Rev. CO067000

Packed Columns for
Normal Phase Chromatography

TSKgel NH_2–100 3μm

INSTRUCTION MANUAL

TOSOH

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 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, 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.

Precautions: Shipping Solvents

First Aid	Inhalation	• Move the person to an area with fresh air. Rinse the mouth with plenty of water. • Call for medical attention immediately.
	Skin exposure	• Wash exposed area with plenty of soap and water.
	Eye exposure	• Open eyes as wide as possible and rinse with clean water for at least 15 minutes. • Call for medical attention immediately.
	Ingestion	• Rinse the mouth with plenty of water and call for medical attention immediately.
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 gas mask, additional protective clothing or rubber boots could be appropriate when handling this product.
	Hazardous substance storage	• If any flammable solvents are used for shipping or storage, keep away from fire and open heat.
Waste Disposal	Disposal methods	• Follow local guidelines for disposal. This product can be incinerated safely.
	General considerations	• Please pay attention to all safety precautions with respect to the handling and storage of this product.
	Disposal precautions	• Assure that appropriate countermeasures are taken when incinerating solvents that contain acetonitrile. Fumes produced during incineration may contain nitrogen oxides.

☐ Shipping solvent : CH_3CN/H_2O=85/15

Precautions: Packing Material

First Aid	Inhalation	• Move the person to an area with fresh air. Rinse the mouth with plenty of water. • Call for medical attention immediately.
	Skin exposure	• Wash exposed area with plenty of soap and water.
	Eye exposure	• Open eyes as wide as possible and rinse with clean water for at least 15 minutes. • Call for medical attention immediately.
	Ingestion	• Rinse the mouth with plenty of water and call for medical attention immediately.
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 gas mask, additional protective clothing or rubber boots could be appropriate when handling this product.
	Hazardous substance storage	• If any flammable solvents are used for shipping or storage, keep away from fire and open heat.
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	• Fumes produced during incineration may contain nitrogen oxides.

☐ Aminoalkyl group functionalized silica gel

Table of Contents

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1. General Information

TSKgel NH_2-100 3μm packed columns have been optimized for high performance NPC and HILIC. 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

1) Analysis column

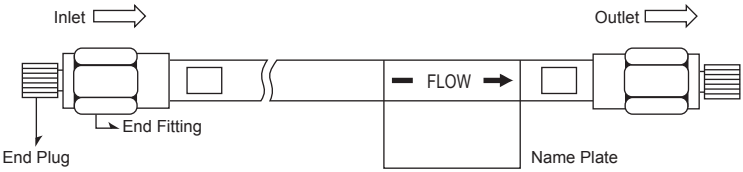
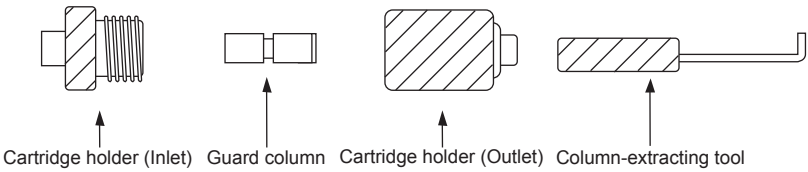


Figure 2 Column Parts (1)

2) Guard column

3.2 mm(I.D.)× 1.5 cm(L)



2.0 mm(I.D.)× 1 cm(L)

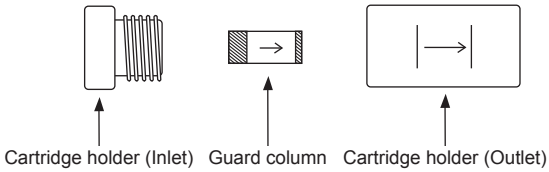


Figure 3 Column Parts (2)

4. Column Installation

(1) Confirm the correct product name is listed on the column label.
(2) 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.
(3) Confirm the flow direction on the column label or on 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) 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.
(5) Initially set the solvent flow rate at one-half of the intended flow rate. Make sure that the solvent is flowing freely out of the end of the tubing from the injector.
(6) 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.
(7) After the solvent flows from the outlet of the column, connect the column to the detector.

