Rev. CO061000

Packed Columns for Polar Organic Solvent
Gel Permeation Chromatograpy

TSKgel® α Series

INSTRUCTION MANUAL

TOSOH CORPORATION

Safety Precautions

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

[Notation Conventions]

Notation	Explanation
⚠ WARNING	Indicates a potentially hazardous situation which could result in death or serious injury.
⚠ CAUTION	Indicates a potentially hazardous situation which could result in 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, and corrosion.
Wear appropriate protective gear when cleaning up a spill.

■**Wear protective eye gear and gloves**
Organic solvents and acids should not come in 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 as intended**
This product is for separation and purification. Do not use for any other purpose.

■**Make sure compounds are safe**
Check that obtained 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.

Cautions in Handling (Packing Material)

First Aid	Inhalation	・Move to fresh air and gargle.
	Skin contact	・Rinse with water.
	Eye contact	・Immediately flush eyes with clean water for at least 15 minutes. Assure thorough rinsing by holding eyelids open. ・Get immediate medical attention.
	Ingestion	・Rinse mouth and get immediate medical attention.
	General	・None in particular.
Cautions in Handling and Storage	Fire	・Do not use open flames. Use grounded, explosion-proof tools to prevent the generation of sparks.
	Ventilation	・Ventilate with ventilation apparatus.
	Protective equipment and body washing	・Wear rubber gloves, safety glasses and dust mask. ・Wash adhering to the body, wash away.
Cautions for Disposal	Method for disposal	・Dispose gradually by incinerating at an incineration facility.
	General points to consider	・Conduct disposal by considering flammability and storage precautions.

☐ Packing material: flammable packing material (vinyl copolymer)
☐ Solvent during shipping: ion-exchanged water
(pay attention to storage temperature, as solvent may freeze near 0 ℃)

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1. Introduction

TSKgel®α Series columns are solvent-resistant, solvent-exchangeable, high-performance and high-speed GPC-packed columns in which a crosslinked hydrophilic synthetic polymer is used as a packing material, and the columns are usable with aqueous solvent systems and various polar organic solvent systems (mixtures of water and organic solvents).
Please read this Instruction Manual carefully for the correct and effective use of these high-performance columns.

2. Unpacking

Confirm that the package and column appearance are normal.

Fig. 1. Appearance of the Package

Confirm that the following documents are included in addition to the column.

1) Instruction Manual 1 copy
2) Inspection Data 1 copy

3. Column Parts

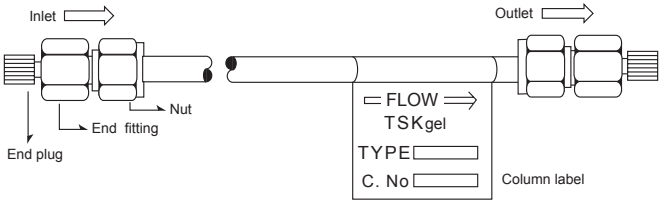

Fig. 2. Column Parts

4. Installation

4−1 Connection of Column Parts
All connections are of the Swagelok type, and the specifications are in inches.

4−2 Flow Direction
Flow direction should be as indicated by the arrow on the column label (Fig. 2). Long-term reverse flow will reduce column performance.

4−3 Prevention of Bubble Entry into the Column
Be careful not to introduce bubbles into the column during installation or removal from the system. Always install the column after removing bubbles from the entire piping system. The introduction of bubbles into the column will cause channeling, resulting in deterioration of column performance.

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
4−5−1 If Solvent Oozes from the End Fitting
The column should be connected to the system after confirming the removal of bubbles from the entire piping system. If solvent oozes from the end fitting when the end plug on the inlet side is removed, carefully connect the column to the system, as described above, so that no bubbles will be introduced into the column.

4−5−2 If Solvent Does Not Ooze from the End Fitting
If solvent does not ooze from the end fitting on the inlet side, connect the end fitting on the outlet side to the system and pump the solvent in the opposite direction in order to push out bubbles in the vicinity of the end fitting on the inlet side (be certain to pump the solvent slowly, as rapid pressurization or pumping may reduce column performance).

4−5−3 After Confirming That No Bubbles Are Present
After confirming that no exiting bubbles are present, and that the solvent oozes from the end fitting on the inlet side, arrange the column in the direction of normal flow and connect the end fitting on the inlet side to the system.

4-6 Connection of Multiple Columns

With the end fitting on the outlet side of the column, which has been connected as above, as the column connection end of the piping system, connect additional columns in sequence, as described above (use 1/16 inch tubing for column-to-column connections). To reduce dead volume, use short tubing, and insert the tubing fully into the end fitting before tightening with ferrules. If there is space between the tubing and end fitting, the flow of solvent will be disturbed, and resolution will be decreased.

