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

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
⚠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 should not come in direct contact with the skin.

■ **Handle package with care.**

Inappropriate handling may cause rupturing and splattering.

■ **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 for future reference.

Table of Contents

1. Introduction

TSKgel Chelate－5PW is a packed column for high performane metal chelate affinity chromatography.
This column has been designed for high resolution.
This Instruction Manual contains crucial information on haw 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

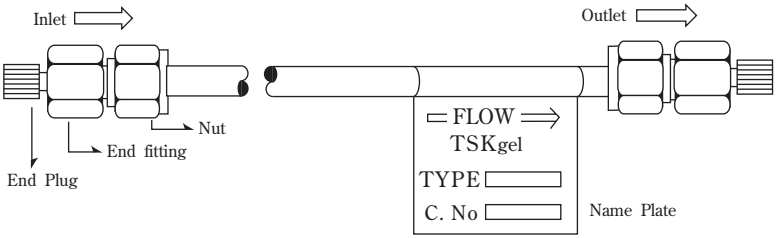
Check that nothing is the matter with the appearance of the package or the column.

Fig. 1　Appearance of the package

Then check that the following documents are attached to the column:
1 copy Instruction Manual
1 copy Inspection Data

3. Column Parts

A) Stainless Steel Column



B) Glass Column

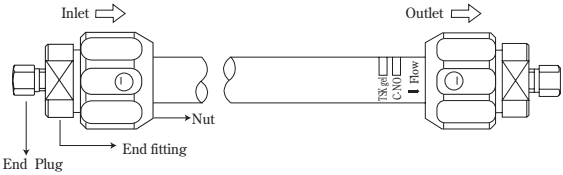


Fig. 2 Column Parts

4. Installation and Safety Considerations

4-1 Connections

Stainless steel column can be connected with swagelock type screw and 1/16" stainless steel tube. Glass column can be connested 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 name plate 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 bubble into the column during its installation or removal from the equipment. 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 end plugs 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 solvent.

A pump without fluctuation should be used.

When a pump with pulsation is 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.

4-6 Measurement

Repid 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 used again soon, replace the solvent in the columns with the buffer of neutral pH containing 0.02% sodium azide.
The volume of the buffer should be larger than the total capacity of column and flow path. Then detach the column from the equipment and seal both ends of column with the end plugs.

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 30℃.
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 . Solvent

6-1 Replacement with solvent

The column is filled with 10 mmol/L sodium acetate buffer for shipment. Wash the column with distilled water first, then replace the water with other solvent. TSKgel Chelate-5PW is porous polymer resin so that frequent organic solvent replacement must be avoided.

6-2 pH Range

Use the column in pH range between 2.0 and 12.0

6-3 Viscosity

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

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

6-5 Impurity

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

6-6 Filtration

Use HPLC grade solvent or filter all solvents with ca 0.5 μm filter prior to chromatography, which reduces the problem of filter-plugging, and prolong column life. Vacuum filtration or sonification would be effective to remove dissolved gasses which may cause trouble to your solvent delivery system.

6-7 Salt Concentration

Salt concentration in eluent should be used below 3mol/L.

6-8 Aqueous Organic Solvent

Aqueous organic solvent cat be added in the solvent at most 20%.

6-9 Degassing

Bubbles may be generated in the solvent during solvent replacement (especially at switching to a system containing an organic solvent).
The solvent should be thoroughly degassed before use to avoid bubble formation.

7. Application Method

7-1 Selection of Metal

TSKgel Chelate-5PW does not contain any metal to make chelation. Load metal ion before sample solution is injected.

Common metal ions are Cu^{++} and Zn^{++}.

Cu^{++} has a strong affinity for proteins and some samples bind only with Cu^{++} although they have weak interaction with metal ion. The binding strength of Zn^{++} is weaker than that of Cu^{++}. Zn^{++} is used to elute the samples selectively.

Ni^{++} and Co^{++} can be also used.