(8) Start pumping the solvent at a flow rate less than one-half of the final flow rate. Avoid a sudden pressure surge to the column.
(9) The columns are very sensitive to pressure pulsing. A pulseless pumping system should be used.
(10) The columns should be equilibrated before use by allowing at least 10 column volumes of solvent to pass through the column.
(11) If a mixture of water or aqueous acetate solution with water-soluble organic solvent (e.g., acetonitrile) is used as a mobile phase, install a guard column and routinely clean the main analytical column in order to prevent the accumulation of impurities on the column. Replacing the guard column and cleaning the analytical column are described in section **11-3 Guard Column Replacement**.
(12) Elution times depend on the counter-anion associated with the amino group on the packing material. Take care so that the counter-anion is sufficiently exchanged when the mobile phase is changed, especially when used for LC/MS. The procedure for exchanging the counter-anion is described in section **6 Solvent Selection and Preparation**.

5. Column Maintenance

(1) If the column is used in routine daily operation, it is permissible to leave the mobile phase in the column overnight if the mobile phase is not corrosive. If halides are included in the mobile phase, it is better to replace the mobile phase with a suitable solvent (for example "packed solvent" shown in the INSPECTION DATA sheet) even for one night.
(2) If the column will not be used for several days, it should be stored as follows:
   a) Purge the system with the "packed solvent" shown in the INSPECTION DATA sheet at a flow rate one-half of the operating flow rate as shown in Table 1 (purge the system with distilled or ion-exchanged water if you have used a buffer solution, as a mobile phase, which contains salt in considerably high concentrations).
   b) Remove the column from the system and keep the ends of the column tightly capped with the end plugs supplied with the column.
   c) Store the column at a relatively constant temperature in its original shipping container. Take care not to allow the column to freeze during storage.
(3) The performance of the cartridge column may be decreased by repeated removal from the cartridge holder. TOSOH recommends that the cartridge column is kept in the cartridge holder with the both ends capped with end plugs.

6. Solvent Selection and Preparation

(1) The shipping solvent is 85 % acetonitrile in water. Before using the column, the

solvent should be replaced with an appropriate mobile phase for analysis. When salt-containing buffers are used as a mobile phase, purge the column for at least 1 h with salt solution containing 5 % of a water-soluble organic solvent (e.g., 200 mmol/L ammonium formate/ acetonitrile= 95/5, v/v) to exchange the counter-anion associated with the amino group on the packing material. When used for LC/MS, special care must be taken so that the counter-anion is sufficiently exchenged. Solvent replacement should be performed at a flow rate one-half of the normal operating flow rate, or at a pressure below the maximum pressure shown in **Table 1**. Note that a drastic change of solvent composition or frequent solvent replacements may shorten the lifetime of the column.

(2) pH range : 2.0-7.5

The pH range should be selected based on the stability of both the packing material and the column itself. At a pH below pH 2.0, the ligand binding sites on the silica-based support are subject to hydrolysis by acidic solutions. Above pH 7.5, the silica backbone may dissolve, leading to rapid column failure. Additionally, the stainless steel of the column is subject to corrosion at a low pH particularly when using halides.

(3) The solvent should be filtered through a 0.5 μm filter in order to prevent the accumulation of small particles. The performance of semi-micro columns quickly deteriorates when exposed to small-particle contamination. Thus, it is highly recommended that an in-line filter containing a membrane of 0.2 ～ 0.5 μm pore size is inserted between the pump and the sample injector.

Line filter

Part No. 0014594 Filter assembly

Part No. 0006280 Fluoropore filter (0.45 μm, package of 100)

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

7. Flow Rate

The flow rate should be selected based on the desired resolution, column life and assay time. Although the TSKgel NH_2-100 3 μm is designed for high-speed analysis, TOSOH recommends that this column is operated at a rather low flow rate because better resolution and extended column life can be expected. A suitable flow rate and the maximum flow rate depend on the organic solvent in the mobile phase. When using the TSKgel NH_2-100 3 μm for the first time, the flow rate should be set at a linear velocity of 6 cm/min (0.20 mL/min for 2.0 mm(I.D.) and 1.00 mL/min for 4.6 mm(I.D.)). The column life may be reduced if the column is operated near the maximum pressure. The maximum pressure for the TSKgel NH_2-100 3 μm is shown in Table 1. The viscosity of the solvent must be considered when selecting the flow rate, too.

Table 1 Maximum Pressure

Part No.	Type	Column Size mm(I.D.)×cm(L)	Maximum Pressure (MPa)
0021967	TSKgel NH_2-100 3μm	2.0×5	15
0021968	〃	2.0×15	20
0021969	〃	4.6×5	5
0021970	〃	4.6×15	15

8. Temperature

The column should be operated in a temperature range of 10 - 50 ℃.