4-7 Order of General Columns

In order to separate higher molecules, which tend to cause an overloading effect, and to decrease their concentration in the column, connect columns in descending order of pour size. Connect the outlet end of the last column to the detector.

4-8 Before Measurement

Measurement is started after the installation of columns. As described above, avoid rapid pressurization or pumping, as it may cause reduced column performance. Be particularly careful when pumps capable of rapid pressure increases are used.

4-9 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.

4-10 After Measurement

4-10-1 Measurement at Temperatures above Room Temperature

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

4-10-2 When the Same Column Is Used on the Following Day

If there is no leakage from the piping system, leave the column connected and use it on the following day. If the next measurement is 3 or more days later, store the column according to Section 4-10-4.

4-10-3 When Salt Solution Was Used As Solvent
Rinse the entire piping system with distilled water or ion-exchanged water. Rinse the system at a rate slower than that shown in Table 1. The amount of water for rinsing should be more than the amount necessary for replacing the column volume and the entire piping system.

Table 1. Flow rate for rinsing

Column	Column size: inner diameter (mm) × length (cm)	Flow rate
α Series	7.8 × 30	0.3 mL/min

4-10-4 Long-Term Storage (3 days or more)
When the column is not used for a long period, replace the solvent with ion-exchanged water. Remove the column from the system and seal both ends with end plugs in the same way as a newly purchased column.

5. Storage of Column

5-1 Storage Method
Store the column after treating as described in Section 4-10.

5-2 Storage Temperature
Store the column where temperature fluctuations are small (constant temperature room).

5-3 Direct Sunlight
Avoid exposure to direct sunlight.

5-4 Corrosive Gas
Store the column in a safe place without corrosive gases.

6. Selection of Solvents

6-1 Solvent Replacement
α Series columns are shipped in ion-exchanged water. Replace the water with the solvent to be used.
Conduct solvent replacement at the flow rate shown in Table 3.
Frequent solvent replacement will deteriorate column performance; therefore, avoid changing the solvent whenever possible.

6-2 Organic Solvents

The solvent in α Series columns can be replaced with various polar organic solvent systems (mixtures of water and organic solvents), as shown in Table 2. There is a solvent replacement method for this.

In Table 3, the flow rates for the replacement of ion-exchanged water by various polar organic solvents are listed. When the solvent is replaced with various polar organic solvents, pay special attention to the following points.

(1) When the column pressure exceeds the maximum pressure (Table 4) during replacement, decrease the flow rate or increase the temperature in order to carry out solvent replacement below the maximum pressure.
(2) After 100 % replacement with a new solvent, continue pumping at a slow flow rate until 2-3 times column volume of solvent is passed.
(3) For replacement with an immiscible solvent, use ethanol as an intermediate solvent.
(4) Never introduce bubbles into the column.

6-3 Degassing

At the time of solvent change (particularly to organic solvent systems) and during measurement, bubbles may be generated from the solvent, and may be introduced into the column. To prevent the generation of bubbles, sufficiently degas the eluent.

Table 2. Exchangeable organic solvents for TSKgel® α Series

Usable (exchangeable) organic solvents
Methanol (MeOH), Ethanol (EtOH), Acetone, Tetrahydrofuran (THF), Acetonitrile (CH_3CN), Dimethyl formamide (DMF), Dimethyl sulfoxide (DMSO), Isopropanol, Hexafluoroisopropanol (HFIP), Water/Methanol, Water/DMSO, Water/CH_3CN, Water/THF

Table 3. Flow rates for replacement of water with various polar organic solvents for TSKgel® α Series

Organic solvent	Flow rate for replacement by recommended solvent (mL/min)	Viscosity (CP)	Temperature (℃)
MeOH	0.8	0.54	30
EtOH	0.3	1.06	25
Acetone	1.0	0.32	20
THF	0.5	0.55	20
CH_3CN	1.0	0.33	30
DMF	0.4	0.80	25
DMSO	0.2	2.00	25
Isopropanol	0.2	2.43	20
HFIP	0.1	5.70	20

6-4 Operable pH Range

The operable pH range of the mobile phase at room temperature is wide (2.0-12.0). The operable pH range at 50-80 ℃ should be kept 6.5 to 7.5.

7. Flow Rate

Flow rates used for the α Series are shown in Table 4. Suitable flow rates and pressures for measurement with aqueous solutions vary depending upon the grade. Never use flow rates or pressures higher than those listed for each column (Table 4).