7-2 Addition of Metal Ion

TSKgel Chelate-5PW can adsorb ca. 60 μmole of metal ion per column.

Dissolve metal ion in distilled water, then inject it with sample loop and saturate the column with the metal ion. When the bleeding of metal ion causes problems during chromatography (i. e. UV detectable metal ion interferes detection), load ca. 30 μmole (50% capacity of the column) of the metal ion.

7-3 Selection of Buffer and pH

After loading metal ions, equilibrate the column with starting buffer.

Most proteins will bind to chelated metal ions at pH 7-9, so tris or phospthate buffer is effective.

Be careful not to cantain chelating reagents such as EDTA or citric acid.

Add 0.5-1.0 mol/L NaCl to the buffer in order to minimize nonspecific ionic interaction.

7-4 Elution Method

1) pH Gradient

The binding strength between metal ion and protein is weaker at lower pH. The protein can be eluted by pH gradient from 7.0 to 3.0

2) Affinity eluent

Elute the sample with increasing concentration of glycine, histamine, imidazole or ammonium chloride.

Protein can be eluted by the gradient of salt in buffer as follows :

Salt	Gradient
glycine	0 — 0.2 mol/L
imidazole	0 — 20 mmol/L
histidine	0 — 0.2 mol/L
ammonium chloride	0 — 0.5 mol/L

Since elution of protein with imidazol does not desorb metal ion, chromatography cat be repeted several times without regeneration.

3) Chelating reagent

Elute the sample with the chelating reagents such as EDTA or EGTA.

The chelating reagents make the metal strip off from gel, so the adsorbed proteins cannot be separated each other.

Affinity elution mentioned above are the most effective.

The followings are the typical examples :

(A) Initial Buffer : 20 mmol/L Tris—HCl Buffer + 0.5 mol/L NaCl(pH 8.0)

Final Buffer : 20 mmol/L Tris—HCl Buffer + 0.5 mol/L NaCl + 0.2—0.5 M glycine

(pH 8.0)

(B) Initial Buffer : 20 mmol/L Phoshate Buffer + 0.5 mol/L NaCl (pH 7.0)

Final Buffer : 20 mmol/L Phosphate Buffer + 0.5 mol/L NaCl + 20 mmol/L imidazole

(pH 7.0)

Linear gradient or Stepwise gradient is applied.

7-5 Regeneration

To regenerate the column at first take off metal ion with the aqueous solution containing 50 mmol/L EDTA and 0.5 mol/L NaCl, then load the new metal ion.

8. Flow Rate

8-1 Choice of Flow Rate

Factors such as resolution, analysis time and column life should be carefully considered in selecting flow rate.
A higher flow rate results in a shorter analysis time.
On the contrary, a 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 at suitable flow rate.
Do not use these columns at flow rates over the maximums shown in Table 1.

8-3 Viscosity of Solvent

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

Table 1 Suitable Flow Rate

TSK-GEL type	Cat. No.	Column Sizes (mmID×cmL)	Suitable Flow Rate (mL/min)	Max. Flow Rate (mL/min)	Max. Press. Drop (MPa)
TSKgel Chelate-5PW	08645 08646	7.5 × 7.5 21.5 × 15.0	0.5 − 1.0 4.0 − 6.0	1.2 8.0	1.0 1.5
TSKgel Chelate-5PW Glass	14440 14441	5.0 × 5.0 8.0 × 7.5	0.5 − 0.8 0.5 − 1.0	1.0 2.0	2.0 1.5

Note : These flow rate are attainable with the viscosity of distilled water.

9. Operating Temperature

9-1 Optimal Operating Temperature

The optimal operating temperature for the columns is below 45℃.

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 increses 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. Guard Column

It is better to use in—line frit filter and a guard column to protect column. Supports in guard column should be exchanged with new ones after every 30—40 sample injections, or when peak shape in chromatogram becomes worse. Guard column is available as TSKguardgel kit. TSKguardgrl kit Chelate-5PW (Cat. No. 08647) consits of a column holder, ten filters and packing materials.