9. Sample Preparation

(1) Preparation of Sample Solution

Prepare the sample solution immediately prior to injection by dissolving the sample into the solvent that is used as an eluent. The eluent should be optimized by adjusting the pH, salt concentration, etc. so that the sample is completely dissolved, otherwise the column lifetime may be reduced by unexpected precipitation of sample on the column.

(2) Filtration of Insoluble Particles

The sample solution should be filtered with a micropore-filter (0.5 μm). Even though no particles can be detected by the naked eye, insoluble particles may exist in the sample.

10. Measurement of Number of Theoretical Plates and Asymmetry Factor

(1) Number of theoretical plates (N)

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

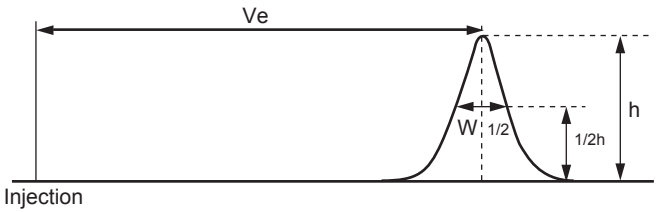


Figure 3 Calculation of Number of Theoretical Plates

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

where:

Ve : Elution time
$W_{1/2}$: Width of peak at half-height
h : Peak height
N : Number of theoretical plates/column

(2) Asymmetry factor (As)

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

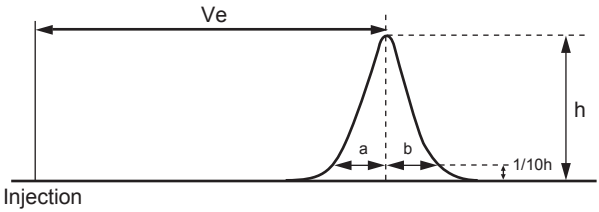


Figure 4 Calculation of Asymmetry Factor

As=b/a

(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 Cartridge Column

Part No.	Type	Column Size mm(I.D.)×cm(L)	Applied Column mm(I.D.)×cm(L)
0021971	TSKguardgel NH_2-100 3μm	2.0×1	TSKgel NH_2-100 3μm (2.0×5, 2.0×15)
0021972	TSKguardgel NH_2-100 3μm	3.2×1.5	TSKgel NH_2-100 3μm (4.6×5, 4.6×15)

Note : Three cartridge columns are packed in a box.

Cartridge holders are shown in Table 3.

Table 3 Cartridge Holder

Part No.	Type	Column Size mm(I.D.)×cm(L)
0019308	Cartridge holder (2.0×1)	2.0×1
0019018	Cartridge holder (3.2×1.5)	3.2×1.5

Note : Two nuts, two ferrules and two small pieces of tubing are attached to the cartridge holder as accessories.

In addition, column-extracting tool is attached to Part No. 0019018

11-3 Guard Column Replacement

Since the guard column has limited adsorbtion 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.

If a mixture of water or aqueous acetate solution with water-soluble organic solvents (e.g., acetonitrile) are used as a mobile phase, the guard column should be replaced after approximately 70 h of usage (for 4.6 mm(I.D.) columns) or 40 h of usage (for 2.0 mm(I.D.) columns). Additionally, the main analytical column should be cleaned when the guard column is replaced (for 4.6 mm(I.D.) columns) or every 20 h of usage (for 2.0 mm(I.D.) columns). Cleaning should be done by purging the column for 0.5 h with a 5% water-soluble organic solvent (e.g., 5% acetonitrile in water) at a flow rate of one-half the operating flow rate, or at a pressure below the maximum pressure as shown in **Table 1**.

12. Troubleshooting

(1) Clogging of the inlet filter

Increased pressure or decreased flow rate are indicative of a clogged inlet filter. In this case, clean the end fitting by reversing the flow direction through the column (The flow rate must be kept below one-half of the operating flow rate as shown in Table 1).

(2) Contamination

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 a different polarity from the operating mobile phase.

(3) Bed Compression

Failure to properly clean the analytical column may result in the formation of a void at the column head due to bed compression.

This failure can be confirmed by carefully removing the column end and inspecting the bed.

13. Quality Specification and Warranty

13-1 INSPECTION DATA

The inspection conditions and the results of each individual column are shown on the INSPECTON 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

TSKgel NH_2-100 3μm are delivered according to the specifications as shown in Table 4.

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 4 can not be obtained, contact a local TOSOH representative within 30 days. Note that the column lifetime is not guaranteed.