Table 4. Maximum flow rates and pressures for the TSKgel ®α Series

Column Type	Column size: inner diameter (mm) × length (cm)	Suitable flow rate (mL/min)	Maximum flow rate (mL/min)	Maximum pressure (per column) (MPa)
TSKgel α-2500	7.8 × 30	0.5-0.8	1.0	4.0
TSKgel α-3000				4.0
TSKgel α-4000		0.3-0.6		3.0
TSKgel α-5000				3.0
TSKgel α-6000				2.0
TSKgel α-M				2.0

8. Temperature for Usage and Storage

8-1 Temperature for Use
Use all α Series columns at 10 ℃-80 ℃.

8-2 Measurement at Elevated Temperatures
Degas the solvent sufficiently before use. After measurement at elevated temperatures, always follow the procedure described in Section 4-10-1 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 when the viscosity of a solvent or sample is high.
(2) The number of theoretical plates is higher than that at room temperature, and resolution is improved.
(3) Adsorption is lowered by heating.

8-4 Measurement at Temperatures Lower than Room Temperature
There are various disadvantages associated with measurement at low temperatures. These directly oppose the advantages listed above for measurement at elevated temperatures. In addition, it is necessary to lower the flow rate when compared to that used at room temperature (25 ℃), as solvent and sample viscosities will be higher.

8-5 Storage Temperature
Store the column at room temperature. (It is made 25 ℃ as much as possible.)
If the column is stored at less than 0 ℃, the column may freeze and the deterioration of the column may take place. Never store the column at below 0 ℃.

9. Preparation of Sample Solutions

9-1 When There Is Insoluble or Gelatinous Material in the Sample
Make sure to centrifuge or filter with a micropore filter (e.g., 0.45 μm). Even when nothing can be seen with the naked eye, it is possible that insoluble material is present. We recommend filtering the sample with a micropore filter.

9-2 Composition of Sample Solutions
Adjust the salt concentration, pH and amount of organic solvent in a sample to

those of the eluent.
When gradient elution is performed, match the sample solution to that of the initial eluent. If a 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

The measurement conditions for the number of theoretical plates and asymmetry factor in a column are described in the Inspection Data.

10−1 Calculation Method for Number of Theoretical Plates

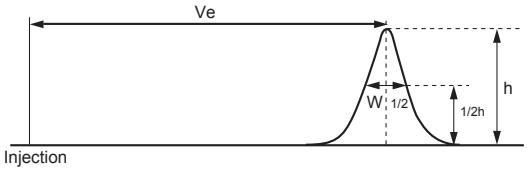

Fig. 3. Calculation method for number of theoretical plates in TSKgel® columns

The number of theoretical plates for a column is calculated by the half-width method, and is expressed in terms of the number of plates per column.

$N = 5.54 (Ve/W_{1/2})^2$
Ve: elution time (min)
$W_{1/2}$: half width of peak
h: peak height
N: number of theoretical plates per column

10−2 Calculation Method for Asymmetry Factor

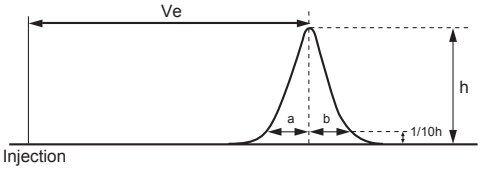

Fig. 4. Calculation method for asymmetry factor in TSKgel® columns

The asymmetry factor for a column is calculated by the 1/10h method.
As = b/a
As: asymmetry factor
On column inspection, measurements are performed using our HLC equipment with a small dead volume.

When equipment with a large dead volume is used or when the injection volume is large, lower numbers of theoretical plates may be obtained.

11. Guard Column

In Sections 4 to 9, basic cautions are given. If a material that can be adsorbed onto the packing material is present in the sample, the material is adsorbed on the inlet side of the column and gradually accumulates there. As a result, the number of theoretical plates may be lowered and the column performance may be altered. In such cases, if a guard column is installed on the inlet side of the analytical column, the performance deterioration due to adsorbed materials can be remedied, and the original state can be restored by replacing the guard column. We therefore recommend the installation of a guard column in order to prevent such problems.
Because a guard column is not an analytical column, resolution will not be improved. A guard column should only be used to prevent the above-described problems. The recommended guard column for the α Series is shown in Table 5.

11−1 Effects of Installation of Guard Column

1) Prevention of top-off from main column, which is caused by pulsed flow, abnormal flow rate and pressure changes.
2) Prevention of main column contamination by cutting off adsorbing material.
3) Protection of main column by cutting off insoluble materials.

11−2 Replacement of Guard Column

A guard column has a limited adsorption capacity; therefore, it has a limited life. It is necessary to replace the guard column sooner than the contamination reaches the main column. The exchange frequency depends upon factors such as the purpose (analytical or preparative), properties of a sample (properties of the main component, properties of impurities, and their amounts), sample loading, eluent and flow rate. Thus, there is no standard exchange frequency.
An increase in column pressure reflects clogging of the end fitting of the guard column and contamination by gelatinous material. It is a good idea to replace the guard column when such pressure increases are seen.

