SW	05104		≥16,000	
TSKgel G2000SW	06727	21.5×30	≥10,000	0.7~1.6
TSKgel G3000SW	06728		≥10,000	
TSKgel G4000SW	06729		≥8,000	
TSKgel G2000SW	05146	21.5×60	≥20,000	0.7~1.6
TSKgel G3000SW	05147		≥20,000	
TSKgel G4000SW	05148		≥16,000	
〈Glass Column〉				
TSKgel G2000SW Glass	08799	8.0×30	≥10,000	0.7~1.6
TSKgel G3000SW Glass	08800		≥10,000	
TSKgel G4000SW Glass	08801		≥8,000	
TSKgel G3000SW Glass	14464	20.0×30	≥6,000	0.7~1.6

### 13.3 Warranty

Immediately after receipt, check the appearance of the column and test its performance according to Section 13.1.

If the guaranteed specifications in Table 7 can not obtained, contact your TOSOH representative within two weeks.

Note that column lifetime is not guaranteed.



**TOSOH**

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## **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](http://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](mailto: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.tbgs@tosoh.com](mailto:info.tbgs@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](mailto: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](mailto:info.tsas@tosoh.com)  
Web site: <http://www.separations.asia.tosohbioscience.com/>

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