Table 4 Guaranteed Specifications

Part No.	Type	Column Size mm(I.D.)×cm(L)	Number of theoretical plates (TP/Column)	Asymmetry factor
0021967	TSKgel NH_2-100 3μm	2.0×5	4,000	0.90-1.35
0021968	〃	2.0×15	15,000	0.90-1.35
0021969	〃	4.6×5	6,000	0.90-1.30
0021970	〃	4.6×15	18,000	0.90-1.30

14. Column Cleaning Solutions

(1) Sample property : Hydrophobic compounds
Water soluble organic solvents such as 70-95 % acetonitrile and methanol in aqueous buffer

(2) Sample property : Hydrophilic compounds
Water soluble organic solvents such as 5-10 % acetonitrile and methanol in aqueous buffer

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Supplemental Information related to TSKgel NH_2-100 3μm columns

INSTRUCTION MANUAL for TSKgel NH_2-100 DC 3μm (4.6 mm(I.D.), 5 cm(L))

To help protect you and/or your property from potential damage and ensure personal safety, please read the “Precautions” which are printed in the beginning of the “TSKgel NH_2-100 3μm” INSTRUCTION MANUAL thoroughly before using the above TSKgel NH_2-100 DC 3μm column.
For this TSKgel NH_2-100 DC 3μm column, please read section 1, section 3 (1), section 7 (Table 1), and section 13 (Table 4) on this supplemental information sheet instead of the corresponding sections you find on the “TSKgel NH_2-100 3μm” INSTRUCTION MANUAL. Please read section 4 (13) in addition to those in each section on the “TSKgel NH_2-100 3μm” INSTRUCTION MANUAL.

1. General Information

This TSKgel NH_2-100 DC 3μm column has the outlet end fitting with an external male connector so that it can be easily connected to another HPLC column.
In this supplemental information sheet, only those instructions different from the other TSKgel NH_2-100 3μm columns are included. Please refer to the “TSKgel NH_2-100 3μm” INSTRUCTION MANUAL regarding instructions which are not mentioned in these document.

3. Column Parts

1) Analysis column

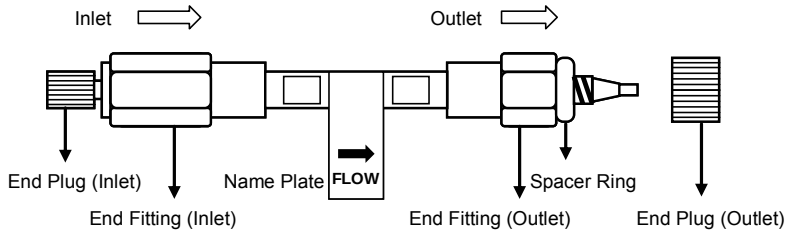

Figure 2　Column Parts

4. Column Installation

(13) To directly connect the TSKgel NH_2-100 DC 3μm column to another HPLC column, finger-tighten the connection between the outlet of the TSKgel NH_2-100 DC 3μm column and the inlet of the second column, and then tighten with appropriate wrenches until you feel some resistance. Check for solvent leaks from the connection. The following operations may cause permanent deterioration in column efficiency.

a) Damage to the tip of the outlet end fitting caused by mechanical force such as accidentally dropping the column.

b) Tightening the connection between the two columns to more than 2.0 Nm of torque (more than about 30 degrees).

c) Repeated connections and disconnections of the two columns. It is recommended that the two columns are kept connected during storage.

d) Connection to other companies' columns not compatible with TOSOH's.

7. Flow Rate

Table 1 Maximum Pressure

Part No.	Type	Column Size mm(I.D.)×cm(L)	Maximum Pressure (MPa)
0021999	TSKgel NH_2-100 DC 3μm	4.6×5	5

11. Guard Column

11-2 Type and Selection of Guard Columns

Table 2 Cartridge Column

Part No.	Type	Column Size mm(I.D.)×cm(L)	Applied Column mm(I.D.)×cm(L)
0021972	TSKguardgel NH_2-100 3μm	3.2×1.5	TSKgel NH_2-100 DC 3μm (4.6×5)

Note: Three cartridge columns are packed in a box.

13. Quality Specification and Warranty

13-3 Warranty

Table 4 Guaranteed Specifications

Part No.	Type	Column Size mm(I.D.)×cm(L)	Number of Theoretical plates (TP/Column)	Asymmetry factor
0021999	TSKgel NH_2-100 DC 3μm	4.6×5	6,000	0.90～1.30

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