If changes are observed in measured data, the guard column should be replaced immediately.

Table 5. Guard column

Part No.	Column Type	Column size: inner diameter (mm) × length (cm)	Solvent in the column	Applicable column
18345	TSKgel guardcolumn α	6.0 × 4	H_2O	α-2500-α-M

12. Troubleshooting

If the following problems occur during the use of TSKgel® 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 bubbles, drying, or freezing, the original resolution will not be recovered. Therefore, handle these columns with care.

12-1 Problems with Column End Fitting

If a sudden decrease in flow rate is observed after the injection of a sample, the back pressure at the same flow rate becomes higher than that of the original column, or the Swagelok of the end fitting is damaged, follow the procedures in Sections 12-1-1 to 12-1-3.

12-1-1 Removal of Clogging Material or Replacement of End Fitting

Disconnect the column from the system and connect the end fitting of the outlet side to the piping on the pump side. Pump at a normal flow rate to push the column the clogging material out of the end fitting of the inlet side. If the clogging material cannot be removed by this procedure or the end fitting is damaged, replace the end fitting as described in Section 12-1-2.

12-1-2 Replacement of End Fitting

Prepare a new end fitting and remove the clogged end fitting from the column. Be certain to prevent any gel leakage. Transfer the remaining gel in the removed end fitting into the new end fitting, and install the new fitting on the column.

12-1-3 After Replacement of End Fitting

Remove bubbles from the new end fitting side by referring to Section 4-5-2. Measure the number of theoretical plates to confirm whether there is any decrease.

12-2 If There Is a Drastic Decrease in Resolution

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

If the number of theoretical plates 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 bubbles are not introduced into the respective columns. If you find a column with decreased performance, follow the procedure in Section 12-2-2.

Drastic decreases in the resolution of a column system may be due to any of the causes listed in Sections 12-2-1 to 12-2-2.

12-2-1 When Flow Path Is Disturbed by Clogging of End Fitting with Debris

Clean or replace the end fitting by referring to Section 12-1, and then measure the number of theoretical plates.

12-2-2 Accumulation of Adsorbed Material

Refer to Section 12-3.

Use of a guard column is strongly recommended to prevent adsorption of samples.

12-3 When a Sample Cannot Be Eluted or Elution Is Substantially Delayed

After repeated use, elution behavior may change drastically. This is though to be caused by surface changes due to trace accumulation of adsorbed material. In these cases, resolution may be recovered by flushing with various solvents having different properties.

Typical examples of adsorption are shown below.

Adsorption phenomena and flushing methods

(1) Ionic adsorption (removal of ionic materials)
    Increase salt concentration to obtain an appropriate ionic strength.
(2) Hydrophobic adsorption (removal of adsorbed hydrophobic materials)
    Use an eluent containing a water-soluble organic solvent.
(3) Hydrogen bond adsorption (removal of proteins, etc., with low solubility)
    Use an eluent containing urea.

(4) 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 the frequent solvent changes. Examine the sample and use the most suitable flushing method.

13. Quality Specifications and Warranty

13−1 Inspection Data
Inspection conditions and results are shown in the Inspection Data.
Among these, the number of theoretical plates is per column and the pressure at the inspection flow rate are listed.

13−2 Quality Specifications
The shipping specifications for TSKgel® α Series columns are as follows.

Table 6. TSKgel® α Series

Part No.	Colum Type	Column size: inner diameter (mm) × length (cm)	Number of theoretical plates (N/30 cm)	Asymmetry factor	Solvent in the column
18339	TSKgel α-2500	7.5 × 30	16,000	0.70 × 1.60	H_2O
18340	TSKgel α-3000		16,000		
18341	TSKgel α-4000		10,000		
18342	TSKgel α-5000		10,000		
18343	TSKgel α-6000		7,000		
18344	TSKgel α-M		7,000		
18345	TSKgel guardcolumn α	6.0 × 4	—	—	H_2O

13−3 Warranty
(1) After receipt of a product, check the number of theoretical plates and the asymmetry factor under the conditions described in the Inspection Data and this Instruction Manual. If the product does not meet these specifications, the product will be replaced at our cost.
(2) If damage to the column is caused by an accident during delivery, the product will be replaced.
(3) Please report the above defects within two weeks after receipt of the

product. After two weeks, we will assume that you have received a satisfactory product.

(4) Column life is not included in the warranty.

(5) Specifications may be changed, for the purpose of improvement, without prior notice.

14. Closing

If you have any questions concerning the contents of this Instruction Manual, please contact us at the address below.

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