11. Preparation of Sample Solution

11-1 Preparation of Sample Solution

Dissolve the sample in the eluent as for as possible.

11-2 Insoluble Matter in Sample Solution

Pretreat 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.

11-3 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 the injection volume of the sample solution is large, it is better to dissolve the sample in initial buffer.

A sample solution cannot be applied when it forms insoluble matters when it is mixed with the eluent.

12. Calculation of Column Efficiency

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

12-1 Method of Calculating the Number of Theoretical Plates

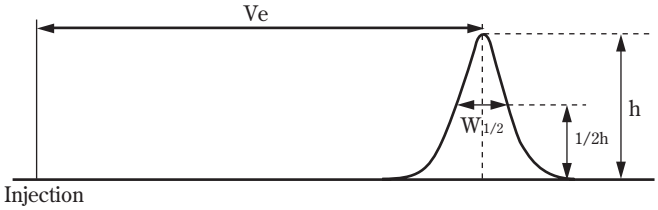


Fig. 3 Method of calculating the number of theoretical plates

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

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

Ve : Elution volume

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

h : Peak height

12-2 Method of Calculating the Asymmetry Factor

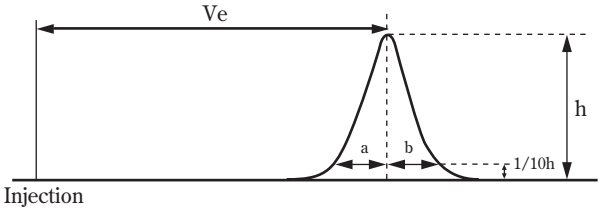


Fig. 4 Method of Calculating the Asymmetry Factor

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

As ＝ b／a

12-3 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.

13. Troubleshooting

In using TSK—GEL column, some problems can be avoided by following these instructions.
However, the problems (such as those due to column life, adsorptive materials, production of air bubbles, dry of the packings, or freezing of solvent, which occur in the column) can not be corrected once they occur, so care should be taken in handling these columns.

13-1 Solvent Leakage from Glass Column

Seal off the column with end fitting and nut by wrench at the torque lower than 3 N · m.

13-2 Clogging of the End Fitting

In case the pressure-drop increases, the end fitting should be cleaned by reversing the flow through the column.
If the clog cannot be removed, the end fitting should be replaced by a new one.
Prepare a new end fitting and remove the old end fitting from the column, being very careful to loose any of the packed gel underneath.
Then expel air from the inlet side referring item 4—4.
Confiem the column's number of theoretical plates and asymmetry factor.

13-3 Cleaning of Column

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 followings are the typical cleaning solvent.
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

Wash the column with the solvent from (a) to (d) solvent.
After washing, confirm the column efficiency.
Generally the column will be cleaned by the solvent (a) or (b).

14. Quality Specifications and Warranty

14-1 Inspection Data

The inspection method and the results of each column column are described in the Inspection Data enclosed in the column package.
In the Inspection Data, N is expressed as that per column.

14-2 Quality Specifications

The shipping specifications of these columns are shown in Table2.

Table 2 Specifications

TSK-GEL type	Cat. No.	Column Sizes (mmID×cmL)	Number of Theoretical Plates (per column)	Asymmetry Factor
TSKgel Chelate-5PW	08645 08646	7.5 × 7.5 21.5 × 15.0	≧ 1,300 ≧ 2,500	0.8 — 1.6 0.8 — 1.6
TSKgel Chelate-5PW Glass	14440 14441	5.0 × 5.0 8.0 × 7.5	≧ 500 ≧ 1,300	0.7 — 1.6 0.7 — 1.6

14-3 Warranty :

Immediately after receipt, check the appearance of the column and test its performance according to the method described in the Inspection Data.
If the guaranteed specifications in Table 2 cannot be obtained, contact your TOSOH representative within two weeks.
Note that column lifetime is not guaranteed.
The specifications of these columns may change without notice for their improvement.

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