TOSOH

TOSOH CORPORATION

SCIENTIFIC INSTRUMENT DIVISION

• Head Office
1－7－7, Akasaka, Minato－ku, Tokyo 107 Japan
Tel : (03)－586－9139
Fax : (03)－587－6986

• Nanyo Plant
4560 Kaisei, Shin Nanyo-shi Yamaguchi 746 Japan
Tel : (0834)－63－9863
Fax : (0834)－63－9879

M010-1

Packed Column for High Performance
Affinity Chromatography
TSKgel Tresyl-5PW

INSTRUCTION MANUAL

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1. Introduction

TSKgel Tresyl-5PW is a packed activated column for high performance affinity chromatography.

This column has been designed for high resolution.

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 the package or the column.

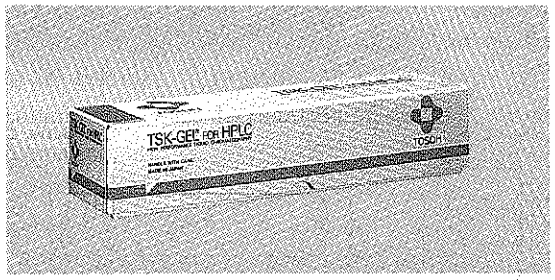


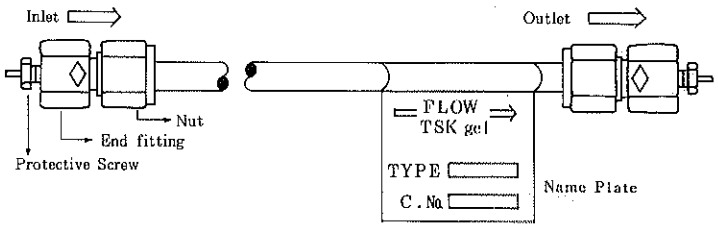
Fig. 1 Appearance of the package

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

1 copy Instruction Manual

3. Column Parts

A) Stainless Steel Column



B) Glass Column

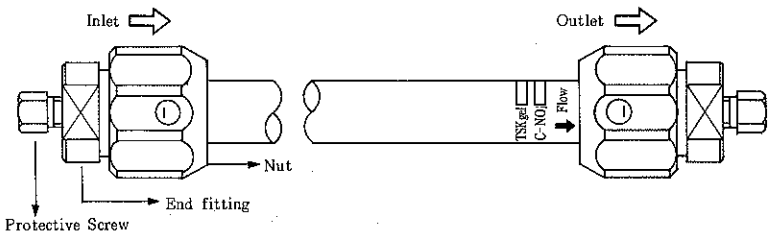


Fig. 2 Column Parts

4. Installation and Safety Considerations

4-1 Connections

Stainless steel column can be connected with swage-lock type screw and 1/16" stainless steel tube. Glass column can be connected with 1/4"-28UNF screw and 1/16" teflon tube.

4-2 Flow Direction

Use the column in the direction shown by the arrow on the tag in Fig. 2.
Operation of 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 tubing before installing the column.
Bubbles in the column will cause degradation of its performance through the occurrence of channeling, etc.

4-4 Installation

Remove the protective screws and confirm the leakage of solvent from the end-fitting of the inlet side of column. When the leakage is confirmed, connect the column to the equipment.
When the leakage is not confirmed, connect the column in the reverse direction to the equipment. Then, feed the solvent at below half of ordinary flow rate not to degrade the column performance. After confirming the leakage of solvent without bubble, connect the column in the correct direction.

4-5 Prevention of Pulsatory Flow

This type of column is easily affected by pulsatory flow of the solvent.
A pump without fluctuation should be used.
When a pump with pulsation is used, connect a pulse damper (accumlator) to the outlet side of the pump in order to compensate for the pulsation.
The damper must be highly resistant to corrosion.

4-6 Measurement

Rapid pressure-up or solvent feeding must be avoided to keep column performance. When the measurement is done at higher temperature than room temperature, do not stop the pump immediately after finishing the measurement. Continue feeding solvent until the column temperature decrease to the room temperature. If the pump is stopped without cooling, air may be sucked into the column by contraction of the solvent.

4-7 Long-term Storage

When the columns are not to be used again soon, replace the solvent in the columns with the buffer of neutral pH containing 0.02% sodium azaide. The volume of the buffer must be larger than the total capacity of column and flow path. Then remove the column from the equipment and seal both end of each column with the protective screws.

5. Storage of Column

5-1 Storage Method

Refer to Section 4-7.

5-2 Storage Temperature

Store the columns at temperature between 4 and 10℃.
The columns may freeze to degrade their efficiency if they are left below 0℃.

5-3 Exposure to Direct Sunlight

Avoid exposing the columns to direct sunlight.

5-4 Corrosive Gasses

Store the columns in a safety place from corrosive gasses.

6. Solvents

6-1 Replace with solvent to be used

The column is filled with acetone for shipment. Wash the column with distilled water first, then replace it with the coupling buffer.

6-2 pH Range

Buffer in pH range between 2.0 and 12.0 can be suitable when the immobilized ligand is stable.

6-3 Viscosity of Solvent

When high viscosity solvent is used, pressure-drop becomes high and tends to make troubles on column, pump, tubing and so on.

6-4 Boiling Point of Solvent

When the column is used over room temperature (25℃), pay attention to the boiling point of solvent.

6-5 Impurity in Solvent

Use the pure solvent as far as possible, because impurity in solvent might cause the ghost peak.

6-6 Aqueous Organic Solvent

Aqueous organic solvent can be added in the sovent at most 20% when the immobilized ligand is stable.

6-7 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 bubble formation.

7. Coupling of Ligand

7-1 Washing of Column

Wash the column with distilled water first, then with coupling buffer.
The solvent volume and flow rate for washing are shown in Table 1.

Table 1

Cat. No.	Column Sizes (mmID×cm)	Volume for Washing		Flow Rate (ml/min)
		Water (ml)	Coupling Buffer(ml)	
014455	6.0×4.0	3	3	0.5
014457	5.0×5.0			
014456	7.5×7.5	10	10	0.8
014458	8.0×7.5			

7-2 Selection of Coupling Buffer

0.5-1.0 potassium phosphate (pH 7-9) or other non-amine containing buffers.

7-3 Coupling of Ligand

Feed and recycle the ligand solution by pumping for over night at temperature between 4 and 25℃.
Flow rates for feeding are as follows:

Column Sizes (mmID×cm)	Flow Rates (ml/min)
6.0×4.0, 5.0×5.0	0.2—0.5
7.5×7.5, 8.0×7.5	0.5—0.8

7-4 Bloking of Remaining Active Groups

After finishing the coupling, remaining active groups on the column should be blocked with 0.1M Tris-HCl buffer (pH 8.0) as the same manner as the coupling of ligand.
Blocking time is as follows:

Column Sizes (mmID×cm)	Blocking Time (hr)
6.0×4.0, 5.0×5.0	1.0
7.5×7.5, 8.0×7.5	3.0

It is also applicable for blocking to use glycine-NaOH buffer (pH8.0) or ethanolamine-HCl buffer (pH 8.0).

8. Flow Rate

8-1 Choice of Flow Rate

Factors such as resolution, analytical time and column life should be carefully considered in selecting flow rates.
Higher flow rate results in shorter analytical time.
On the contrary, lower flow rate results in improved column efficiency.
Furthermore, lower flow rate tends to extend column life and to decrease the occurrence of top-off.

8-2 Suitable Flow Rate

As shown in Table 1, use the column with suitable flow rate.
Do not use these columns with flow rates over the maximum shown in Table 2.

8-3 Viscosity of Solvent

The higher flow rate can be applied with the lower viscosity of solvent. With the higher viscosity of solvent, keep the flow rate at the lower level.

Table 2 Suitable Flow Rate

TSK-GEL type	Cat.No.	Column Sizes (mmID×cmL)	Max. Flow Rate (ml/min)	Suitable Flow Rate (ml/min)	Max.Press. Drop (kg/cm²)
TKSgel Tresyl-5PW	014455	6.0×4.0	1.0	0.5−0.8	10
	014457	5.0×5.0	1.0	0.5−0.8	10
	014456	7.5×7.5	1.2	0.5−1.0	10
	014458	8.0×7.5	1.2	0.5−1.0	10

Note: These flow rates are attainable with the buffer with the same viscosity as distilled water.

9. Operating Temperature

9-1 Optimal Operating Temperature

The optimal operating temperature for the columns is above 0℃. (Operating temperature is dependent on the stability of ligand immobilized to the column)

9-2 Measurement at High Temperature

Use the solvent after sufficient degassing. After finishing the measurement at high temperature, follow the instruction of item 4-6.

9-3 Advantages of Measurement at High Temperature

1) The viscosity can be reduced by elevating the solvent temperature.
2) The number of theoretical plates increases and resolution improves in comparison with measurement at room temperature.

9-4 Measurement at Temperature below Room Temperature

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.

10. Preparation of a Sample Solutions

10-1 Preparation of a Sample Solution

Dissolve the sample in the eluent as far as possible.

10-2 Insoluble Matter in a Sample Solution

Purify the sample solution either by centrifugation or preferably by micro-pore filtration.
Even if nothing can be seen, insoluble matters exist in the sample solution in many cases.

10-3 Composition of Sample Solution

Adjust the pH and concentrations of salt and organic solvent in a sample solution as closely as possible to those of the eluent.
When gradient elution is applied, the sample had better to be dissolved in the first buffer.
A sample solution cannot be applied when it forms insoluble matters with eluent.

11. Troubleshooting

Some problems can be resolved by the following instructions.
However, the problems such as those due to column life, adsorptive materials, production of air bubbles, dried gel, or frozen solvent cannot be remedied once they occur, so take care for handling these columns.

11-1 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.

11-2 Replacement of the End Fitting

Prepare a new end fitting and remove the old one from the column, be careful not to loose any of the packed gel.
Then expel air from the inlet side referring item 4-4.

11-3 Cleaning of Columns

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

The following are the typical cleaning solvents.
a) 0.1-0.2 N NaOH aqueous solution
b) 20-40% Acetic Acid aqueous solution
c) Buffer added Aqueous Organic Solvent
d) Buffer added Urea and Neutral Surfactant
Cleaning solvent, however, is dependent on the stability of ligand immobilized to column.
Wash the column with the above solvent from top to bottom solvent one by one.
Confirm the column efficiency after every washing.